Synopsis of PhD Thesis

SPECTROSCOPY AND FUNCTION OF MACROAGGREGATES OF THE CHLOROPHYLL A/B LIGHT HARVESTING ANTENNA

Virginijus Barzda

Institute of Plant Biology
Biological Research Center
Hungarian Academy of Sciences

Szeged
1996

1. Introduction

Spectroscopy is a powerful non-destructive tool which is frequently applied to investigate biological objects. However, investigations on highly organized biological samples are met with a number of difficulties due to complex inhomogeneous structures which very often appear when the size of the molecular assemblies becomes commensurate with the wavelength of measuring light (Tinoco et al. 1987). The decrease in the absorbance named as flattening and increase in scattering of the incident light appear in the absorption spectra (Duysens, 1956; Bustamante & Maestre, 1988). Large, "anomalous" so called polymerization and salt induced (psi) type circular dichroism (CD) signals are given rise (Lerman, 1973). Psi-type CD signals were observed in viruses, DNA-polylysine complexes, DNA-aggregates, chromatin, nucleohistones, sperm heads, erythrocytes, aggregates of pigment protein complexes and chloroplasts (Tinoco & Williams, 1984; Bustamante et al. 1991; Maestre et al. 1982; Finzi et al. 1989). The theory for psi-type CD was developed by Keller & Bustamante (1986a,b). Theory shows that in large densely packed aggregates, which posses global chirality, long-range coupling between chromophores can become significant and the macroaggregate can act as a coupled entity which is capable to differentiate between the left and right circularly polarized light. Up to now psi-type theory is considered to offer the most comprehensive explanation for the giant, "anomalous" CD signals of macroaggregates (Tinoco et al. 1987).

The most remarkable prediction of the psi-type CD theory is the delocalization of the excitation energy over large distances or over the entire macroaggregate due to long-range coupling between chromophores (Keller & Bustamante, 1986b). This mechanism differs very significantly from those in smaller aggregates in which, depending on the distance, orientation and coupling strength of chromophores, the excitation energy migration has been described to occure via: exchange mechanism involving electron exchange between donor and acceptor molecules, coherent (exciton) processes or "hopping" type of non-radiative energy transfer mechanisms (Dexter, 1953; Davidov, 1971; Förster, 1949). Usually, excitation energy migration for long distances in the macroaggregates is considered to occur via multistep hopping mechanism (Förster, 1949). According to the theory for psi-type aggregates (Keller & Bustamante, 1986b) all elements of the interaction tensor between chromophores must be taken into account, i.e. in addition to the short range interaction, intermediate and radiative coupling terms can play a role. This can result in an instantaneous delocalization of the excitation energy over a large domain or the entire aggregate. The aggregate can act as a single entity via series of collective modes of excitation.

Theoretical models for simplified systems have been elaborated to qualitatively explain psi-type CD features in chirally organized macroaggregates. However, many assumptions and predictions of the theory remain to be tested experimentally. Spectroscopic characterization of psi-type aggregates is far from being complete and relatively little is known about the dependence of the spectroscopic features on the macrostructural parameters of large molecular assemblies. Systematic investigations on the structural and functional parameters are scarce, partly because of the lack of a suitable model system in which both energy migration mechanisms and spectroscopic features can be studied as a function of size of aggregates and of other macroorganizational parameters.

Photosynthetic membranes and aggregates of pigment-protein complexes can readily be used to investigate psi-type CD features. In chlorophyll-containing systems the structural parameters can be varied in a broad interval, between the monomeric solution of chlorophylls (Chls) and large arrays of the pigment dipoles in macroaggregates of pigment-protein

complexes (Garab, 1996). The measurements can be performed in the visible range and not only in UV as for most psi-type aggregates. The Chl a/b light harvesting complex of photosystem II, LHCII has been shown to exhibit macroaggregation induced anomalous CD features (Gregory et al. 1980; Garab et al. 1988a), and the 3-dimensional structure of this complex is known at nearly atomic resolution (Kühlbrandt et al. 1994). This opens a possibility to interpret spectroscopic data in relation to exact structural parameters. This prompted us to conduct systematic studies on LHCII in different aggregation states and macroaggregates with different ultrastructure. It is considered an equally important task to understand spectroscopy and functional role of large chirally organized macroaggregates in granal thylakoid membranes.

By using CD technique it has been shown that PSII particles in granal thylakoid membranes are assembled into chirally organized macrodomains (Garab et al. 1988a; Garab et al. 1988c). This type of macroorganization of particles has been shown to be sensitive to different physico-chemical factors and the composition of the membranes (Garab et al. 1991). The chirally organized macrodomains have been suggested to play a structural role: they are proposed to be involved in the spacial separation of the photosystems in thylakoid membranes (Garab et al. 1988a). It has also been suggested that chiral macrodomains constitute the structural basis for the excitation energy migration over large distances in the antenna system (Garab et al. 1991). However, direct experimental evidence for the migration (or delocalization) of the excitation energy over large distances had not been presented and no comparative studies had been conducted on the mechanism of energy migration in aggregates of different sizes.

In chloroplasts under 'normal conditions', the antenna system minimizes quantum losses and supplies the excitation energy to the reaction centers. On the other hand, it has been demonstrated that under high light intensities, when excess energy is absorbed, controlled energy dissipation can occur in the antenna (for review see Horton et al. (1994)). This plays a protective role against photoinhibitory damages of the photosynthetic machinery. Various adaptational mechanisms are likely to be involved in the regulation of the photophysical pathways. Factors affecting the structure of antenna are very likely to be involved in these processes. The mechanism of non-photochemical quenching of Chl fluorescence, which indicates the transient operation of dissipative pathways in the antenna, has been proposed to originate from changes in the state of aggregation of the antenna complexes (Horton et al. 1991). Indeed, aggregation induces quenching of Chl fluorescence in purified LHCII (Ruban & Horton, 1992). Light-induced changes in the chiral macroorganization of the chromophores in thylakoid membranes have also been observed by Garab et al. (1988b). However, the physical mechanism of the aggregation-dependent fluorescence quenching in LHCII and the light-induced structural changes in chiraly organized macrodomains had not been clarified.

In this work we report the results of systematic studies by steady state and time resolved spectroscopic investigations in thylakoid membranes and LHCII of different aggregational states. The aim of this work was to contribute to the understanding of the spectroscopy and function of psi-type aggregates and their role in the chloroplast thylakoid membranes. Our investigations were focused on the following questions: (i) the anomalous psi-type CD features of the macroaggregates, (ii) excitation energy migration in LHCII of different aggregational state, (iii) fluorescence quenching mechanisms induced by aggregation in LHCII, (iv) physical origin of light-induced structural changes in thylakoid membranes and LHCII.

2. Materials and Methods

Thylakoid membranes were isolated according to Garab et al. (1988a). LHCII was isolated according to Krupa et al. (1987) with minor modifications. Three dimensional microcrystalline, stacked lamellar and unstructured aggregates of LHCII were obtained. Structure of the samples was investigated by negative staining and thin section electron microscopy.

Absorption, and CD and LD was measured in a Shimadzu UV3000 spectrophotometer and a Jobin Yvon CD6 dichrograph, respectively. For the measurements of light-induced changes, the set-ups were equipped with side illumination attachments. Fluorescence spectra were measured in a home built fluorimeter described by Garab et al. (1980). Absorbance transients under singlet-singlet annihilation conditions were measured in a set-up described by Andriunas et al. (1985).

3. Results

3.1. Spectroscopic properties and macroorganization of chromophores

By means of systematic comparative study of the absorbance and CD of thylakoid membranes and LHCII-preparations we have provided direct experimental evidence on the dependency of the magnitude of psi-type CD bands on the size of macroaggregates. Size-dependency of psi-type CD bands had been predicted in a theoretical model (Keller & Bustamante, 1986a,b), but to our knowledge had not been verified experimentally.

By investigating LHCII macroaggregates with different ultrastructure we have also shown that the intensity of the psi-type CD bands depends not only on the size but also on the long-range order in the aggregates: psi-type CD is absent in macroaggregates with undefined structure. Furthermore, the intensity and shape of the excitonic CD bands have been shown to be largely invariant on the size and ultrastructure of macroaggregates. As shown by the effects of different treatments (polyacrylamide gel, photoinhibitory light) on the intensity of psi-type CD bands, the chiral macroorganization of chromophores was found to be significantly more vulnerable than the structure of the constituent particles, the excitonic CD bands of which were invariant to the treatments.

3.2. Excitation energy migration in LHCII aggregates of different sizes

We investigated the picosecond transient absorbance kinetics under singlet-singlet annihilation conditions in large, 3-dimensional, stacked lamellar aggregates of the purified LHCII and its form of small aggregates. Our data strongly suggest that the macroorganizational parameters significantly influence the energy migration pathways in the aggregates. In small aggregates ($d \approx 100$-200 nm) of trimers the excitation energy migration can be characterized by a percolation type of excitation migration in a small cluster of chromophores. In the macroaggregates ($d \approx 2$-4 μm) the annihilation kinetics were consistent with a model predicted for (infinetly) large three dimensional aggregates. Although from these investigations we did not get an answer on the question whether or not instantenous delocalization of the excitation energy occurs in psi-type aggregates, our data provided experimental evidence that LHCII macroaggregates can constitute a structural basis for a long-range migration of the excitation energy.

3.3. Fluorescence quenching in LHCII macroaggregates

Based on our studies of the variations of the fluorescence yield as a function of the size of LHCII aggregates we proposed that two quenching mechanisms must be considered:

1. The dramatic (5-10 times) increase in the fluorescence yield of non-aggregated LHCII (trimers and monomers) is explained by the gradual degradation of the excitonic interactions as judged from CD and absorption spectra. An excitonic band can serve as a quencher if the optically allowed state of the excitonic band is situated not on the lowest energy level of the band. Conversely, upon disrupting the excitonic interactions in the complexes the quenching ceases and the fluorescence yield increases.

2. If the size of aggregates remains in the range between about 5 μm and 100 nm, decrease ($\sim$50%) in the fluorescence yield upon macroaggregation can be explained with a model involving 'impurity' like quenching centers. In small aggregates these centers are disconnected from the bulk pigments, whereas in macroaggregates, because of the long-range migration of the excitation energy, they can quench the fluorescence. Minor components, e.g. the 29 kDa pigment-protein complex (cf. (Bassi et al. 1990)) or complexes with high xanthophyll content (Gilmore et al. 1995), can act as 'impurities'. We propose that this latter mechanism plays a role in quenching the excess excitation energy in the antenna of photosystem II in granal thylakoid membranes.

3.4. Light-induced changes in thylakoid membranes and LHCII

By means of CD, LD and fluorescence investigations, we have shown that stacked lamellar aggregates of LHCII and granal thylakoid membranes are capable of undergoing light-induced reversible structural changes which are associated with charactersitic light-induced reversible changes in the photophysical pathways.

In granal thylakoids, the light-induced reversible structural changes, detected as ΔCD, bring about reversible changes in the fluorescence yield that indicate an increased dissipation of the excitation energy. These changes become gradually more significant in excess light compared to non-saturating light intensities, and can be eliminated by suspending the membranes in hypotonic, low-salt medium in which the chiral macroaggregates are absent.

In lamellar aggregates of LHCII, light-induced reversible ΔCD and ΔLD are also accompanied by reversible changes in the fluorescence yield. In small aggregates and trimers no light-induced ΔCD occurs and the fluorescence changes are irreversible.

It is proposed that the structural changes are induced by thermal effects due to the excess light energy absorbed by the pigments, a mechanism similar to that in liquid crystals (Janossy, 1991).

The presently available data suggest that the structural flexibility of macroaggregated LHCII, i.e. the capability of aggregates to undergo light-induced reversible structural changes is lended by their macroorganization state into lamellar aggregates also enriched in lipids. Our data also show that the structure and function of the antenna system of chloroplasts can be regulated by the absorption of excess light energy with a mechanism independent of the operation of the photochemical apparatus. The exact nature of the structural changes and the underlying physical mechanisms are not known, and thus remain the subject of further investigations.

4. References

Andriunas, K., Viscekas, J., Kabelka, V., Michailov, V.I., Pavliuk, A.A., Petrovsky, G.T. & Syrus, V. (1985) *Sov. J. E. T. P.* 42, 333-337.

Bassi, R., Rigoni, F. & Giacometti, G.M. (1990) *Photochem. Photobiol.* 52, 1187-1206.

Bustamante, C. & Maestre, M.F. (1988) *Proc. Natl. Acad. Sci. USA* 85, 8482-8486.

Bustamante, C., Samori, B. & Builes, E. (1991) *Biochemistry* 30, 5661-5666.

Davidov, A.S. (1971) *Theory of molecular excitons.* Plenum Press, New York.

Dexter, D.L. (1953) *J. Chem. Phys.* 21, 836-850.

Duysens, L.N.M. (1956) *Biochim. Biophys. Acta* 19, 1-12.

Finzi, L., Bustamante, C., Garab, G. & Juang, C. (1989) *Proc. Natl. Acad. Sci. USA* 86, 8748-8752.

Förster, T. (1949) *Z. Naturforsch.* 4a, 321-327.

Garab, G., Sundqvist, C., Mustardy, L. & Faludi-Dániel, A. (1980) *Photochem. Photobiol.* 31, 491-494.

Garab, G., Faludi-Dániel, A., Sutherland, J.C. & Hind, G. (1988a) *Biochemistry* 27, 2425-2430.

Garab, G., Leegood, R.C., Walker, D.A., Sutherland, J.C. & Hind, G. (1988b) *Biochemistry* 27, 2430-2434.

Garab, G., Wells, K.S., Finzi, L. & Bustamante, C. (1988c) *Biochemistry* 27, 5839-5843.

Garab, G., Kieleczawa, J., Sutherland, J.C., Bustamante, C. & Hind, G. (1991) *Photochem. Photobiol.* 54, 273-281.

Garab, G. (1996) In: *Biophysical Techniques in Photosynthesis* (Amesz, J. & Hoff, A. eds.), Linear and Circular Dichroism, 11-40, Kluwer Academic Publishers, Amsterdam.

Gilmore, A.M., Hazlett, T.L. & Govindjee (1995) *Proc. Natl. Acad. Sci. USA* 92, 2273-2277.

Gregory, R.P.F., Demeter, S. & Faludi-Dániel, A. (1980) *Biochim. Biophys. Acta* 591, 356-360.

Horton, P., Ruban, A.V., Rees, D., Pascal, A.A., Noctor, G. & Young, A.J. (1991) *FEBS Lett.* 292, 1-4.

Horton, P., Ruban, A.V. & Walters, R.G. (1994) *Plant Physiol.* 106, 415-420.

Janossy, I. (1991) *Optical effects in liquid crystals.* Kluwer Academic Publishers, Dordrecht.

Keller, D. & Bustamante, C. (1986a) *J. Chem. Phys.* 84, 2961-2971.

Keller, D. & Bustamante, C. (1986b) *J. Chem. Phys.* 84, 2972-2979.

Krupa, Z., Huner, N.P.A., Williams, J.P., Maissan, E. & James, D.R. (1987) *Plant Physiol.* 84, 19-24.

Kühlbrandt, W., Wang, D.N. & Fujiyoshi, Y. (1994) *Nature* 367, 614-621.

Lerman, L.S. (1973) In: *Physico-Chemical Properties of the Nucleic Acids* (Duchesne, J. ed.) Vol 3, 59-76, Academic, New York.

Maestre, M.F., Bustamante, C., Hayes, T.L., Subirana, J.A. & Tinoco, I.J. (1982) *Nature* 298, 773-774.

Ruban, A.V. & Horton, P. (1992) *Biochim. Biophys. Acta* 1102, 30-38.

Tinoco, I.J. & Williams, A.L.J. (1984) *Ann. Rev. Phys. Chem.* 35, 329-355.

Tinoco, I.J., Mickols, W., Maestre, M.F. & Bustamante, C. (1987) *Ann. Rev. Biophys. Biophys. Chem.* 16, 319-349.

5. List of publications

Barzda, V., Mustárdy, L., and Garab, G. (1994) Size-dependency of circular dichroism in macroaggregates of photosynthetic pigment-protein complexes, *Biochemistry*, 33, 10837-10841.

Barzda, V., Garab, G., Gulbinas, V. and Valkunas, L. (1995) Long distance migration of the excitation energy and fluorescence quenching mechanisms in chiral macroaggregates of LHCII. In: *Photosynthesis: from Light to Biosphere* (Mathis, P., Ed.) Vol. I, 319-322, Kluwer Academic Publishers, Dordrecht.

Barzda, V., Simidjiev, I., Vianelli, A., Jennings, R., and Garab, G. (1995) Light induced reversible structural changes in chirally organized macroaggregates of purified chlorophyll a/b protein complexes. In: *Photosynthesis: from Light to Biosphere* (Mathis, P., Ed.) Vol. I, 315-318, Kluwer Academic Publishers, Dordrecht.

Barzda, V., Garab, G., Gulbinas, V. and Valkunas, L. (1996) Evidence for long-range excitation energy migration in macroaggregates of the chlorophyll a/b light- harvesting antenna complexes, *Biochim. Biophys. Acta*, 1273, 231-236.

Barzda, V., Istokovics, A., Simidjiev, I. and Garab, G. Inherent structural flexibility of chiral macroaggregates of light harvesting chlorophyll a/b pigment protein complexes. Light-induced reversible structural changes associated with energy dissipation (1996) *Biochemistry* (in press).

Istokovics, A., Lajkó, F., Liker, E., *Barzda, V.*, Simidjiev, I., Garab, G. (1992) Inhibition of the light induced reversible structural rearrangements of the macrodomains and the phosphorylation of membranes by quinone antagonists. In: *Research in Photosynthesis* (ed. N. Murata) Vol. II, 631-634. Kluwer Acad. Publ.

Istokovics, A., *Barzda, V.*, Lajkó, F., Simidjiev, I. and Garab, G. (1995) Characterization of the light-induced reversible structural changes in granal thylakoid membranes. In: *Photosynthesis: from Light to Biosphere* (Mathis, P., Ed.) Vol. III, 265-268, Kluwer Academic Publishers, Dordrecht.

Osváth, S., Meszéna, G., *Barzda, V.* and Garab, G. (1994) Trapping magnetically oriented chloroplast thylakoid membranes in gels for electric measurements, *J. Photochem. Photobiol. B: Biol.*, 26, 287-292.

Simidjiev, I., *Barzda, V.*, Horváth, I. and Garab, G. (1995) Role of thylakoid lipids in the assembly and structural flexibility of chirally organized macroaggregates of the light-harvesting chlorophyll a/b proteins. In *Photosynthesis: from Light to Biosphere* (Mathis, P., Ed.) Vol. I, 115-118, Kluwer Academic Publishers, Dordrecht.

6. Congress and Conference Abstracts

Barzda, V., Mustárdy, L.A. and Garab, G. (1992) Book of Abstracts, FESPP Workshop, Szeged, p.43.

Barzda, V., Mustárdy, L., Valkunas, L., Gulbinas, V., Tóth, Zs. and Garab, G. (1993) Abstracts of 11th International Biophysics Congress, Budapest, Hungary p. 193.

Barzda, V., Mustárdy, L., Valkunas, L., Gulbinas, V., Vianelli, A., Jennings, R.C., and Garab, G. (1993) 4th Nordic-Baltic Workshop on Photochemistry. Program and Abstracts, Tartu, Estonia.

Barzda, V., Simidjiev, I., Vianelli, A. and Garab, G. (1994) ESF Workshop on Pigment-Protein Complexes: Structure and Spectroscopic Properties, Program and Abstracts, Preila, Lithuania.

Barzda, V., Garab, G., Gulbinas, V. and Valkunas, L. (1994) ESF Workshop on Pigment-Protein Complexes: Structure and Spectral Properties, Program and Abstracts, Preila, Lithuania.

Barzda, V., Garab, G., Gulbinas, V. and Valkunas, L. (1995) ESF Workshop on Light-Harvesting Systems, Program, Nant, France.

Barzda, V., Simidjiev, Garab, G., Zucchelli, G., Vianelli, A., Finzi, L., Garlaschi, F. M. and Jennings, R. C. (1995) ESF Workshop on Light-Harvesting Systems, Program, Nant, France.

Barzda, V., Istokovics, A., Simidjiev, I. and Garab, G. (1995) ESF Workshop on Light-Harvesting Systems, Program, Nant, France.

Barzda, V., Garab, G., Gulbinas, V. and Valkunas, L. (1995) Photosynthesis Research, Abstracts Xth International Photosynthesis Congress, Kluwer Academic Publishers, The Netherlands.

Barzda, V., Garab, G., Gulbinas, V. and Valkunas, L. (1996) Abstracts of 40th Annual Meting of Biophysical Society, Biophys. J., 70, A355.

Barzda, V., Istokovics, A., Simidjiev, I. and Garab, G. (1996) Abstracts of 40th Annual Meting of Biophysical Society, Biophys. J., 70, A355.

Garab, G., *Barzda, V.*, Istokovics, A. and Simidjiev, I. (1995) Photosynthesis Research, Abstracts Xth International Photosynthesis Congress, Kluwer Academic Publishers, The Netherlands.

Istokovics, A., *Barzda, V.*, Lajkó, F., Simidjiev, I. and Garab, G. (1995) Photosynthesis Research, Abstracts Xth International Photosynthesis Congress, Kluwer Academic Publishers, The Netherlands.

Simidjiev, I., *Barzda, V.*, Horváth, I. and Garab, G. (1995) Photosynthesis Research, Abstracts Xth International Photosynthesis Congress, Kluwer Academic Publishers, The Netherlands.

Vianelli, A., *Barzda, V.*, Jennings, R.C. and Garab, G. (1993) Abstracts of 11th International Biophysics Congress, Budapest, Hungary, p. 189.