SZERKEZETI VIZSGÁLATOK KLOROFILL KOMPLEXEKEN ÉS GASZTRIN ANALÓGOKON SPEKTROSZKÓPIAI MÓDSZEREKKEL

Doktori értekezés

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S z e g e d

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STRUCTURAL INVESTIGATION OF CHLOROPHYLL COMPLEXES AND GASTRIN ANALOGUES USING SPECTROSCOPIC MEGHODS

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THESIS

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I. INTRODUCTION

I.1. INTRODUCTORY REMARKS

One of the major trends discernible in the biological sciences over the past few decades has been the increased utilization of physical methods in the study of complex biological systems. The discovery of many new bioactive molecules has led on to questions concerning their mode of action, and hence their structure within the living environment - i.e. the exact molecular shape they occupy in three dimensional space. Physical techniques are of particular value in the probing and elucidation of the molecular structures of bioactive compounds and a wide range of methods have been described [1]. Often these techniques have the added advantage that they are non--destructive, or require only minute samples, and so compounds may be subjected to further examination. Luminescence, visible-ultra violet spectroscopy and circular dichroism (CD) spectropolarimetry are physical techniques that have gained widespread use in the elucidation of molecular structure and conformation [2-4], and these techniques were employed in this study.

1.2. THE AIMS OF THE THESIS

The aim of the work was to investigate the structure and composition of two groups of compounds using the physical techniques already mentioned. The compounds studied fell into two groups: (i) chlorophyll-polypeptide and chlorophyll-amino acid complexes, which closely model the behaviour of plant pigment complexes, and (ii) analogues of the peptide hormone fragment, tetragastrin, which exhibits all the biological properties of the parent hormone, gastrin. These two classes of compounds may be grouped together for the purposes of this work because the methodological approach used in the study of both groups of compounds was identical.

1.3. LITERATURE SURVEY

1.3.1. Circular Dichroism Spectropolarimetry

CD spectropolarimetry complements absorption spectroscopy by providing information on the stereochemistry of molecules. Two types of physical methods are capable of providing stereochemical assignments of molecules; the first and most direct is X-ray crystallography, the second, and generally more applicable, encompasses the chiroptical method, i.e. optical rotatory dispersion and CD spectroscopy [4-6]. CD is a phenomenon in which a chiral molecule exhibits dissimilar absorption coefficients for left and right-handed circularly polarized light. In order to show a CD effect, known as a Cotton effect, a molecule must be chiral (i.e. have neither a plane of symmetry nor be point symmetric), and secondly, there must be an absorption band in the spectral range of the detecting instrument. The effect will only be apparent close to the absorption maximum. CD is measured by determining the difference in extinction coefficients for left and right-handed circularly polarized light, $\Delta\varepsilon$.

$$\Delta\varepsilon = \varepsilon_L - \varepsilon_R.$$

ε_L and ε_R are the molecular extinction coefficients for left and right-handed circularly polarized light respectively, measured in $mole^{-1}cm^2$ or $M^{-1}cm^{-1}$.

However, CD data are usually reported in terms of molecular ellipticity [θ]. The differential absorptivity

of left and right-handed circularly polarized light results in circularly polarized light becoming elliptically polarized. Therefore the CD effect may be determined by the ellipticity, θ, induced by the medium.

$$\tan \theta = \frac{\text{minor axis of ellipse}}{\text{major axis of ellipse}}$$

$$\theta = \log 10. \frac{180}{4\pi} .C.l(\varepsilon_L - \varepsilon_R) \qquad [22]$$

C = concentration in moles per litre, l = path length in dm, θ = ellipticity in degrees.

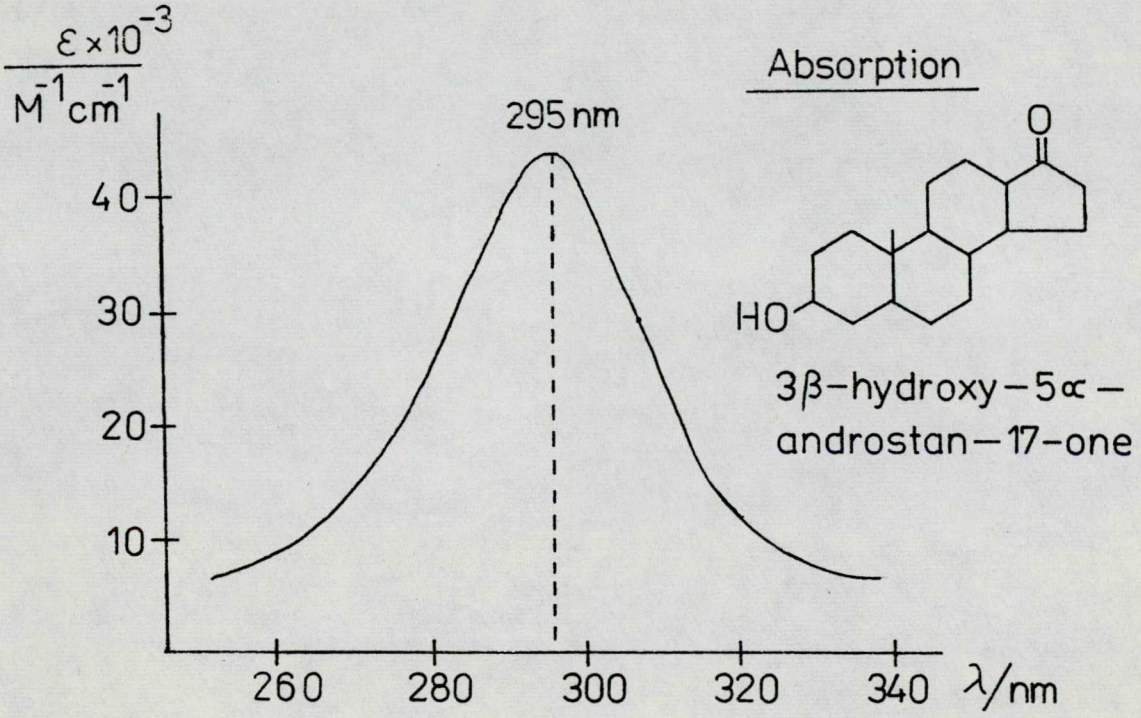
The molar ellipticity is given by: $[\theta] = \frac{\theta .100}{C.l}$

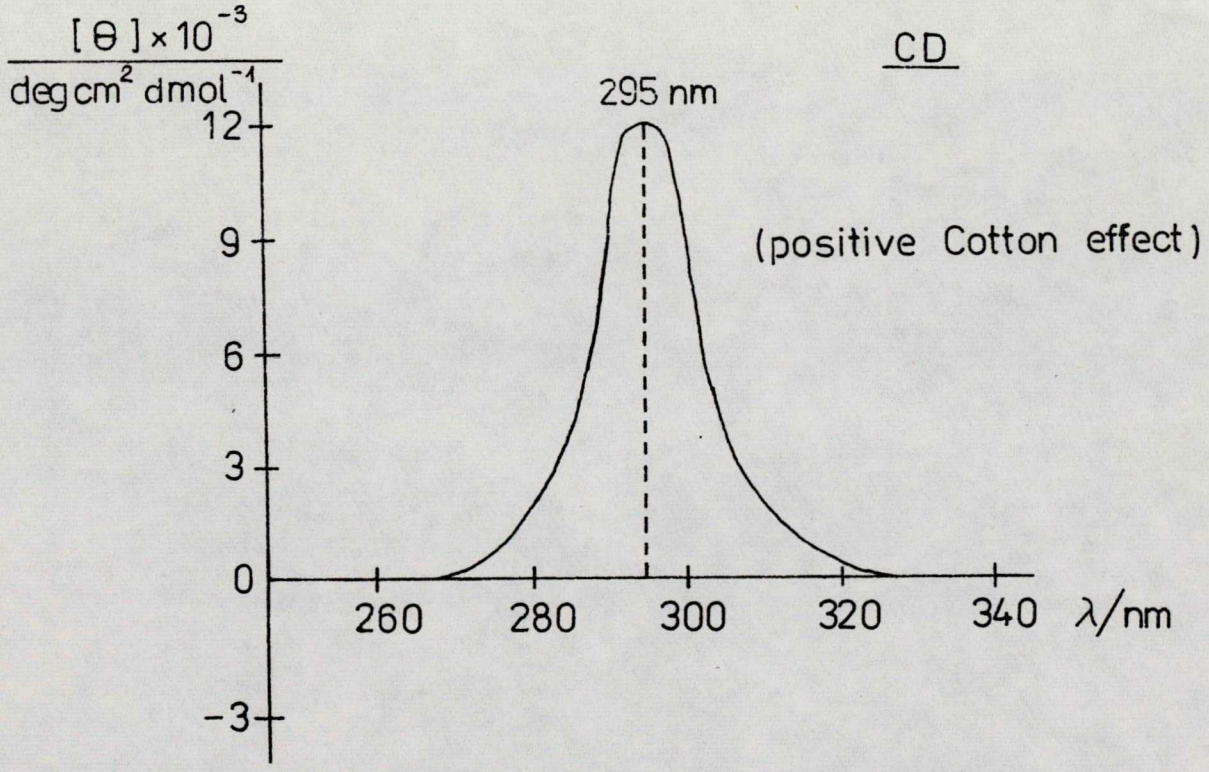
hence $\frac{[\theta].C.l}{100} = \log 10. \frac{180}{4\pi} .C.l(\varepsilon_L - \varepsilon_R)$

$[\theta] = 3299\ \Delta\varepsilon$, $[\theta]$ is in degree cm^2/decimole.

The relationship between absorption and CD spectra is illustrated in Fig. 1.

The spectra shown represent the simplest case of a single transition of an optically active chromophore. Often a simple absorption spectrum will give rise to a more complicated CD picture, for example, an electrically forbidden transition may be clearly visible in the CD spectrum but not be apparent in the absorption spectrum (e.g. the n-π^* transition of the amide chromophore at





redrawn from [7]

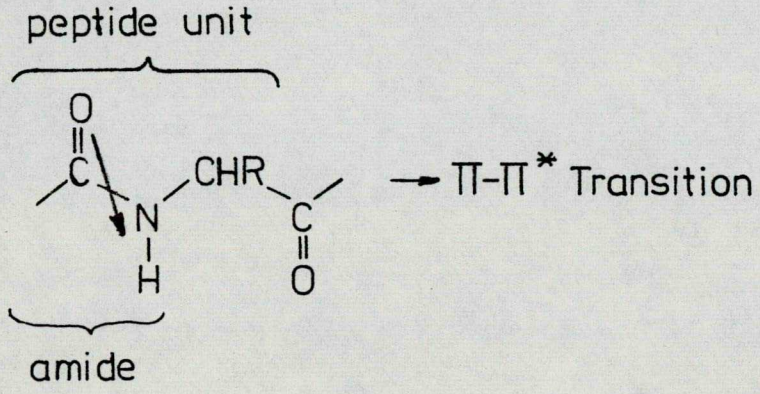
Fig. 1. Relationship Between Absorption and CD Spectra

around 225 nm is electrically forbidden but is magnetically allowed [8]). Another example of an effect giving rise to a more complicated CD spectrum is if the molecule is in a dimer or multimer state, this will result in the CD being split into maxima and minima signals. The spectral characteristics of the split signal can yield information concerning the geometry of the dimer. For example the orientation of the porphyrin rings relative to one another may be derived by examining the CD spectrum of chl-a dimer [54]. The maximum-minimum signal from a chl dimer will be conservative (i.e. the integral areas of the maximum and minimum peaks will be equal) unless there is an extrinsic CD effect whereby chirality is conferred onto a chromophore by a nearby asymmetric molecule. In the case of isolated chl--protein complexes the CD signal observed arises primarily as a result of the chirality conferred to chl from the complexing protein molecule. The intrinsic CD of chl (arising from the 5 chiral centres of chl) is small in comparison to the extrinsic effect.

1.3.2. Use of Circular Dichroism in Determining Peptide Conformation

For an electronic transition to exhibit a Cotton effect it must have both an electric and a magnetic dipole associated with it [9-10]. The CD spectrum of a molecule will be affected by the symmetric state of the molecule,

and hence the conformation. The main chromophore present in the "backbone" of any peptide or protein is the amide chromophore.



The Amide Chromophore within the Peptide "Backbone"

The ultra-violet (UV) absorption spectrum of the amide group has an intense band around 195 nm which is due to the π-π^* transition. The n-π^* band is rarely discernible as this transition is electrically forbidden and its extinction coefficient is two orders of magnitudes smaller. CD shows both transitions [11], but the precise spectrum will be determined by the conformation adopted by the molecule, e.g. poly-L-lysine (pH 11) has a random coil conformation [12], while poly-L-glutamate (pH 3) adopts an alpha-helical structure [13]. The geometric characteristics of the alpha-helix and the other conformations frequently encountered in proteins are described in [1]. The conformation of any peptide backbone may be

described in terms of the dihedral angles of the peptide chain. For a complete description of a molecular conformation it is also necessary to specify the torsional angles of the side chain groups. (I do not mean to suggest that a peptide molecule adopts a rigid, set conformation, bond rotation will always occur but some bonds will allow complete rotional freedom, while others will be severely restricted, e.g. the C-N peptide link). Fig. 2. shows a typical examples of CD-spectra of helical and random proteins.

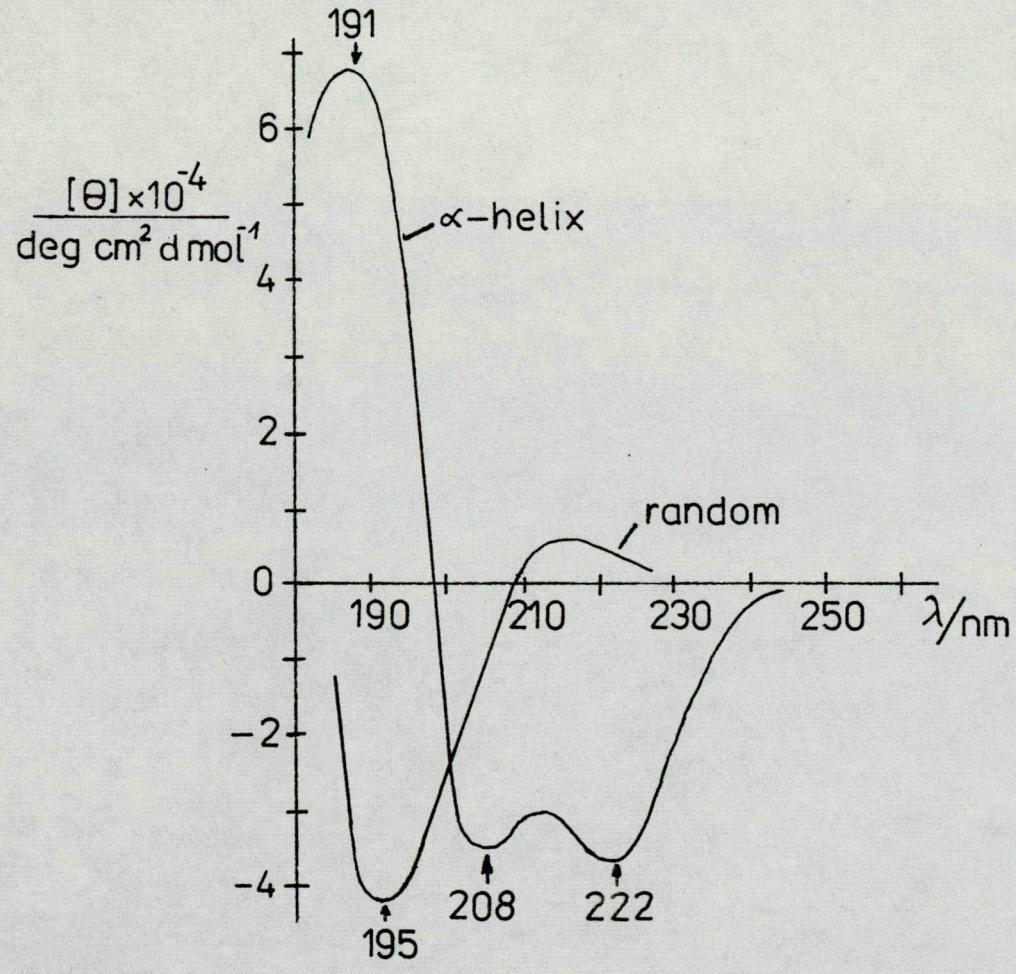
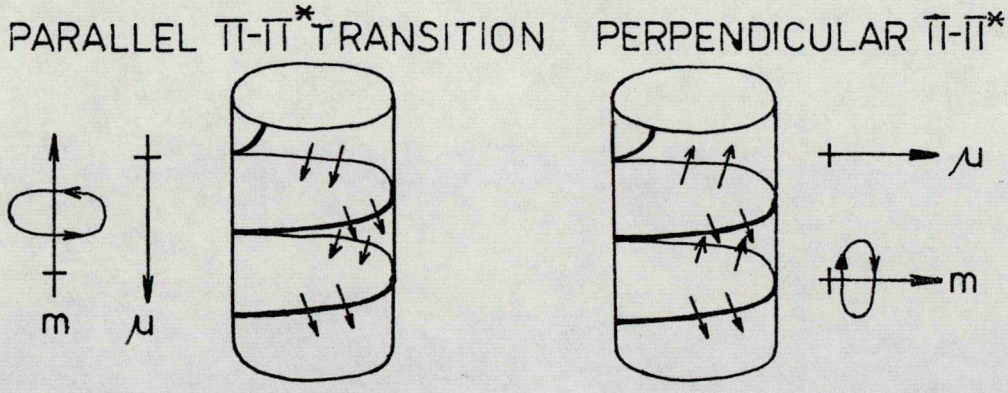


Fig. 2. CD Spectra of alpha-helical and random coil proteins [20].

Proteins adopting the alpha-helical conformation have their amide groups arranged according to the geometry of the helix. Moffitt [14] was the first to recognise that this results in the splitting of the π-π^* transition. When the amide chromophores are coupled together in a helical arrangement two excitation modes are possible: excitation such that the resultant electric and magnetic transition vectors act in a direction perpendicular to the axis of the helix, or such that the resultant electric and magnetic transition vectors act parallel to the axis [15] (Fig.3).



shows electronic moment of π-π^* transition of individual amide groups

shows resultant vector, m magnetic μ electronic

Fig. 3. Splitting of the Amide π-π^* Transition in a Right--Handed Alpha-Helix redrawn from [15]

The CD spectrum of polypeptides with a right-handed alpha helix (i.e. consisting of L amino acids) has a π-π^* parallel band at 208 nm (negative) and the π-π^* perpendicular band at 191 nm (positive). In-fact, any helical conformation, and not just the alpha-helix, will result in a similar splitting of the π-π^* transition. The only difference will be in the position and size of the maxima, indeed with varying degrees of success the π-π^* splitting have been calculated in different helical conformation [16-17].

The discussion above has referred only to polypeptides, however, the same argument applies to short peptides of 4 amino acid residues or more - if the molecule is predominantly in a disordered conformation it will exhibit a random coil-like CD spectrum (small positive peak near 218 nm, large negative peak at around 195 nm). If, however, the molecule exists predominantly in an ordered conformation such as a beta-turn [18] or a gamma-turn [19] then the π-π^* transition will be split and a negative band will be observed above 200 nm, and a positive one below 195 nm. A turn is merely a short helix.

So far only the amide chromophore of the peptide backbone has been considered, however, the side chain groups may also contribute to the CD spectrum. Of special interest are the aromatic amino acid residues (Tyr, Trp and Phe) because additional chromophores are introduced. The

^1L_a and ^1L_b aromatic transitions of Trp, Tyr and Phe occurs in the 250-290 nm region. These transitions are forbidden by symmetry and the molar extinction coefficients are correspondingly low (e.g. Trp 5579 $M^{-1}cm^{-1}$ (279 nm), Tyr 1405 $M^{-1}cm^{-1}$ (275 nm), Phe 195 $M^{-1}cm^{-1}$ (258 nm) [20]). Because the aromatic ring in all these residues is far from the chiral carbon atom (approximately 0·5 nm) it is to be expected that the magnitude of the Cotton effect will be small.

L-Phe $[\theta]_{257nm}$ = 72 deg cm^2/decimole [21]

L-Tyr $[\theta]_{279nm}$ = 1320 deg cm^2/decimole [21]

L-Trp $[\theta]_{276nm}$ = 1900 deg cm^2/decimole [22]

Such small CD signals are difficult to measure accurately and the CD measurements of the aromatic ^1L_a and ^1L_b can only be of qualitative value. A spectropolarimeter will best measure a CD signal where $\Delta\varepsilon/\varepsilon$ is large, a good signal will be produced when this ratio is greater than $1x10^{-2}$ in the 'La transition of Trp at 276 nm

$$\frac{\Delta\varepsilon}{\varepsilon} = \frac{0{\cdot}576\ M^{-1}cm^{-1}}{5579\ M^{-1}cm^{-1}} \simeq 1x10^{-4}$$

and so this transition is not readily measured.

1.3.3. Conformation of Active Gastrin Analogues

The gastro-interstinal hormone gastrin was characterized by Tracy and Gregory [23] as a heptadeca-peptide. Later it was noticised that the entire

physiological action of the hormone is produced by the C-terminal tetrapeptide amide [24]:

H-Trp-Met-Asp-Phe-NH_2

Tetragastrin (TG)

The C-terminal tetrapeptide is the active centre of the hormone and represents the smallest structure able to produce the full range of the biological effects of the parent hormone. Immunological studies [25] indicate that the tetrapeptide adopts a defined conformation. The conformation of TG has been extensively investigated both experimentally [26-28] and theoretically [29-31] [69]. Abbilon et al. [31] performed energy optimization studies on a range of possible conformers and found that the conformation of lowest energy was a gamma–turn at the aspartyl residue (see below). The existence of a gamma–turn at the aspartyl residue results in the aromatic rings of Trp and Phe coming in close proximity to one another, and the conformation is further stabilised by the aromatic-aromatic hydrophobic interaction between them. A gamma–turn contains a hydrogen bond between the amide group of the ith residue and the carbonyl group of the (i+2)th residue. In the proposed model there is a H bond between the -NH- group of phenylalanine and the -CO- group of methionine (Fig. 4).

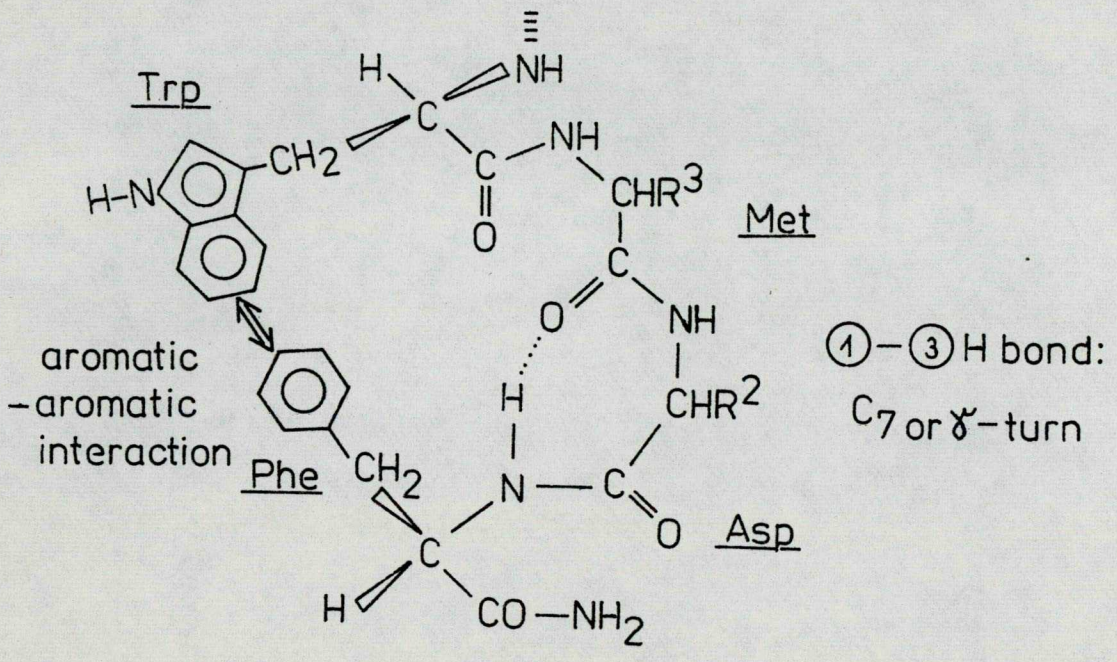


Fig. 4. Proposed Conformation of Tetragastrin

If this conformation of TG is correct the distance separating the aromatic rings is around 0·45 nm. Such an aromatic-aromatic interaction should produce an effect on the CD spectrum. Furthermore, if TG has such an ordered conformation we can expect to observe to splitting of the π-π^* transition.

The CD spectrum of TG has been studied [32], and a positive peak at 193 nm and a negative one at 203 nm were observed showing the presence of an ordered conformation. This, therefore, is in agreement with the proposed structure but does not unambiguously prove it because CD spectra of such a complex molecules are not diagnostic as far as molecular conformation is concerned. Substantial further

evidence that the proposed structure is indeed the conformation adopted by TG would be provided if it were possible to show a positive correlation between CD spectra and biological activity in active analogues of TG. In this study a series of TG analogues, both active and inactive, were examined by CD spectropolarimetry.

1.3.4. Chlorophyll Complexes and their Models

The other group of compounds studied were chlorophyll--amino acid and chlorophyll-polypeptide complexes. These complexes act as models for in-vivo chlorophyll-protein complexes. Chlorophyll (chl) present in chloroplast thylakoid membranes occurs in association with specific protein called chl-binding proteins. It has been shown [33] that practically all the chl present in thylakoid membranes is bound in proteins complexes. The exact photophysical and photochemical properties of a chl molecule is determined by its environment in the thylakoid membrane. In particular the orientation of the Qy porphyrin absorption dipole relative to the membrane plane is important - the plane of the Qy dipole in short wavelength absorbing forms of chl-a is almost parallel to the membrane plane, while in longer wavelength absorbing forms the angle between the planes is greater [34-35]. Recently

it has also been suggested that the presence of charged amino acids close to the porphyrin ring may also affect the absorption properties of chl [36]. Hence the spectroscopic properties of chl-a in-vivo is determined by the interaction of chl with proteins and specific amino acids.

Pigment-protein complexes are one of the major constituents of the photosynthetic apparatus, clearly an understanding of their structure and organization is essential if the photosynthetic process is to be understood. For some time it has been possible to reproducibly resolve two chl-protein complexes from plant material, CPI and CPII [37]. Numerous papers detail the extraction and resolution procedures used in blue-green algae [38-39] and in higher plants [40-41]. Essentially these preparations rely on detergents to solubilize chl--protein complexes out from the photosynthetic membrane followed by separation by SDS-PAGE, sucrose gradient centrifugation, or ion-exchange chromatography. The detergent solubilizes the complexes by disrupting the hydrophobic bonds between lipids and proteins that hold the complex in place in the membrane. However detergent treatment may also give rise to interaction between chl complexes and detergents as noted in [42-43].

One of the trends in modern photophysical research of photosynthetisis has been towards a study of re-

constituted and artificial pigment-protein complexes [44]. Chl-protein complexes isolated from plants show a suprising universality in their spectroscopic properties, for example, the red absorption maximum of isolated CPI alway is found between 674-678 nm [37]. This offers encouragement for the preparation of artificial chl complexes that will act as models of in-vivo complexes. The modelling of chl complexes is not new; milk proteins [45], casein [46], bovine [47] and human serum albumin [48] have been used to study interactions between chl and proteins. In other studies [49-50] chlorophyllide - apomyoglobin complexes have been prepared; the replacement of chl by chlorophyllide enhances complex solubility in water. Following the preparation of the above mentioned chl-protein complexes it seemed appropriate to attempt to prepare chl-a-amino acid complexes. These complexes would also be expected to closely model the behaviour of plant chl complexes. Spectroscopic measurements would yield information on the composition and molecular structure of these complexes, and the effect of detergents could be tested.

2. MATERIALS AND METHODS

2.1. PREPARATION OF CHLOROPHYLL-COMPLEXES

First it was necessary to prepare chl-containing liposomes. Chl-a was extracted from spinach and separated from chl-b and other plant pigments by sucrose column chromatography as described in [51]. It was important to make measurements of prepared complexes in aqueous solution and the main problem was to prevent chl aggregation. Chl-a was solubilized by incorporating it into lecithin liposomes.

0·008 g lecithin (Reanal) was weighed out and dissolved in approximately 2 ml diethyl ether. 1·8 mg of chl-a was added as 1·62 ml of a $1{\cdot}23\times10^{-3}$ M solution in ether. The mixture was evaporated to dryness by bubbling through nitrogen gas. 8 ml of $5{\cdot}0\times10^{-2}$ M Tris-hydroxymethyl-aminomethane, pH 7·2, (Tris) buffer was added to the dry solid. The mixture was subjected to 25x45 sec bursts of ultra sound using a MSE 150 Watt sonicator over a period of 20 minutes. The amplitude setting was 18,000 microns, the sample tube was kept immersed in ice/water to prevent excessive warming. The concentration of chl-a was $2{\cdot}50\times10^{-4}$ M. The absorption spectrum of the sample was taken to check for chl aggregates which absorb around 700 nm. If a peak was present here the sample was subjected to a further period of sonication. Exposure to light of all chl-a containing solutions was avoided wherever possible.

COMPLEX PREPARATION

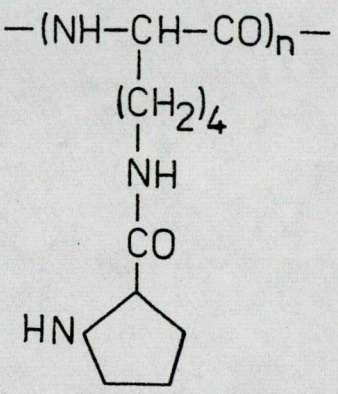
Complexes were prepared by adding the various ligands to the chl-liposome (C-L) preparation. The procedure adopted for the amino acids tryptophan, proline and lysine, and the polypeptide poly-proline-poly-lysine are described below.

Amino Acids

A 1.0×10^{-3} M aqueous solution of D,L-tryptophan, D,L-proline or D,L-lysine (Reanal, Budapest) was prepared. 4 ml of the amino acid solution was mixed with 4 ml of the C-L preparation, the chl–a:amino acid ratio was 1:4 and the concentration of chl–a was now $1{\cdot}25 \times 10^{-4}$ M. After shaking the amino acid-chl–a solution was allowed to stand for 30 minutes, and then was chromatographed on a G25 Sephadex column (V_t = 15 ml, internal diameter = 2 cm). Prior to sample application the column was equilibrated with Tris buffer, and following sample application it was eluted with the same buffer. 1·5 ml fractions were collected and those in which Abs (235 nm)/ Abs (674 nm) = 1·5-2·0 were pooled and used in further studies.

Poly-proline-poly-lysine

The general structural formula of this 'brush'-polymer polypeptide is shown below.



In the compound used n was between 70 and 90, the molecular weight of individual molecules therefore varied between 15 K Da and 20 K Da. (This compound was a gift from the Department of Medical Chemistry, Medical University of Szeged (SzOTE)).

0·014 g ($8·0x10^{-7}$ moles) of poly-Pro-poly-Lys was weighed out and carefully added to 3 ml of C-L solution. The polypeptide was allowed to fully dissolve in the C-L solution, the chl–a:polypeptide ratio was near to 1:1 and the concentration of chl–a remained $2·50x10^{-4}$ M. The solution was allowed to stand for 30 minutes at room temperature and chromatographed on a G100 Sephadex column (V_t = 17 ml, internal diameter = 2 cm). Tris buffer was used both to equilibrate and elute the column. 1·5 ml fractions were collected and those in which Abs (235 nm)/ Abs (674 nm) = 1·5-2·0 were pooled and used in further studies.

DETERGENTS

The effect of two detergents were tested on the prepared complexes:

(1) Sodium Dodecyl Sulphate (SDS) (Fluka AG, Buch SG, Switzerland)

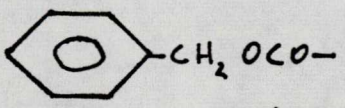
(2) Digitonin (Dig) (Reanal, Budapest)

Both detergents were prepared as a $1 \cdot 0 \times 10^{-2}$ M solution in Tris buffer (above the critical micellar concentration) and mixed at a ratio of 1:1 with the complexes. The mixture was allowed to stand for 60 minutes prior to making measurements.

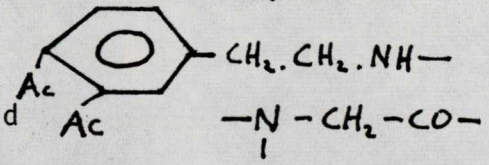
2.2. PREPARATION OF GASTRIN ANALOGUES

Analogues examined are listed below using the standard 3 letter abbreviation for amino acids. Unless otherwise stated all amino acids have an L configuration.

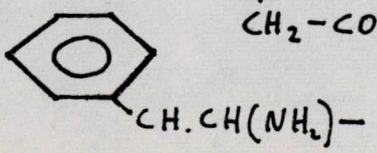
	Analogue Number	Biological Activity
H-Trp-Met-Asp-Phe-NH_2	1	Active
Z-Met-Asp-Phe-NH_2	2	Active
BOC-Trp-Met-Asp-PEA	3	Active
BOC-Trp-Leu-Asp-HVA	4	Inactive
BOC-Trp-Met-Glu-Phe-NH_2	5	Inactive
Ac-Tyr-Met-DAla-Trp-Met-Asp-Phe-NH_2	6	Active
Ac-Tyr-Met-Gly-DTrp-Met-Asp-Phe-NH_2	7	Inactive
Ac-Tyr-Met-Gly-Trp-DMet-Asp-Phe-NH_2	8	Active
BOC-DAla-Trp-Met-Asp-Phe-NH_2	9	Active
BOC-Trp-Met-Asp(OBu^t)-Phe-OH	10	Inactive
BOC-IDE-Trp-Met-Asp-Phe-NH_2	11	Inactive
BOC-Trp-Met-Asp-Tyr(CH_3)-NH_2	12	Active

Z = benzyloxycarbonyl 

BOC = tertiary butyloxycarbonyl $Bu^t OCO-$

HVA = homoveratrilamine 

IDE = iminodiacetic acid

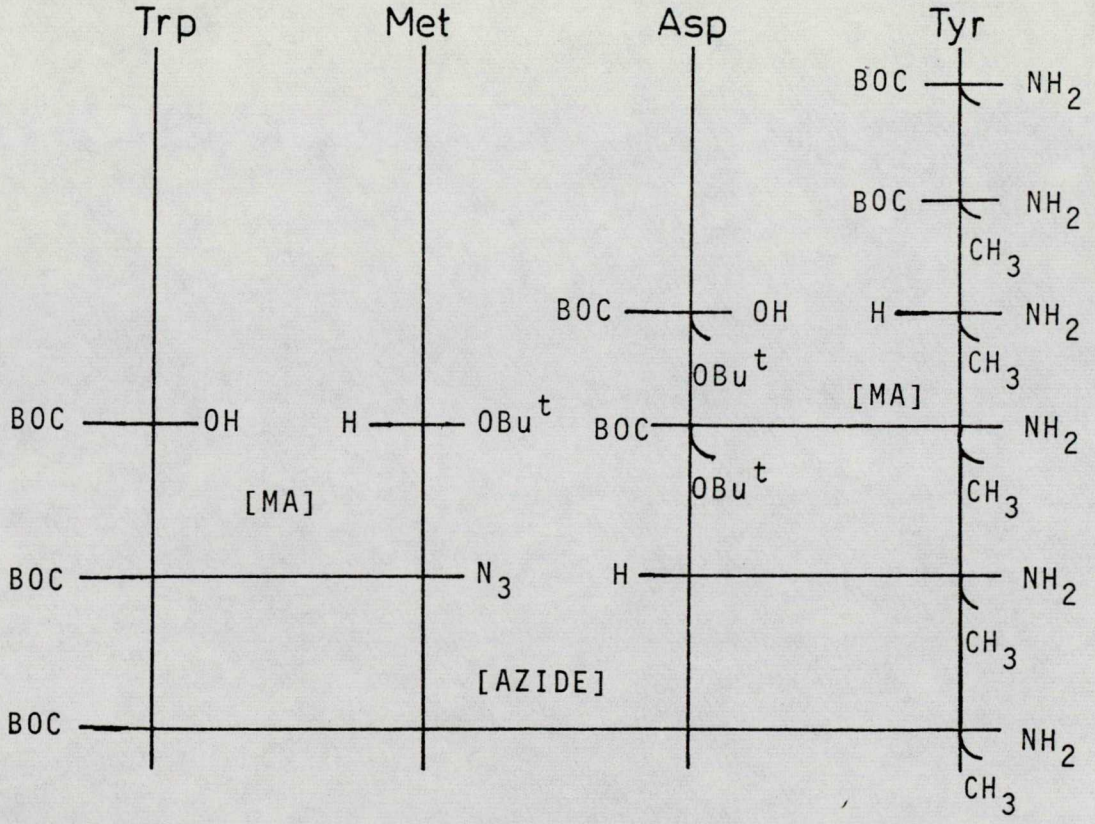
PEA = phenyl-ethylamine 

Ac = acetyl

Biological activities were determined by the bioassay of Halter et al. [21] measuring acid secretion conductometrically in rat stomach. With the exception of analogue no.12 all peptides were kindly provided by the Department of Medical Chemistry, Medical University of Szeged (SzOTE), Szeged, Hungary.

Synthesis of BOC-Trp-Met-Asp-Tyr (CH_3)-NH_2

The synthesis scheme of tertiary butoxylcarbonyl--tryptophyl-methionyl-aspartyl-O-methyltyrosylamide is detailed below.



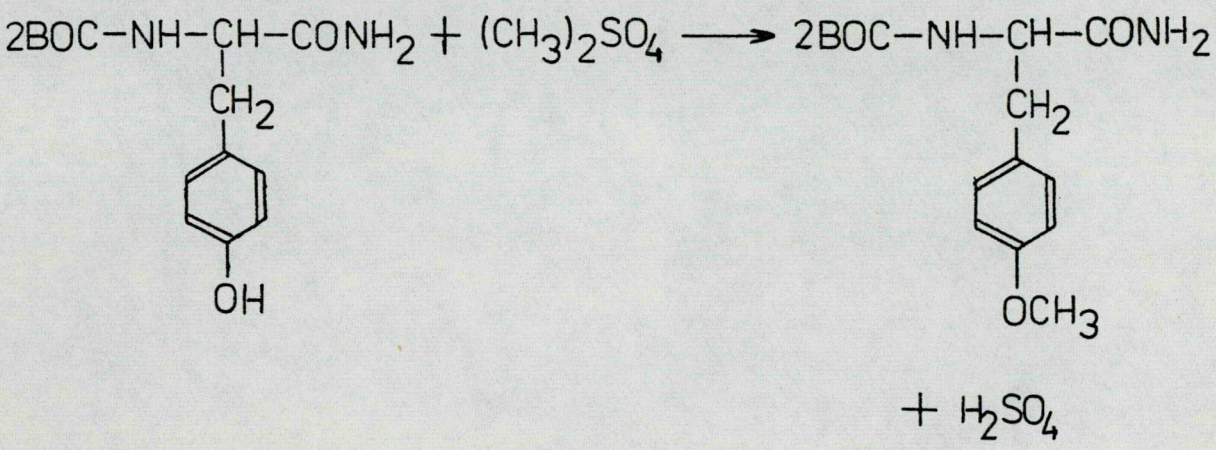
Peptide bond forming methods used were the mixed anhydride method (MA), and the azide method (AZIDE). With the exception of step I, standard methods were used as described in the literature [55]. Step I was a new modification of the O-methylation of tyrosine described in [56].

O-Acylation of tertiany butoxycarbonyl tyrosine amide

2·8 g BOC-Tyr-NH_2 (10 m moles) was dissolved in 15 ml absolute methanol and cooled to 0°C. Sodium methoxide solution was prepared (345 mg (15 m moles) metallic sodium was dissolved in 15 ml dry methanol under cooling) and added to the BOC-Tyr-NH_2 solution. 1·4 ml of dimethyl sulphuric acid (15 m moles) was then added.

The reaction was allowed to proceed for 10 minutes at 0°C and then the reaction mix was adjusted to pH 10 with 2 M sodium hydroxide. The reaction was allowed to go to completion overnight.

TLC in 8:1:1 (chloroform:methanol:acetone) R_f = 0·9 (R_f of BOC-Tyr-NH_2 = 0·6).



Product was precipitated by adding water, filtered, washed and dried.

Yield = 1•9 g or 68 %.

2.3. SPECTROSCOPIC MEASUREMENTS

Absorption Spectra

Absorption spectra were made using either a Unicam SP 1800 spectrophotometer (range 840-200 nm) or a Spectrocord UV-VIS instrument (range 900-200 nm). Spectra were taken against an appropriate blank and a cuvette was chosen such just that the maximum absorbance was always between 0·4 and 1·0. Measurements were made at room temperature.

Fluorescence Spectra

Fluorescence spectra were obtained using a Perkin-Elmer MPF-44A spectrofluorimeter. Measurements were made using a 0·3 cm path length cuvette, and samples were diluted using Tris buffer (typically the chl-a concentration was $1{\cdot}0 \times 10^{-4}$ M) in this way re-absorption effects were avoided. Measurements were made at room temperature.

Circular Dichroism Spectra

CD spectra were made on a Jasco J-40 C (single beam) spectropolarimeter (range 1000-190 nm). Spectra were recorded at the ambient temperature of the instrument, 30^{o}C. The cuvette path length was chosen such that the maximum absorbance of the sample was between 0·7 and 1·2 (ideally 1·0).

Chl complexes requiring dilution were diluted in Tris buffer.

Gastrin analogues are largely insoluble in water so 2,2,2-trifluoroethanol (TFE)(Sigma) was used. This solvent was chosen because of its transparency in the far UV region, and there is some empirical evidence to suggest that TFE mimics the natural environment of membrane proteins, inasmuch the CD spectra of these proteins in 80% TFE were similar to spectra of membrane proteins in their natural environment [52-53]. TFE was distilled once to remove any contaminating alcohols.

3. RESULTS

3.1. CHLOROPHYLL-AMINO ACID COMPLEXES

3.1.1. Absorption Spectra

The absorption spectra of the prepared chl-a complexes are shown in Figs 5-8. Of particular interest is the position of the red absorbance maximum of chl-a in various environments, as shown in table 1.

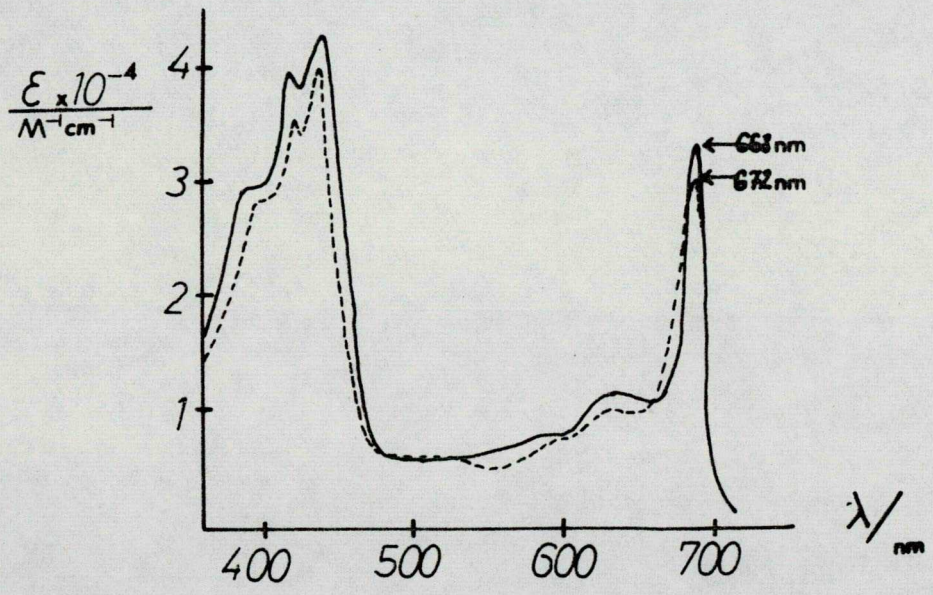


Fig. 5. Absorption spectra of chl-a containing liposomes —— and chl-a-Trp/liposome ---

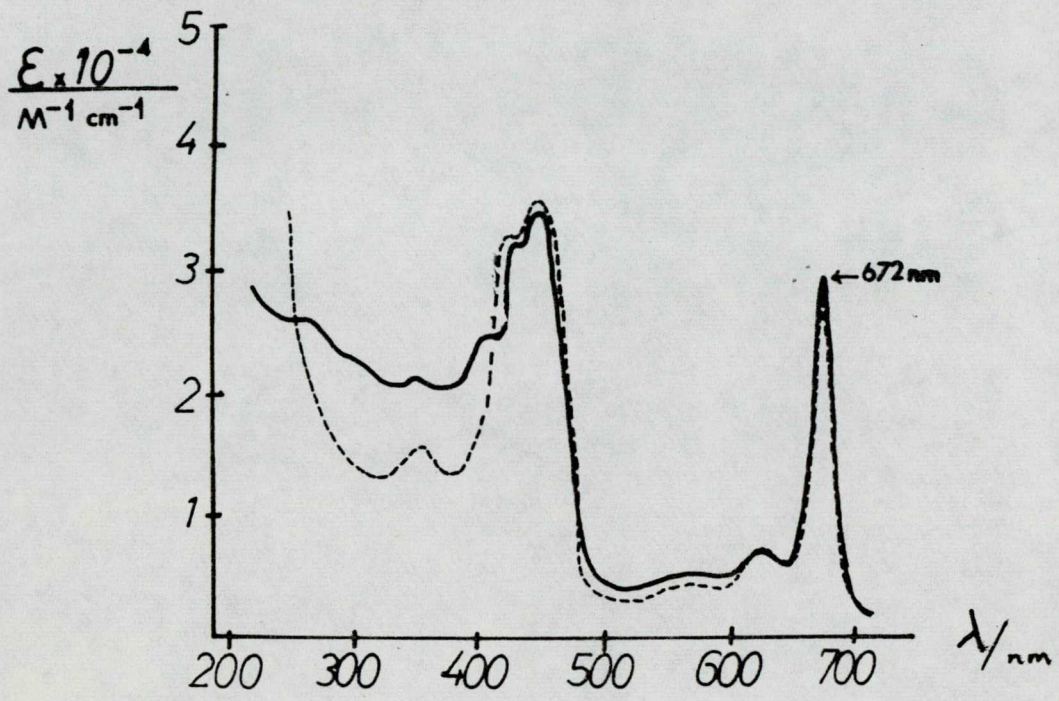


Fig. 6. Absorption spectra of chl-a-Lys/liposome and chl-a-Pro/liposome ——; and Chl-a-poly-Pro-poly-Lys/liposome ---

Table 1. POSITION OF RED ABSORPTION MAXIMUM OF Chl-a IN VARIOUS ENVIRONMENTS

Material	λ_{max}/nm	
chl-a in dry acetone	663	[57]
chl-a in-vivo	678	[58]
chl-a containing liposomes	668	
chl-a-Trp/liposome	672	
chl-a-Lys/liposome	672	
chl-a-Pro/liposome	672	
chl-a-poly-Pro-poly-Lys/liposome	673	
chl-a-HSA/liposome	674	[48]

The red absorption maximum of chl-a shows a definite shift towards longer wavelengths when compared to uncomplexed chl-a as existing in dry acetone solution. The red shift to 672 nm cannot be explained merely by the inclusion of chl-a in a liposome system because the red absorption maximum of chl-a in a liposomic environment is found at a significantly lower wavelength at 668 nm. The existence of such a large bathochromic shift is strong evidence that the chl-a molecule is complexed [49]. Further evidence indicating that chl-a is part of a complex is provided by the fact that in the samples chl-a is present in an aqueous medium; free, uncomplexed chl would immediately be precipitated out of such an environment.

The effect of detergents on the absorption spectra is of interest because of the extraction techniques used in the isolation of plant chl-protein complexes. Two detergents were tested, SDS and Dig. Fig. 7 and 8 shows the absorption spectra of complexes following detergent treatment.

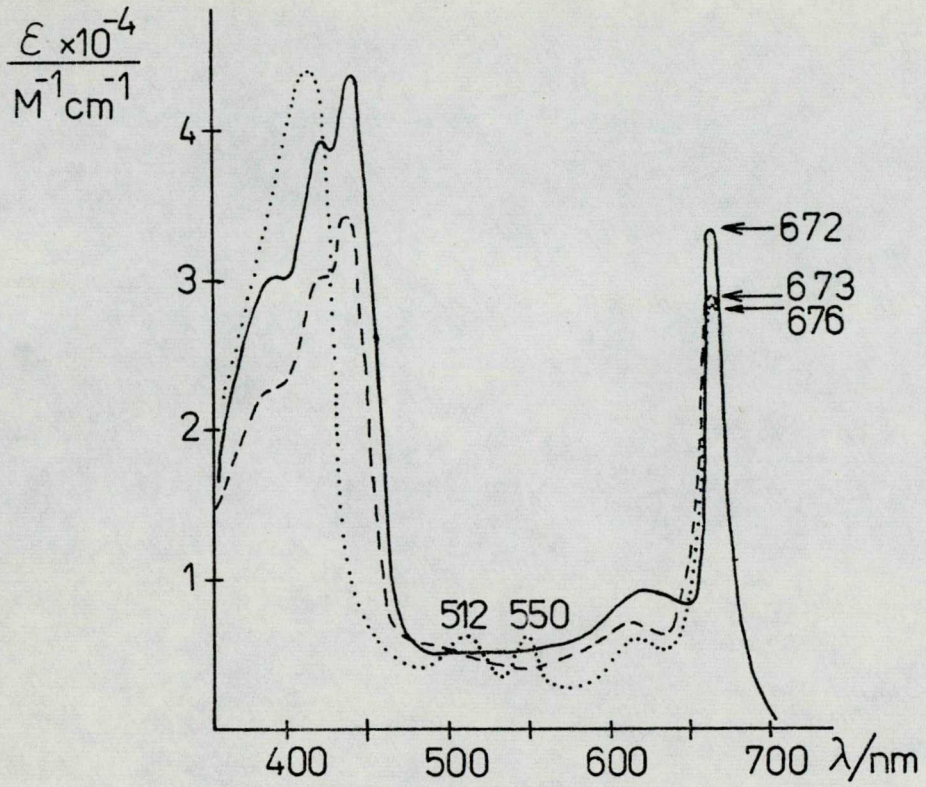


Fig. 7. Absorption spectra of chl-a-Lys/liposome ——; + $1{\cdot}0\times10^{-2}$ M SDS ··· ; + $1{\cdot}0\times10^{-2}$ M Dig – – –

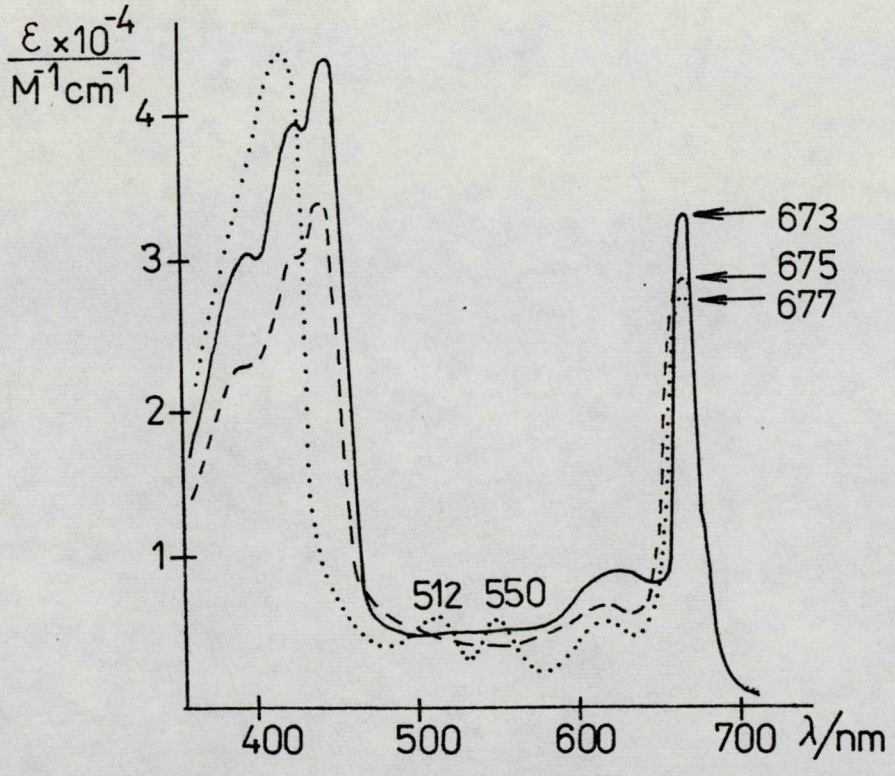


Fig. 8. Absorption spectra of chl-a-poly-Pro-poly-Lys/liposome ——; + $1{\cdot}0\times10^{-2}$ M SDS ···; + $1{\cdot}0\times10^{-2}$ M Dig – – –

Table 2. POSITION OF RED ABSORPTION MAXIMUM OF Chl-a IN VARIOUS ENVIRONMENTS AFTER TREATMENT WITH DETERGENTS

Material	λ_{max}/nm	
	SDS	Dig
chl-a in dry acetone	663	663
chl-a containing liposomes	669	669
chl-a-Trp/liposome	675	674
chl-a-Lys/liposome	676	673
chl-a-Pro/liposome	676	674
chl-a-poly-Pro-poly-Lys/ liposome	677	675

What is particularly noticeable is that the non-ionic detergent Dig does not induce a large change in the position of the red maximum, while the anionic detergent SDS does alter its position. A further effect of SDS is to cause the appearance of small peaks at 512 nm and 550 nm. The appearance of these peaks is a clear indication that a significant portion of chl-a in the complex has undergone pheophytinization. Dig does not induce these change, the only effect of this detergent is to cause a general decrease in absorption as well as a broadening of the absorption bands above 350 nm. In the UV region little change is produced by either detergent.

3.1.2. Fluorescence Spectra

Fluorescence emission spectra were obtained exciting the sample at an appropriate wavelength in the UV where, in addition to chl-a the amino acid or polypeptide also absorbs. In the case of chl-a-Trp excitation was at 280 nm (in the aromatic π-π^* band), while all other complexes were excited at 235 nm.

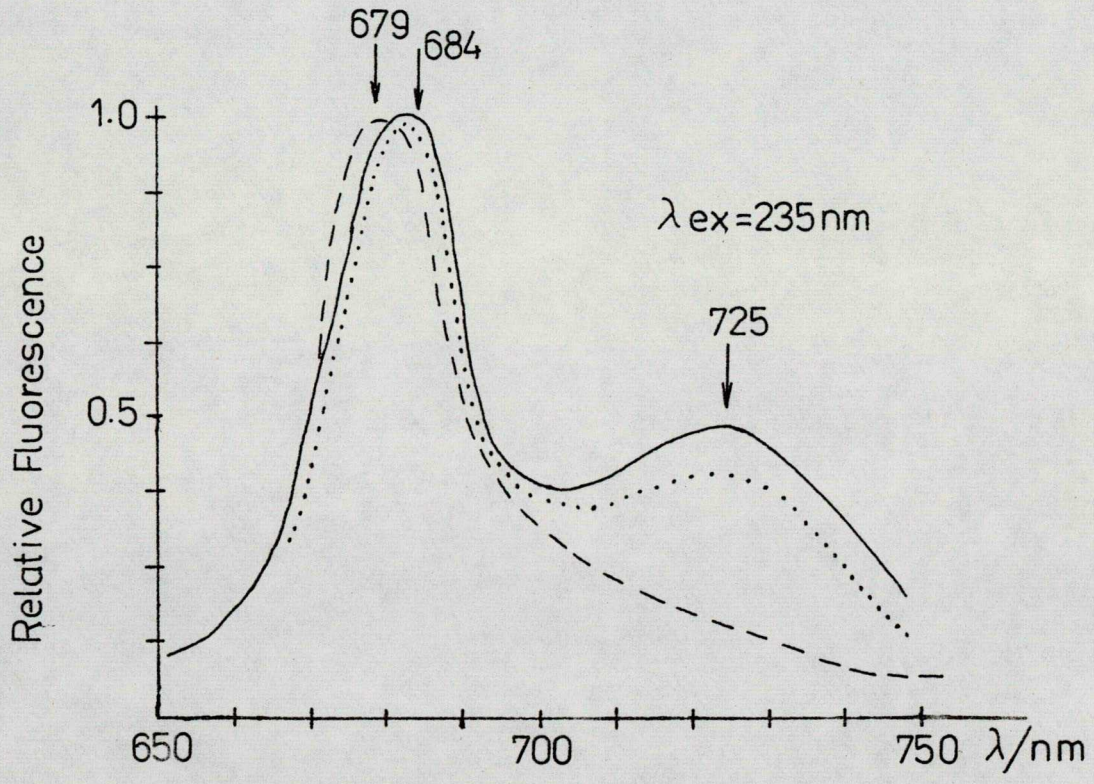


Fig. 9. Fluorescence spectra of chl-a-Lys/liposome and chl-Pro/liposome ——; + $1{\cdot}0\times10^{-2}$ M SDS ---; + $1{\cdot}0\times10^{-2}$ M Dig ...

λ_{ex} = 235 nm

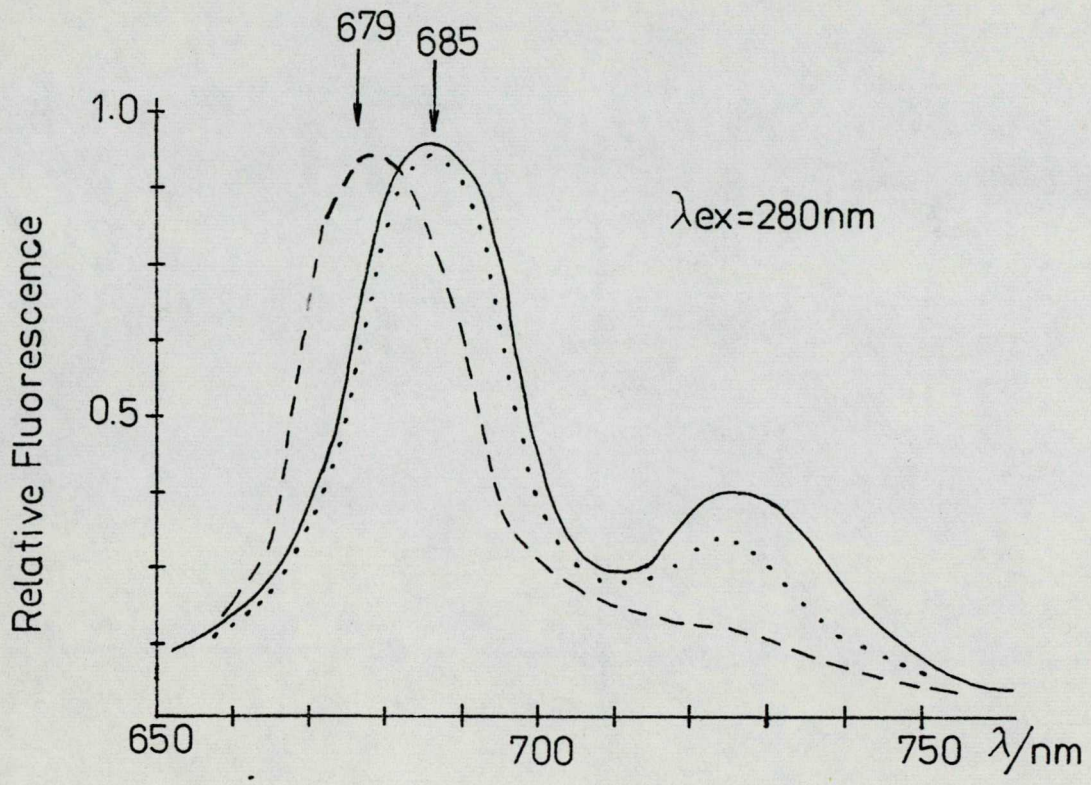


Fig. 10. Fluorescence spectra of chl-a-Trp/liposome ——; + $1{\cdot}0\times10^{-2}$ M SDS ---; + $1{\cdot}0\times10^{-2}$ M Dig ...

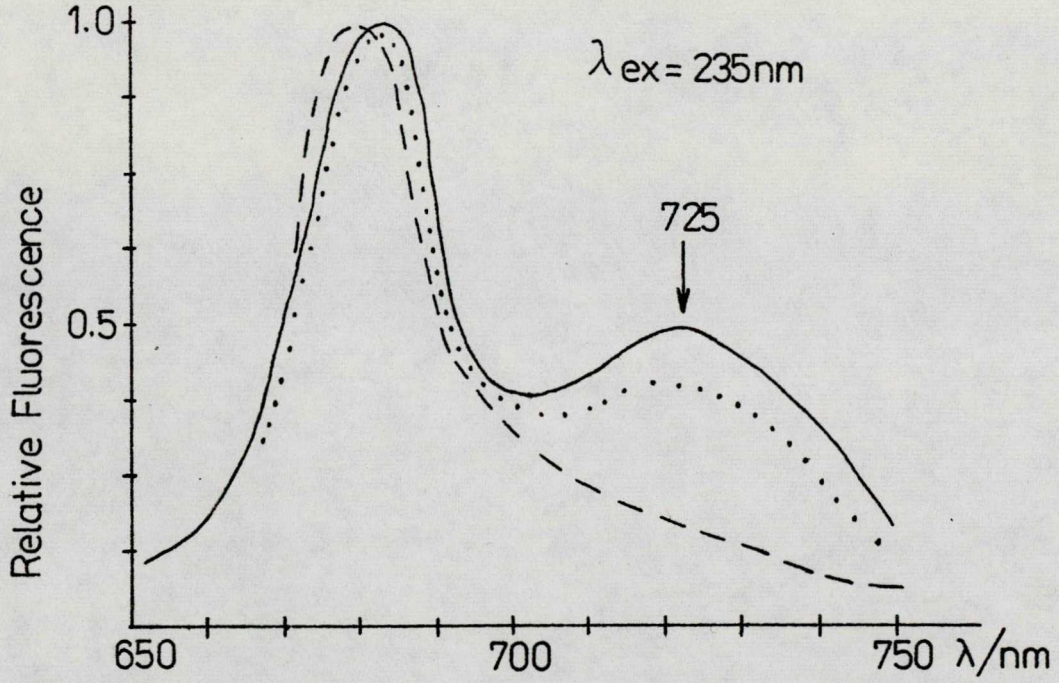


Fig. 11. Fluorescence spectra of chl-a-poly-Pro-poly-Lys/liposome ——; + $1{\cdot}0\times10^{-2}$ M SDS ---; + $1{\cdot}0\times10^{-2}$ M Dig ...

The fluorescence spectra of chl-a complexes shows two bands at 683 and 725 nm. It is significant that the long wave fluorescence is quite pronounced, and is greater than can be accounted for by vibrational long wave fluorescence. The increase in the long wave fluorescence to above the vibrational level when the sample is excited at 235 nm (or 280 nm) is attributable to energy transfer from the amino acid/polypeptide to chl-a, and hence the existence of a complex is strongly supported.

The effect of detergents is of interest; when the complex is excited at 235 nm (or 280 nm) SDS treatment results in the loss of the enhanced long wave fluorescence, and only the residual vibrational component remains. With Dig treatment there is a smaller decrease in this fluorescence.

The fluorescence excitation spectra (Fig. 12) shows that the fluorescence at 686 nm originates primarily from the 440 nm absorption band rather than the 420 nm band.

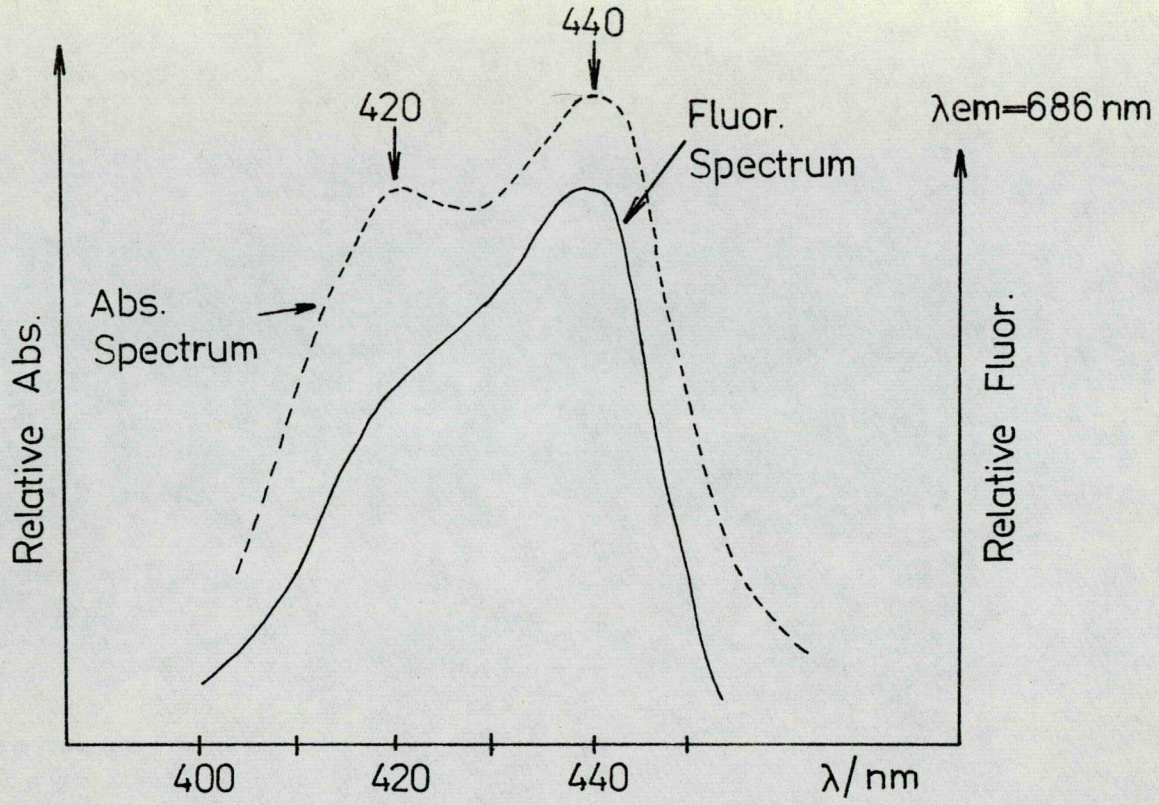


Fig. 12. Fluorescence excitation spectrum of chl-a-poly--Pro-poly-Lys/liposome
λ_{em} = 686 nm. Absorption spectrum also shown.

3.1.3. Circular Dichroism Spectra

Fig. 13 shows the CD spectrum of chl-a-poly-Pro-poly--Lys in the UV range.

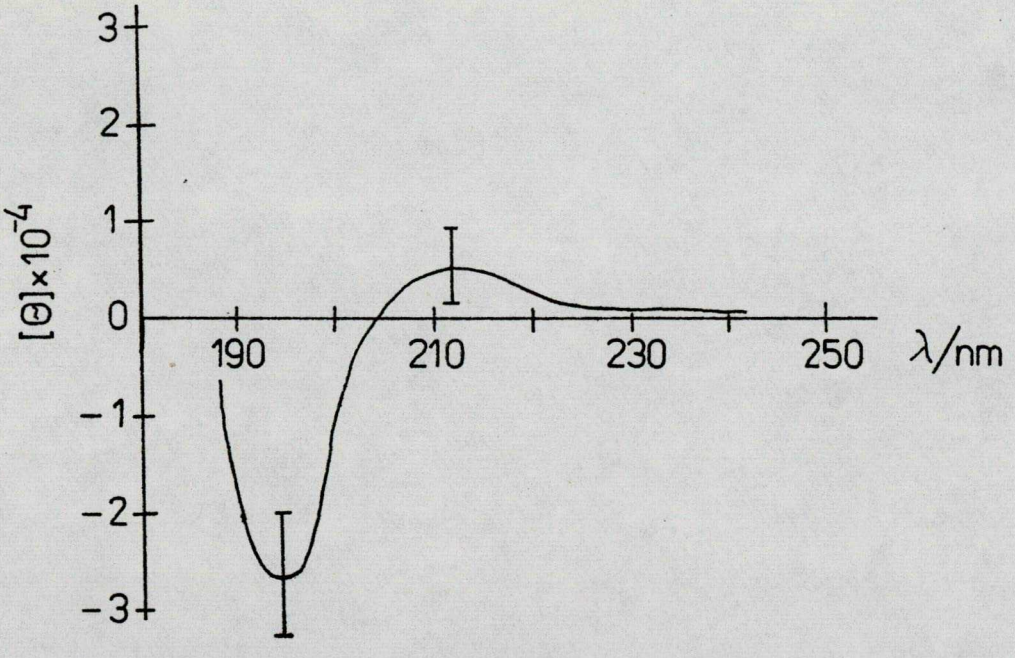


Fig. 13. CD spectrum of chl-a-poly-Pro-poly-Lys/liposome

The negative peak at around 195 nm clearly indicates that the polypeptide adopts a random coil conformation. As expected detergents have no effect on this. The vertical bars show the noise level of the recording instrument, this increases towards lower wavelengths.

Fig. 14 shows the CD spectrum in the red absorption region.

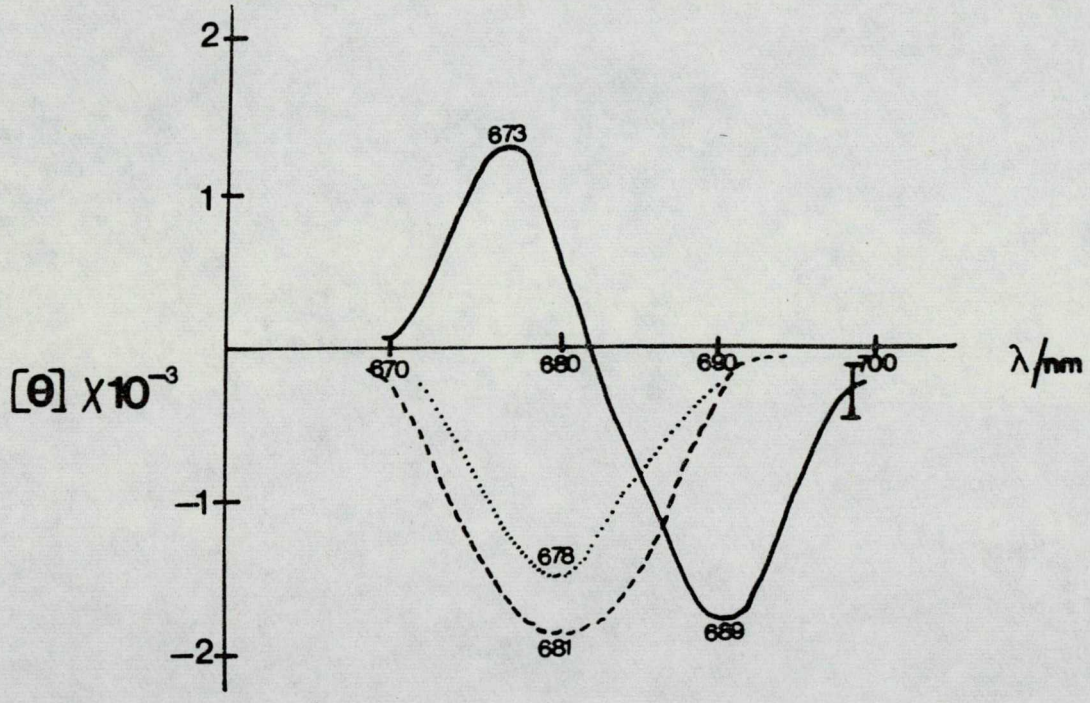


Fig. 14. CD spectrum of chl-a-poly-Pro-poly-Lys/liposome ——; + $1{\cdot}0\times10^{-2}$ M SDS ---; + $1{\cdot}0\times10^{-2}$ M Dig ...

The CD curve of the untreated complex shows a maximum at 673 nm and a minimum at 689 nm. This type of split signal is indicative of a chl-a-chl-a dimer [54]. It is also worth noting that the signal is non-conservative as

the area of the minimum is somewhat larger than the area of the maximum peak. The non-conservative nature of the dimeric signal is accounted for by the existence of an extrinsic CD component that confers chirality onto the porphyrin chromophore. The presence of a nearby polypeptide or amino acid asymmetrically perturbs the environment of the porphyrin nucleus,and this results in a non-conservative signal. The magnitude of the dimeric signal is much greater than could originate from the chiral carbon atoms of chl-a, the enhancement of the CD signal is extrinsic in origin. Hence the presence of a polypeptide/amino acid close to the porphyrin ring affects both the shape and size of the CD spectrum.

Detergent treatment results in the replacement of the dimeric signal by a single negative peat at 678 nm (in the case of Dig) and 681 nm (in the case of SDS). Both detergents cause the dissociation of the chl dimer as the absence of a split signal shows. The effect of the two detergents on the poly-Pro-poly-Lys complex differs somewhat as the CD signal maximum of the Dig treated complex occurs at 678 nm, while that for SDS at 681 nm.

3.2. GASTRIN ANALOGUES

CD spectra of gastrin analogues are shown in Fig. 15-23. All spectra were taken in TFE solution. CD is expressed as molecular ellipticity in deg cm^2/d mol.

Synthetic Human Gastrin 1 (Fig. 15)

H-Glu-Gly-Pro-Trp-Leu-$(Glu)_5$-Ala-Tyr-Gly-Trp-Met-Asp-
-Phe-NH_2

The hormone exhibited a CD curve with a positive band centred around 194 nm and two negative bands at 208 and 222 nm. This curve type is similar to poly-L-glutamate (pH 3), which has been shown to adopt an alpha-helical conformation. The 4-11 peptide fragment(-Trp-Leu-$(Glu)_5$-
-Ala-)is strongly alpha-helix forming as evaluated by Chou and Fasman [59]. The negative band at 222 nm is largely the result of the n-π^* amide transition [11], but the ^1B_b transition of Trp derivatives also shows a signal at this wavelength [8]. The near UV region gives a positive signal.

BOC-Trp-Met-Asp-NH_2 (TG) (Fig. 16)

The absence of the alpha-helix forming 4-11 peptide fragment results in a substantial reduction in the CD signal. The continued presence of a positive band at 193 nm and a negative band at 205 nm, showing that the

π-π^* amide transition is split, indicates that the peptide adopts an ordered conformation. The negative peak at 218 nm is most likely the ^1B_b indole transition. In most tryptophan derivatives (e.g. N-acetyl-L-tryptophan ethyl ester [8] or H-Gly-Phe-$(Gly)_2$-Trp-Gly-OH [60]) the CD signal of the ^1B_b transition is positive, however the same transition contributes a negative signal in the peptide H-Gly-Phe-Trp-Gly-OH [60]. It is thought [60] that the reversal in sign is caused by the presence of an aromatic interaction between Trp and Phe. The appearance of a band with negative ellipticity around 218 nm would appear to lend support to the existence of a conformation in which the Trp and Phe residues are close to one another, such as is illustrated in the introduction (Fig. 4). The signal in the near UV region arises from the L_b and L_a bands of Trp and Phe, the net contribution is negative.

CD spectra broadly similar to Fig. 11 will be referred to as TG-like.

Z-Met-Asp-Phe-NH_2 (Fig. 17)

The CD curve here shows a maximum at 193 nm and a minimum at 206 nm indicating that the peptide adopts an ordered conformation as shown by the splitting of the π-π^* amide transition. As this peptide does not have a Trp residue included in it, no separate peak can be

observed around 220 nm. The near UV region shows negative ellipticity.

BOC-Trp-Met-Asp-PEA (Fig. 18)

The CD curve of this analogue is extremely similar to TG with a positive peak at around 190 nm and two negative peaks near 203 nm and 220 nm. The analogue is an active one.

BOC-Trp-Leu-Asp-HVA (Fig. 19)

This analogue shows an entirely different type of CD curve, negative ellipticity is observed at a peak centred around 195 nm. This is clearly a random conformation, and the analogue is biologically inactive. (It is not entirely clear why the ^1B_b Trp band at 218 nm give rise to a negative CD signal, it may be that inspite of adopting a random conformation some sort of interaction exists between Trp and the aromatic ring in the HVA group).

BOC-Trp-Met-Glu-Phe-NH_2 (Fig. 20)

This analogue shows a TG type CD curve, but is inactive. It may be noticed that this analogue differs from TG only by the substituation of a glutamyl residue in the place of the aspartyl one.

Ac-Tyr-Met-DAla-Trp-Met-Asp-Phe-NH_2 (Fig. 21)

Shows a TG-like CD curve. The addition of the Ac-Tyr-Met-DAla-peptide fragment to TG does not alter the conformation of the C terminal tetrapeptide (TG). The peptide is active.

Ac-Tyr-Met-Gly-Trp-DMet-Asp-Phe-NH_2 (Fig. 22)

This analogue also shows a TG-like CD curve. The conformation of the methionyl residue may be reserved without it affecting the favoured conformation. The analogue shows biological activity.

Ac-Tyr-Met-Gly-DTrp-Met-Asp-Phe-NH_2 (Fig. 23)

If however the configuration of the tryptophyl residue is reversed (Trp to DTrp) then the Trp-Phe aromatic interaction cannot take place. CD spectroscopy shows the conformation to be a random one. This analogue is biological inactive. (Some measurements indicate this analogue may infact be marginally active with around 1 % of the activity of pentagastrin).

BOC-DAla-Trp-Met-Asp-Phe-NH_2 (Fig. 24)

This peptide shows a TG-like CD curve. It is structurally related to the analogue no. 6, shown in Fig. 21. The CD curves are similar, both are biologically active.

BOC-Trp-Met-Asp(OBu^t)-Phe-OH (Fig. 25)

This peptide adopts a TG-like conformation as the CD curve shows. However it is not biological active. Analogue no. 5, shown in Fig. 20, appears to adopt a TG-like conformation but is also inactive. It may be noticed that in both cases the aspartyl residue has been either replaced or modified.

BOC-IDE-Trp-Met-Asp-Phe-NH_2 (Fig. 26)

CD shows that this peptide does not adopt a TG-like conformation. The presence of highly polar carboxylic acid group close to Trp may adversely affect the aromatic interaction between the Trp and Phe residues. The analogue is biological inactive.

BOC-Trp-Met-Asp-Tyr(CH_3)-NH_2 (Fig. 27)

The substitution of Phe by an O-methylated tyrosyl residue results in relatively little change in the CD spectrum, the molecule adopts an ordered conformation and a Trp-Tyr aromatic interaction presumably occurs. The analogue is an active one.

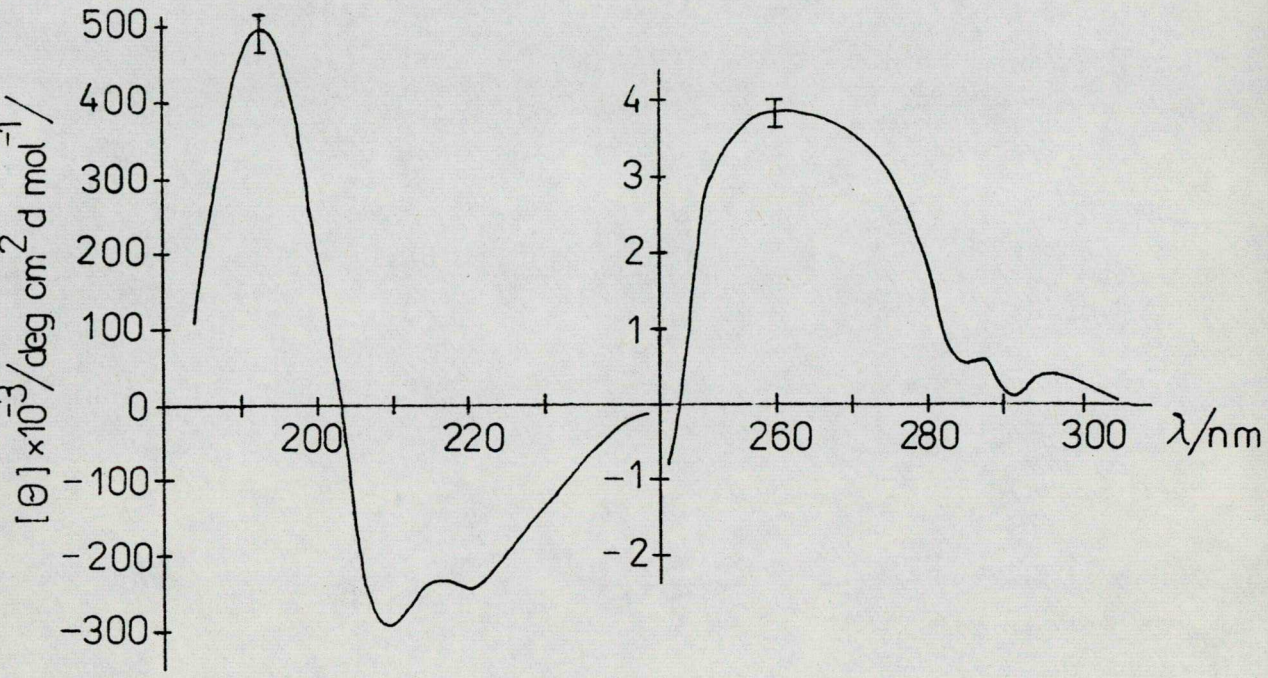


Fig. 15.: Synthetic Human Gastrin 1

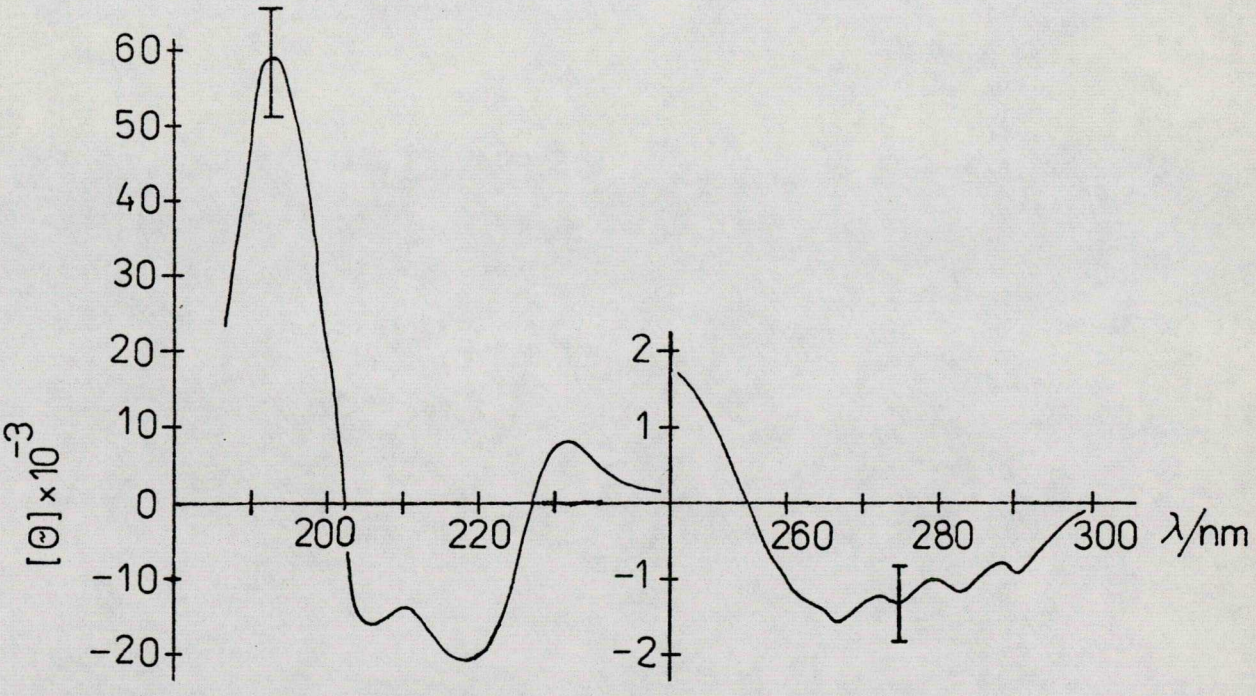


Fig. 16.: BOC-Trp-Met-Asp-NH_2 (TG)

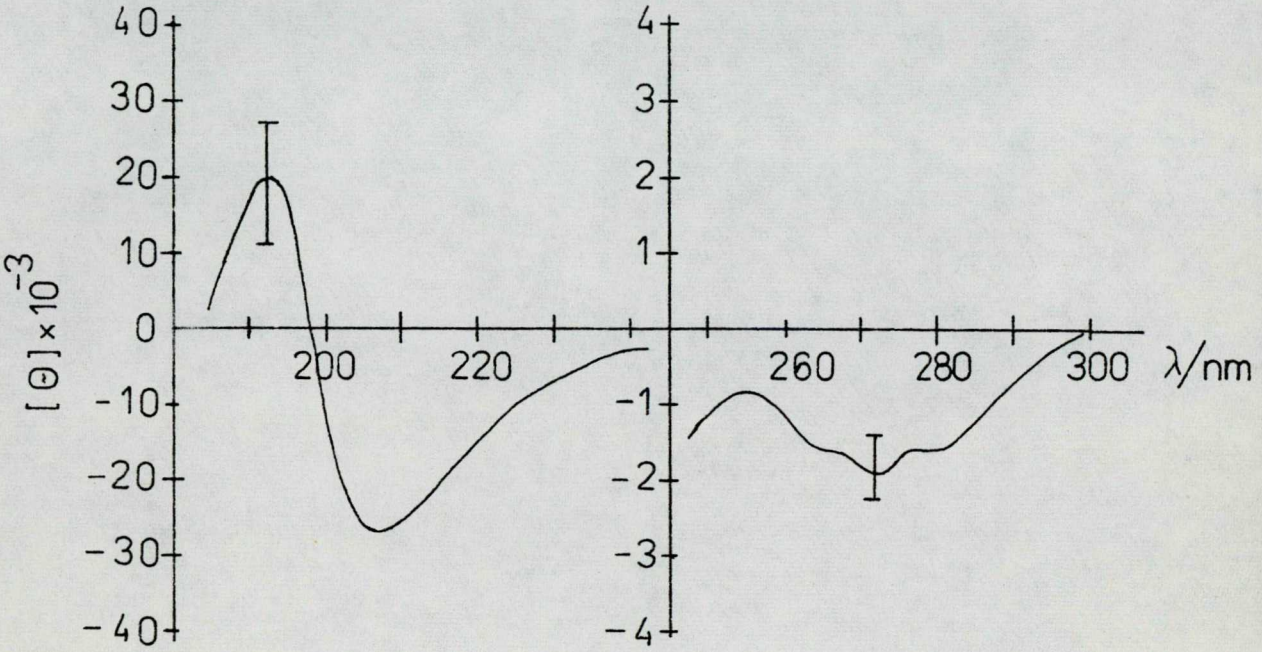


Fig. 17.: Z-Met-Asp-Phe-NH_2

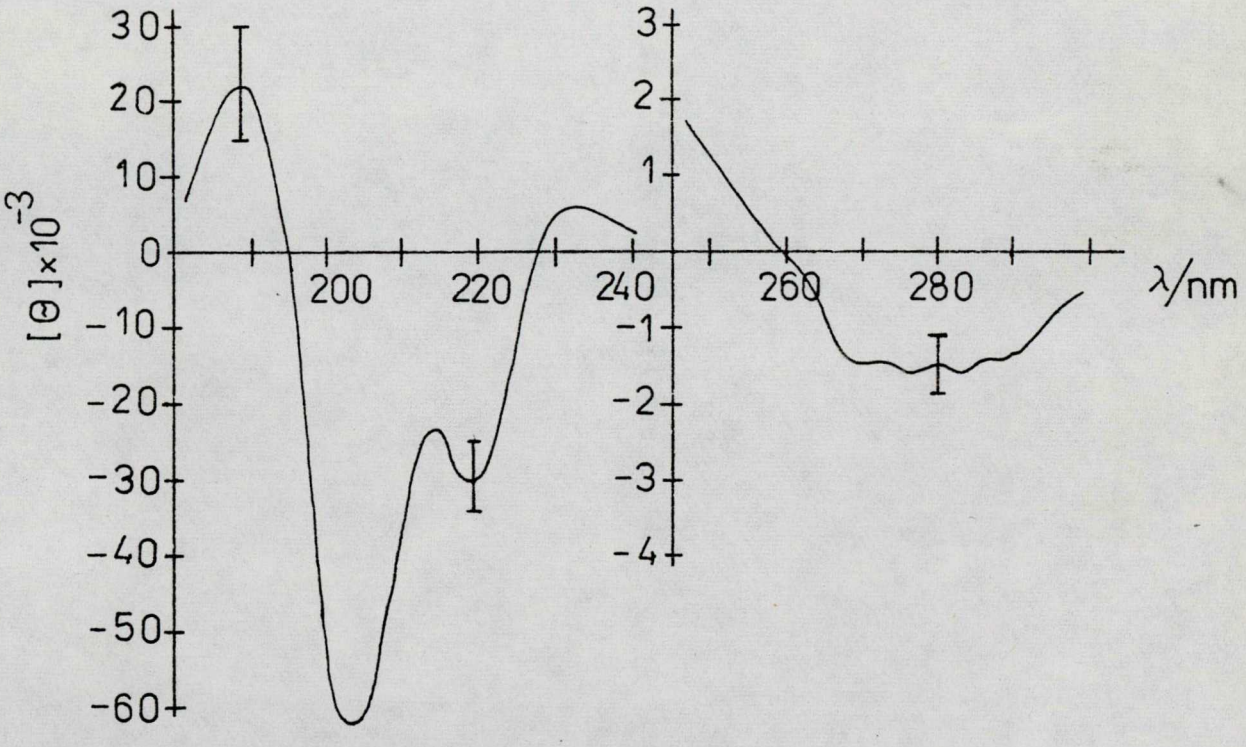


Fig. 18.: BOC-Trp-Met-Asp-PEA

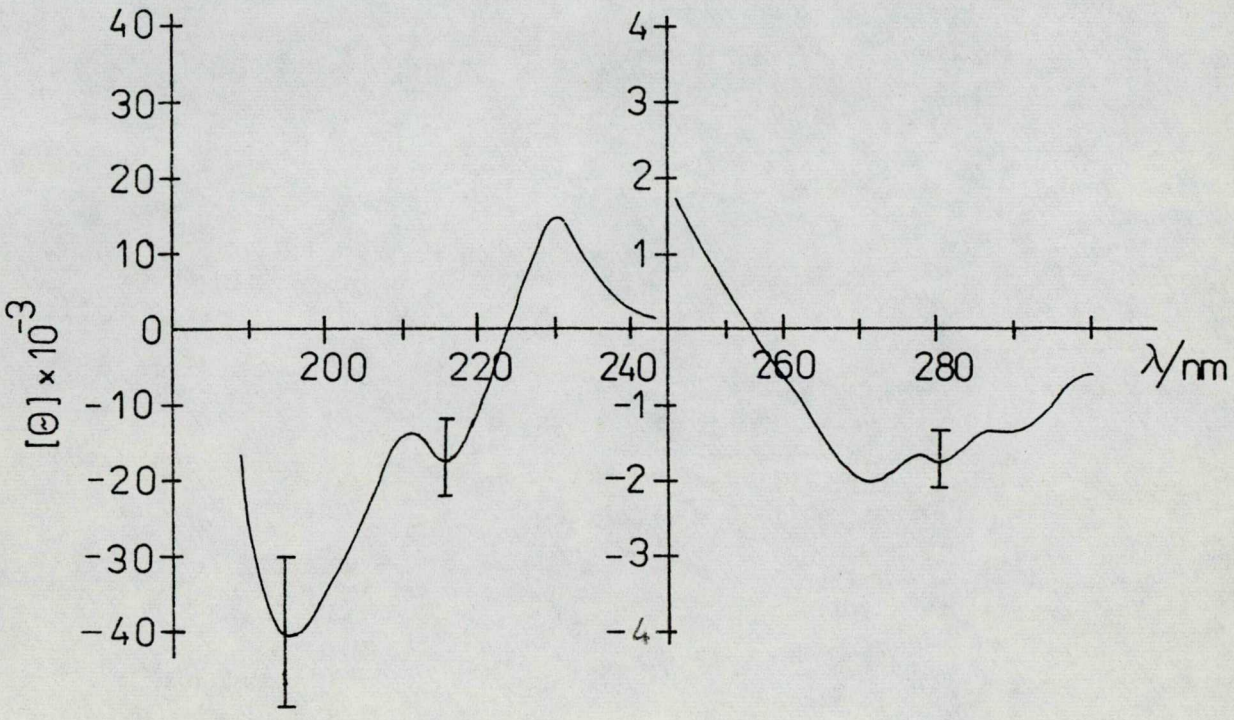


Fig. 19.: BOC-Trp-Leu-Asp-HVA

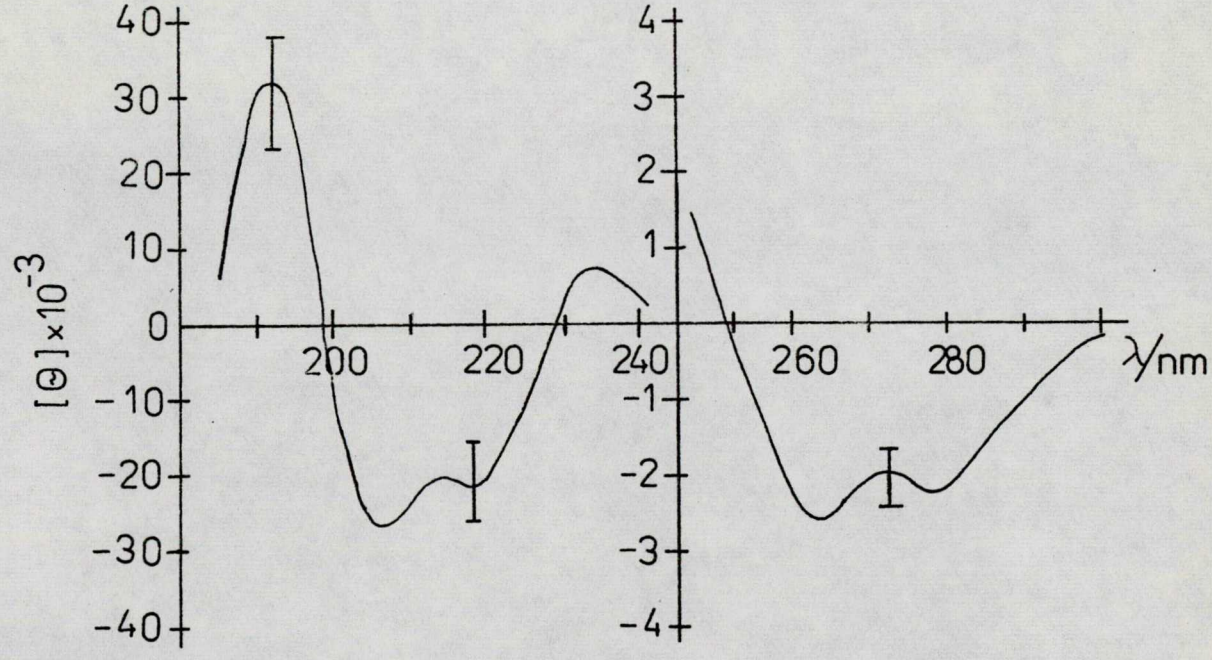


Fig. 20.: BOC-Trp-Met-Glu-Phe-NH_2

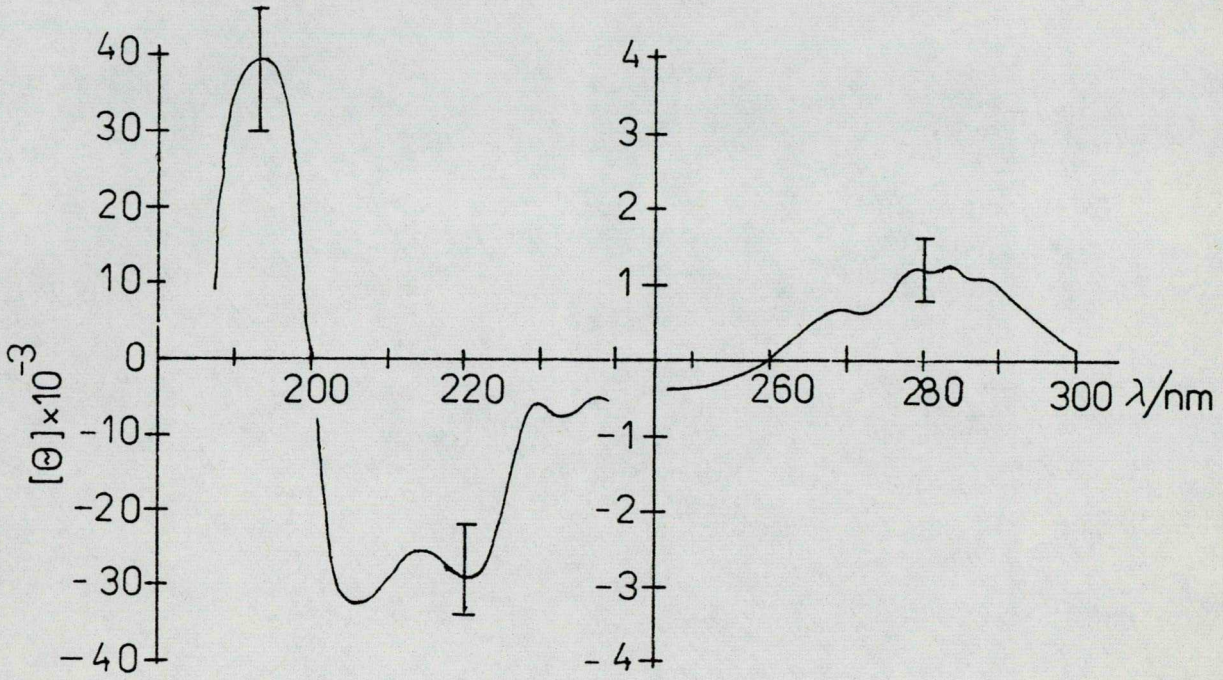


Fig. 21.: Ac-Tyr-Met-DAla-Trp-Met-Asp-Phe-NH_2

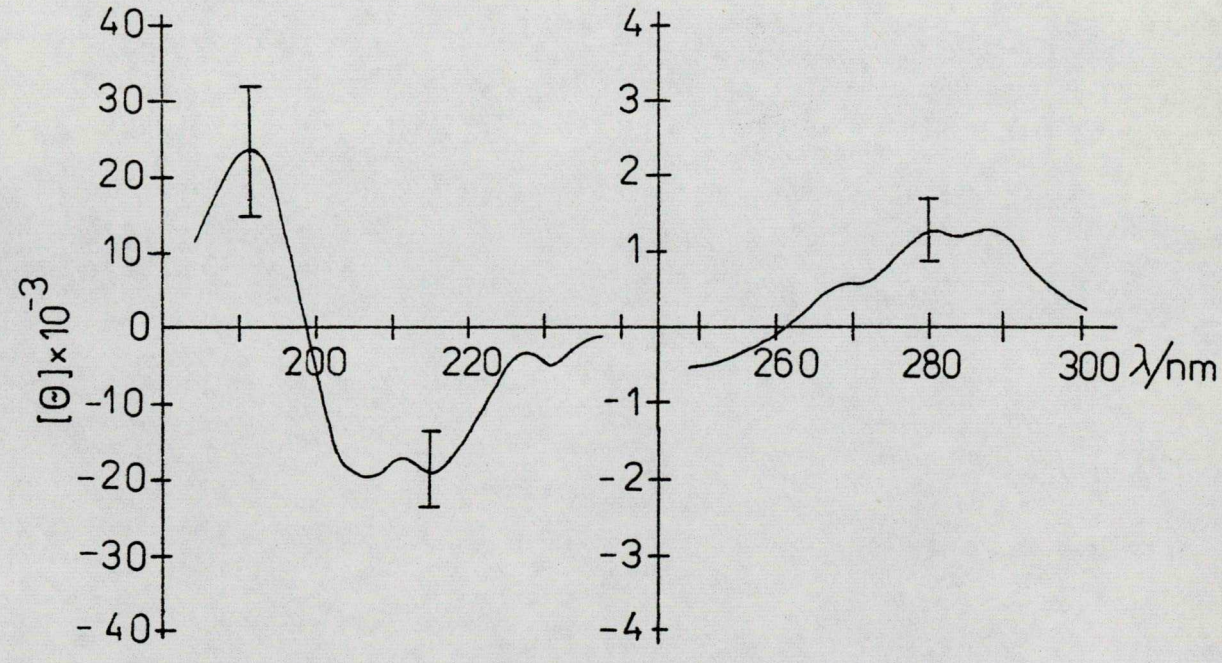


Fig. 22.: Ac-Tyr-Met-Gly-Trp-DMet-Asp-Phe-NH_2

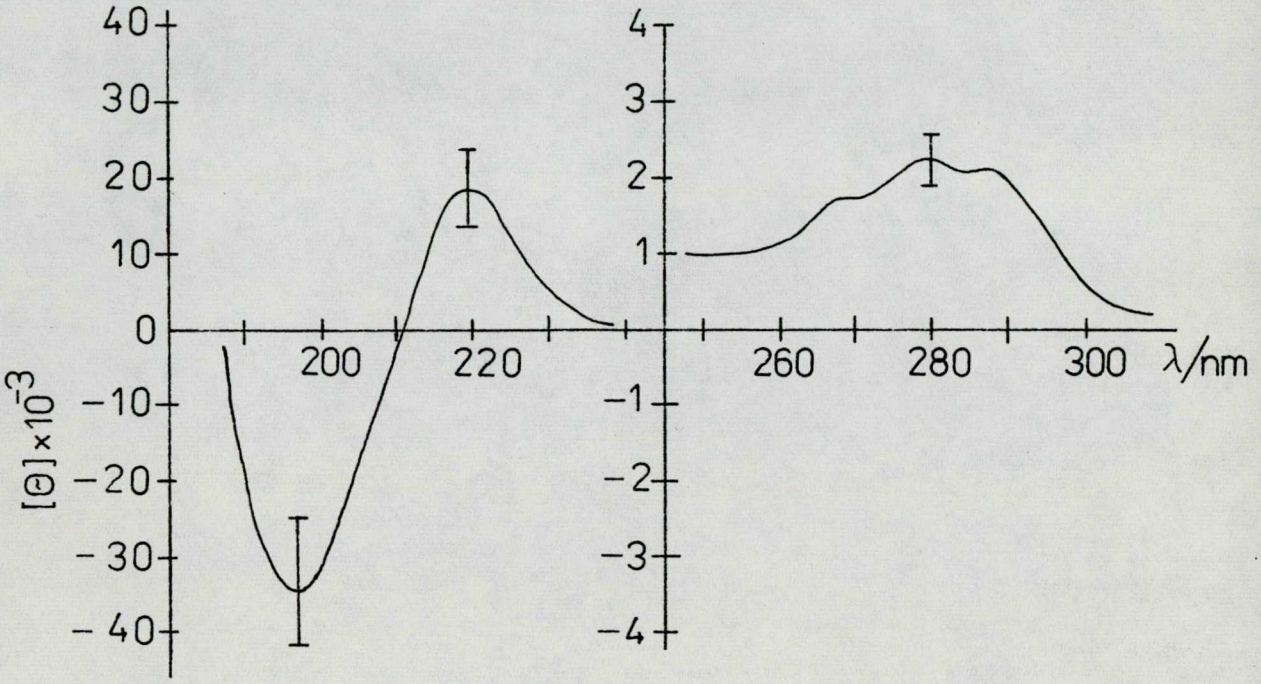


Fig. 23.: Ac-Tyr-Met-Gly-DTrp-Met-Asp-Phe-NH_2

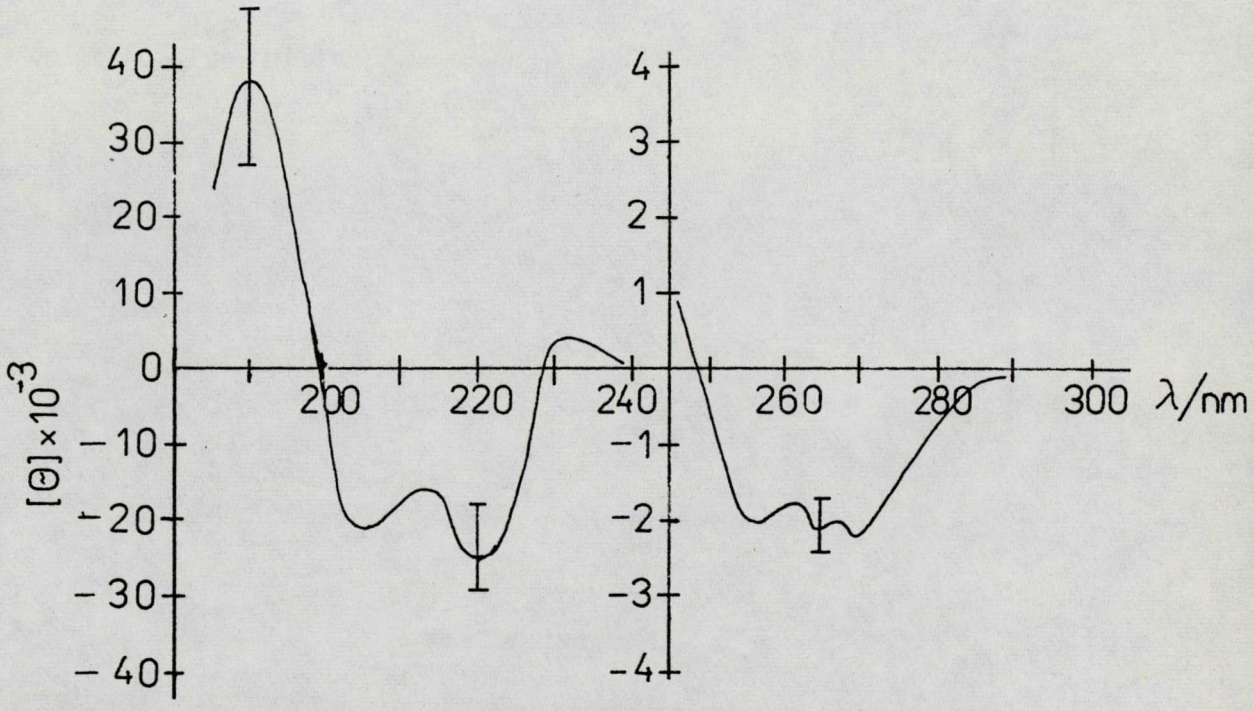


Fig. 24.: BOC-DAla-Trp-Met-Asp-Phe-NH_2

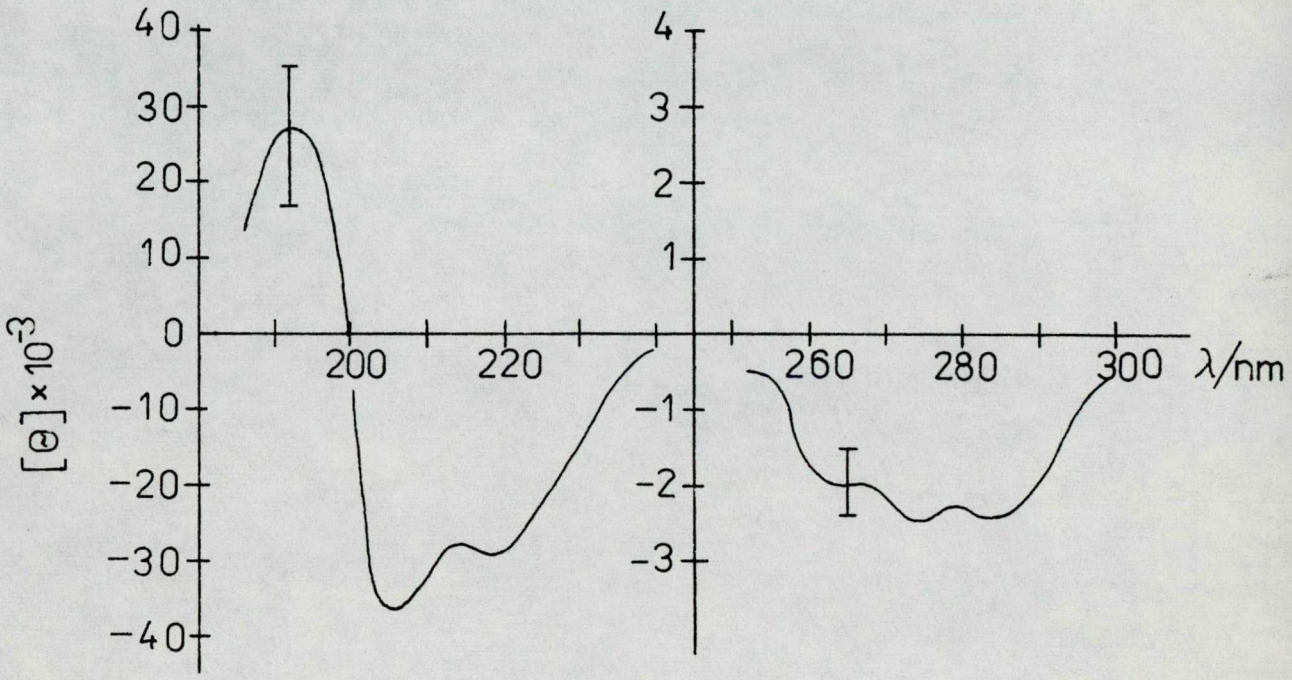


Fig. 25.: BOC-Trp-Met-Asp(OBu^t)-Phe-OH

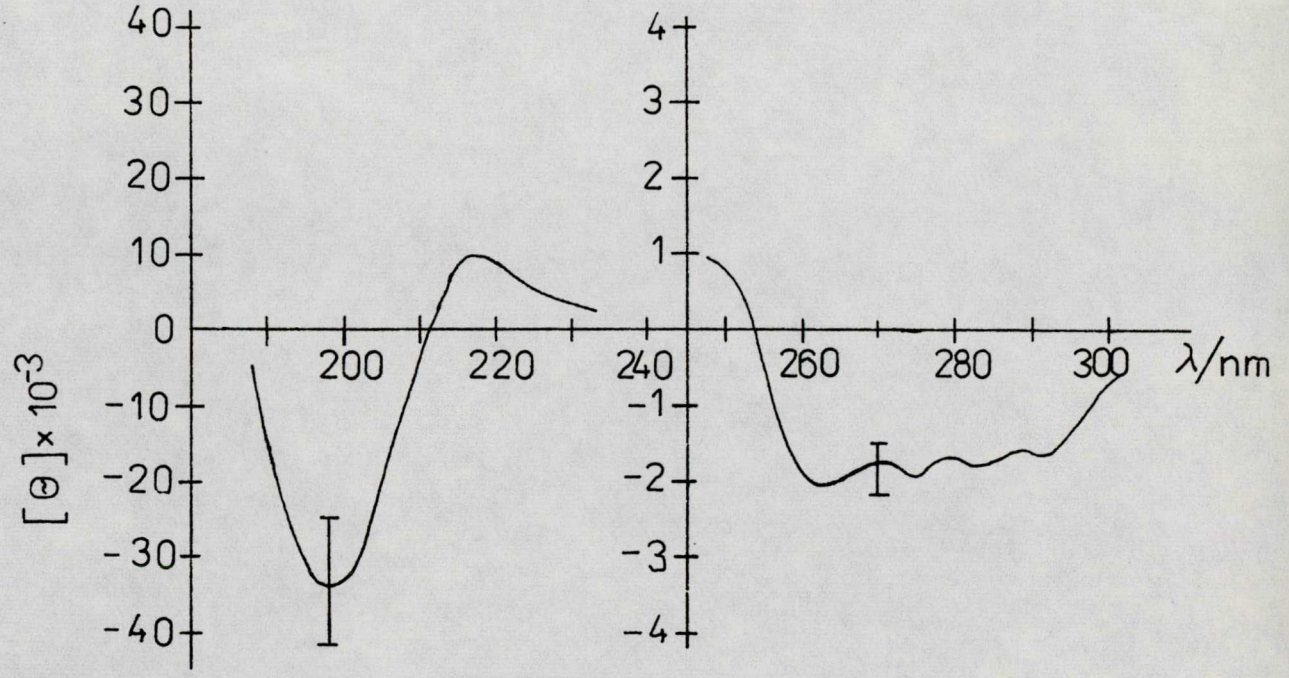


Fig. 26.: BOC-IDE-Trp-Met-Asp-Phe-NH_2

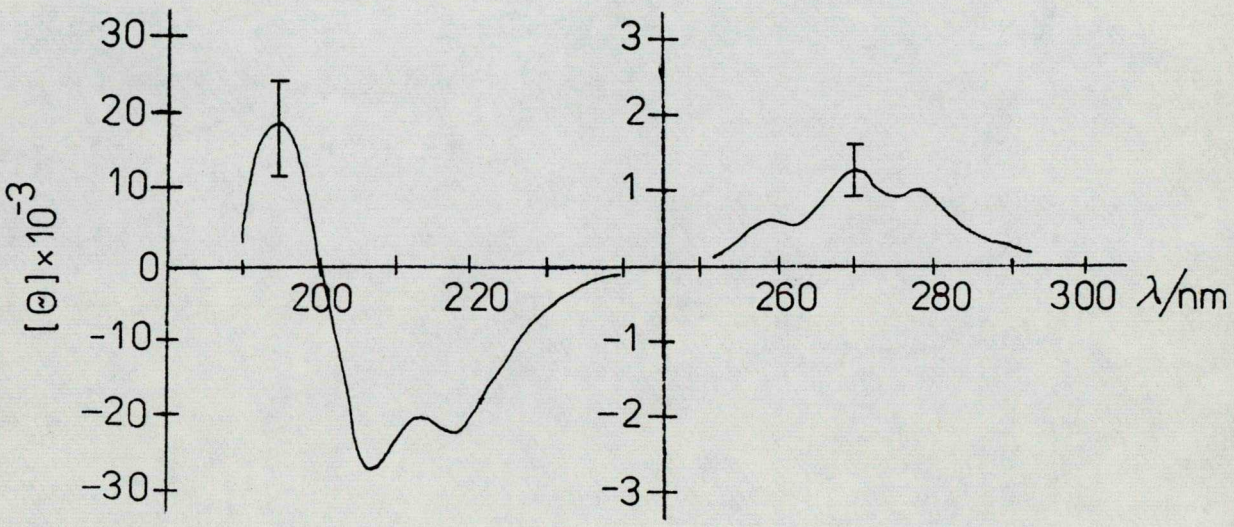


Fig. 27.: BOC-Trp-Met-Asp-Tyr(CH_3)-NH_2

4. DISCUSSION

In the introduction it was mentioned that for a Cotton effect to be observed an electronic transition must occur within the spectral range of the measuring instrument, and secondly this transition must be subject to a chiral environment. A useful distinction concerning the source of chirality and the origin of CD in a chromophore has made by Moffit and Moscowitz [68]. The CD effect may arise from an electronic transition of a chromophore which is inherently chiral (e.g. the peptide amide chromophore). Alternatively the CD effect may arise from an inherently achiral chromophore which is asymmetrically perturbed by the environment, this is known as an induced CD. Chirality conferred onto a chromophore may originate from within the chromophore molecule, which results in an intrinsically induced CD (e.g. CD of a planar ketone), or chirality may originate from outside the chromophore molecule, which results in an extrinsically induced CD. Therefore a distinction may be drawn between the CD of gastrin analogues, which arises as a result of the inherent chiral nature of the peptide molecule, and the CD of prepared chl-a complexes, which is mostly extrinsic in origin where the porphyrin nucleus is asymmetrically perturbed as a result of complexation.

4.1. STRUCTURE OF CHLOROPHYLL COMPLEXES

The CD curves of prepared chl-amino acid and chl-polypeptide conplexes show a negative peak at around 689 nm and 673 nm, and such a split signal indicates the presence of a chl-a-chl-a dimer. The size as well as the non-conservative nature of the split signal indicates that the CD is mostly extrinsic in origin. The CD of chl-a monomers in acetone solution arises entirely from the 5 chiral carbon atoms of chl-a (primarily from the 3 present in the porphyrin nucleus) and the ellipticity measured in 80 % acetone solution is -2·0 deg cm^2/d mol at 662-663 nm [70]. The ellipticity at 689 nm of the prepared chl-a-poly--Pro-poly-Lys complex is $-1{\cdot}8 \times 10^3$ deg cm^2/d mol, this enhancement can only be accounted for by an extrinsic CD effect arising out of an interaction between the polypeptide and the porphyrin chromophore. The observation of a dimer type CD signal in the prepared complexes is of interest because isolated plant chl-protein complexes, inparticular P700 reaction centre complex, produce a similar dimeric CD signal [64]. The possible structure of chl-a dimers has been studied by Fong and Koester [65-66]. The porphyrin nucleus of chl-a contains a nucleophilic keto carbonyl function in ring V, while the central magnesium atom is coordinationally unsaturated (coordination number = 4). It has been postulated [65-66] that the P700 reaction centre dimer is a hydrated chl-a dimer of the form $(\text{chl-a}.H_2O)_2$ as depicted in Fig. 28.

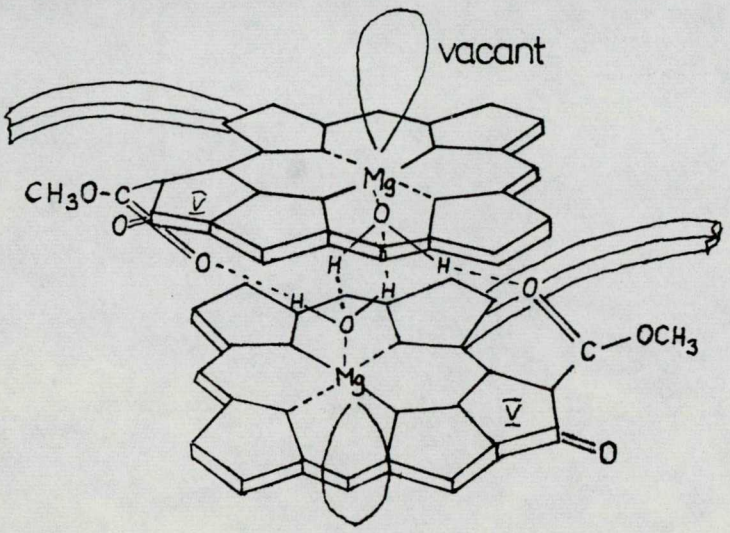


Fig. 28. Proposed structure of dimeric chl-a
(redrawn from [66])

A chl-a dimer such as above would account for the appearance of split CD band in the red absorbance region of chl-a. The porphyrin nucleus (and the phytol chain) are free to interact with the amino acids or polypeptides, indeed the second axial position on the Mg atom may be the site of complexation. Such a dimer would not be expected to be very tightly bound and so it is not surprising that detergent treatment results in its dissociation.

That chl-a and the amino acid/polypeptide are complexed together is strongly supported by other observations. Firstly, during appropriate gel filtration of the sample, chl-a and its ligand appear in the same fraction. Secondly,

the enhancement of the long wave fluorescence to above the residual vibrational level when the sample was excited in the UV can only be readily explained by the existence of a chl-a-amino acid/polypeptide complex. Thirdly, evidence of complexation is provided by the red shift in the red absorption band of chl-a from 668 nm in lecithin liposomic solution to 672 nm following the addition of the amino acid or polypeptide.

The effect of SDS is more severe than that of Dig on the prepared chl-a complexes causing a complete fall in the long wave fluorescence to the vibrational level. Absorption spectra show that SDS treatment results in some degree of pheophitinization.

4.2. CONFORMATION OF GASTRIN ANALOGUES

The CD signal of gastrin analogues arises entirely as a result of the chiral nature of the polypeptide molecule.

On the basis of the CD results it is possible to divide the gastrin analogues examined into two main groups: (1) those analogues which exhibit a TG-like CD curve, and (2) those which show a random type CD curve. The results may be tabulated in the following way.

	Analogue Number	TG-type conformation	Biological Activity
H-Trp-Met-Asp-Phe-NH_2	1	+	+
Z-Met-Asp-Phe-NH_2	2	+	+
BOC-Trp-Met-Asp-PEA	3	+	+
BOC-DAla-Trp-Met-Asp-Phe-NH_2	9	+	+
Ac-Tyr-Met-DAla-Trp-Met-Asp-Phe-NH_2	6	+	+
Ac-Tyr-Met-Gly-Trp-DMet-Asp-Phe-NH_2	8	+	+
BOC-Trp-Met-Asp-Tyr(CH_3)-NH_2	12	+	+
BOC-Trp-Met-Asp(OBu^t)-Phe-OH	10	+	-
BOC-Trp-Met-Glu-Phe-NH_2	5	+	-
BOC-Trp-Leu-Asp-HVA	4	-	-
BOC-IDE-Trp-Met-Asp-Phe-NH_2	11	-	-
Ac-Tyr-Met-Gly-DTrp-Met-Asp-Phe-NH_2	7	-	+/-

All peptides analogues that are biological active have a TG-type conformation, but not all peptides which have the privileged conformation necessarily show biologically activity (e.g. peptides 5 and 10). This study indicates that those peptides which adopt a different conformation to TG are biologically inactive, conformational similarity is a prerequisite for gastrinic activity (peptides 2,3,6,8,9 and 12), but in itself is insufficient to guarantee it (peptides 5 and 10). It may be noticed that in both of these latter analogues the Asp residue has been either replaced or modified. In his studies on the structure-

-function relationship in gastrin-like peptides Morley [61] concluded that while a wide range of substitutions could be performed on the Trp, Met and Phe residues of TG, the presence of the aspartyl residue at position 3 was essential for gastrinic activity. Later Penke et al. [62-63] showed that gastrinic activity could be maintained provided a negatively charged group of the same length as the aspartyl residue, for example serine sulphate ester, is placed at the Asp position. The action of any hormone is governed by 2 processes, firstly interaction occurs between the hormone and its receptor - the conformation adopted by the hormone is important here. Secondly, once bound, the hormone must induce a triggering process in the receptor which ultimately results in the biological change characteristic of the hormone. In the hormone gastrin the Asp residue is responsible for this triggering process, its presence, or that of a suitably negatively ionisable residue, is essential for the "firing" of the hormone [61]. However the Asp residue does not play a large role in determining the conformation of the molecule. Hence gastrin analogues (peptides 5 and 10) in which the Asp residue has been replaced may adopt the privileged conformation, but the analogue will not be active because the triggering process cannot be carried through.

As has already been stated a TG-like CD curve consists of a positive peak at around 190 nm and a region of negative

ellipticity between 200 and 220 nm. The positive peak at 190 nm may be assigned to the perpendicular π-π^* amide transition, [15], while the negative peak at around 218 nm can be assigned to the ^1B_b transition of Trp [8], with the n-π^* amide transition also making a small contribution close to this wavelength [11]. The negative band centred on around 203 nm appears to be a composite band, but predominantly consists of the parallel π-π^* amide transition [15] but also includes contributions from the ^1B_a transition of Trp [8], and where appropriate the ^1B_b transition of Tyr [71]. In all active gastrin analogues the ^1B_b transition of Trp centred on 218 nm is responsible for a negative CD peak, while most other Trp containing peptides show positive ellipticity for this transition. [8] [60]. However in the peptide series H-Gly-Phe-$(Gly)_n$--Trp-Gly-OH the CD signal of the ^1B_b transition shows a progressive invertion from positive to negative as n decreases from 2 to zero [60]. The negative ellipticity of the ^1B_b transition when n = 0 has been attributed to the presence of an aromatic-aromatic interaction between Phe and Trp in the peptide. Therefore it may be concluded that the appearance of a similar negative signal in active gastrin analogues results from the proximity of the Phe and Trp residues to one another. While this does not unambiquously prove that the conformation of active gastrin

analogues consists of a gamma-turn about the Asp residue as depicted in Fig. 4, it is difficult to device another conformation which would result in the two aromatic residues coming in close proximity. Theoretical calculations based on molecular energy minimization studies [31] support the supposition that the gamma-turn conformation is the favoured conformer of TG. Such a conformation is stabilized by hydrogen bonding between the Met carbonyl and the Phe peptide amide groups as well as by the interaction between the Phe and Trp aromatic rings. The energy gained from these interaction more than compensates for the small energy investment required for the establishment of the gamma-turn conformation (primarily the twisting of peptide bonds, $\omega < \pm 10^{o}$).

Recently spin-lattice relaxation studies using two dimentional ^{13}C NMR have been carried out on TG [72]. These results, which will be published concurrently with this work, strengthen the evidence for a gamma-turn conformation at the level of the aspartyl residue as suggested by CD data.

5a. SUMMARY

Two groups compounds were examined using spectroscopic techniques in order to gain information concerning their molecular structure and conformation. The two groups consisted of: (1) chl-amino acid and chl-polypeptide complexes, and, (2) analogues of the peptide hormone fragment tetragastrin.

The existence of complexation between chl-a and amino acid/polypeptide was confirmed by a number of observations Firstly the red absorption maximum of chl-a was red shifted to 672 nm, this degree of red shift strongly suggests chl-a is complexed. Secondly the enhancement of the long wave fluorescence at 725 nm to above the residual vibrational level when the sample was excited in the UV can only be readily explained by complexation; the increased fluorescence is a result of energy transfer between the ligand and chl-a. Finally the CD of the red band showed a split signal, the non-conservative nature of the peaks again indicates complexation of chl-a. The strength of the CD signal ($[\theta]_{689nm}$=-1.8x10^3 deg cm^2/d mol) can only be accounted by an extrinsic CD effect whereby chirality is conferred to the chl-a molecule by the complexing ligand. The split CD signal shows the prepared complexes are dimers, and the possible nature of the chl-a-chl-a interaction is discussed. The effect of detergent treatment is to cause the chl-a dimer to disssociate as the CD of the treated complexes shows.

CD studies on a series of 12 gastrin analogues, including both active and inactive compounds, showed that gastrinic activity depends upon the adoption of a certain privileged conformation. This conformation is a prerequisite for biological activity, but in itself does not guarantee a biological response. It was found that some analogues inspite of adopting the favoured conformation were not active because the C-terminal aspartyl residue had either been replaced or modified. The CD of all <u>active</u> analogues examined showed a split π-π^* amide band with a positive signal at 190 nm (π-π^* perpendicular transition) and a negative signal at 203 nm (π-π^* parallel transition). This shows active gastrin analogues adopt an ordered (non-random) conformation. Theoretical calculation, using energy minimization studies, have suggested that the preferred conformation is a gamma-turn of the level of the aspartyl residue resulting in proximity of the two aromatic rings of Phe and Trp. CD data confirms the existence of an aromatic-aromatic interaction (the ^1B_b transition of Trp is normally <u>positive</u>, but in active gastrin analogues is <u>negative</u>, this is similar to the CD of peptides in which Trp and Phe residues are adjacent). Thus it is argued the conformation adopted by active gastrin analogues is a gamma-turn about the Asp residue with a hydrogen bond between the peptide Phe amide group and the Met carbonyl group; the conformation is further stabilised by an aromatic-aromatic interaction

between the rings of Phe and Trp. On the basis of the CD results a gamma-turn conformation is strongly suggested, and a correlation has been found between biological activity and conformation in the gastrin analogues examined.

5b. ÖSSZEFOGLALÁS

Két vegyületcsoportban vizsgáltuk a molekuláris és konformációs térszerkezetet spektroszkópiai módszerekkel. Az egyik csoportot a-klorofill-aminosav és a-klorofill--polipeptid komplexek képezték, melyek a növényekben levő klorofill-fehérje komplexek modelljeiként szolgálnak; a másik csoportba a gasztrin peptid hormon aktiv centrumát képező tetragasztrin fragmentum szintetikus analógjai tartoztak.

1/ Az a-klorofill és az aminosav ill. fehérje között lejátszódó komplex képzést több megfigyelés igazolta: a/ A komplexekben az a-klorofill vörös elnyelési sávja hosszabb hullámok felé tolódott /672 nm-re/; b/ a fluoreszcencia spektrumban a 725 nm-es sáv intenzitása jóval nagyobb volt, mint ami a vibrációs sáv részvételével magyarázható; c/ a CD spektrum vörös tartományában olyan nem-konzervatív jelfelhasadást észleltünk, amely arra mutat, hogy az a-klorofill komplexkötésbe jutott. Igy pl. az a-klorofill poliprolin-polilizinnel képezett komplexében $[\theta]_{689nm} = -1.8x10^3$ fok $cm^2/dmol$ CD intenzitás olyan külsődleges hatással magyarázható, melynek során a polipeptid az a-klorofill ligandum kiralitását erősen megnövelte. A felhasadt CD jel azt mutatja, hogy a komplexben a-klorofill dimérek vannak. Az a-klorofillok közötti kölcsönhatások lehetséges tipusait számbavéve megvitattuk a

dimérek valószinü tipusait. Ha a komplexeket detergensekkel kezeltük, a CD változás a dimérek szétbomlását jelezte. Az alkalmazott detergensek közül az ionos Na-dodecil szulfát és a nem-ionos digitonin közül az abszorpciós és fluoreszcencia spektrumok változása a Na-dodecil szulfátot mutatta hatékonyabbnak a dimérek lebontásában és bizonyos fokú feofitinizációban is.

2/ Egy 12 gasztrin analógot tartalmazó sorozat CD vizsgálatából arra lehetett következtetni, hogy a biológiai aktivitás egy sajátos "kedvező" molekula konformációhoz kötött. Ez a konformáció a biológiai aktivitás szükséges, de nem elégséges feltétele, mivel vannak "kedvező" molekulakonformációjú gasztrin analógok, melyek inaktívak, mivel a C-végi aszpartil maradék módosult vagy más aminosav helyettesiti. A vizsgálatok körébe vont biológiai aktivitással rendelkező analógok közös jellemzője, hogy CD spektrumok egy hasadt π-π^* amidsávot tartalmaznak, melyek pozitiv /π-π^* merőleges elektronátmenetet tükröző/, része 190 nm-nél, negativ, /π-π^* párhuzamos elektronátmenetből eredő/ része 203-nél található. Ez a CD jel azt mutatja, hogy a biológiailag aktív gasztrin molekulák meghatározott módon rendezett /nem random/ konformációval rendelkeznek. Irodalmi megfontolások és energia minimumot kereső számitások szerint a kedvező konformáció az aszpartil-maradék olyan gamma-fordulata lehet, amely a két aromás fenilalanin és triptofén gyürüt egymás közelébe hoz. A 218 nm körül meg-

figyelhető CD jel szerint ez a konformáció az aromás gyűrűk között kölcsönhatást eredményez. Erre abból lehet következtetni, hogy míg a triptofán ^1B_b elektronátmenete, amely normális körülmények között pozitív CD jelet hoz létre, az aktív gasztrin analógokban negatív CD jelet ad. Hasonló előjel váltás olyan peptidekben tapasztalható, melyekben a triptofán maradék szomszédságába fenilalanin maradék helyezkedik el. A gamma-fordulatot /C_7-es gyűrűt/ egy metionin és fenilalanin maradékot összekötő hidrogénkötés hozza létre. Az aromás-aromás kölcsönhatás és a gamma-fordulat erősítik egymást. Ennek eredményeképpen bőven kompenzálódik a gamma-fordulat létrehozásához szükséges energiabefektetés, amely a peptidkötés elforgatására fordítódik /$\omega < 10^{\circ}$/. A gasztrin analógokon végzett CD mérések eredményeképpen sikerült tisztáznunk a biológiai aktivitáshoz szükséges molekuláris konformációt.

ABBREVIATIONS

Abs	absorbance
CD	Circular Dichroism
chl	chlorophyll
C-L	chlorophyll-liposome solution
Da	daltons
deg	degrees
Dig	digitonin
Fluor	fluorescence
NMR	nuclear magnetic resonance
PAGE	poly acrylamide gel electrophoresis
poly-Pro-poly-Lys	poly-L-proline-poly-lysine
SDS	sodium dodecyl sulphate
TFE	2,2,2-trifluoroethanol
TLC	thin layer chromatography
Tris	tris-hydroxylmethyl-aminomethane
UV	ultra-violet
λ_{em}	emission wavelength
λ_{ex}	excitation wavelength

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