## Introduction and the aim of the work

Aluminium is the most abundant metal in the earth's crust. Despite its abundance, its human toxicity remained disputed until the mid-1970s. The bioavailability of aluminium was rather limited under normal conditions due to the occurrence in the nature only in bound forms with other elements, mostly in insoluble forms. The problem arised when as a consequence of acid rain the pH of natural and soil water decreased and hence, the mobility of the Al(III) ion (the concentration of soluble Al(III) compounds) increased. Other major sources of aluminium exposure, like nutrition, pharmaceutical products, antiperspirants etc. also contribute to the increased level of aluminium. These soluble inorganic and organic Al(III) complexes can easily enter living organisms, which have not thus far developed effective protection mechanisms against the toxicity of this metal ion. A series of clinical and epidemiological studies have demonstrated the relationship between aluminium toxicity and the occurrence of some human disorders such as osteomalacia in the bones, microcytic anemia in the red blood cells or neurodegenerative diseases in the brain (dialysis encephalopathy or dialysis dementia, Alzheimer's disease).

The neurotoxic effect of Al(III) on living organisms is beyond any doubt and aluminium has been shown to interfere with a variety of cellular metabolic processes. The possible damaging effects of Al(III) on tissues may originate in its ability to replace Mg(II) and Ca(II) in their biologically important complexes. An important issue in understanding the toxicity of aluminium is the solubility and speciation of Al(III) compounds, which are strongly pH dependent, whereas the solubility of organically complexed Al(III) is correlated with the concentration and type of dissolved organic ligands. In humans, neither the metabolism of Al(III) nor the mechanism of its toxicity is elucidated yet. In order to get more information about its toxic effects in humans, it is very important to know the speciation of Al(III) in the main biofluids and tissues. In the light of this consideration, the aim of our work was to characterise the interaction of Al(III) with biologically important molecules like peptides, peptide like ligand as salicylglycine and the aminoacid type ligands like iminodiacetate (IDA), nitrilotriacetate (NTA) and their phosphonic derivatives such as N-(phosphonomethyl) glycine (IDAP), imino-bis (methylene-phosphonic acid) (IDA2P) as well as N-(phosphonomethyl) iminodiacetic acid (NTAP), N,N bis(phosphonomethyl)glycine (NTA2P) and nitrilo-tris(methylene-phosphonic acid) (NTA3P).

**Experimental methods applied for the investigations**

The stability constants of the proton and Al(III) complexes of the ligands were determined by pH–potentiometric method. In order to confirm the potentiometric results and to determine the solution structure of the complexes formed multinuclear ($^{1}H$, $^{13}C$, $^{31}P$) NMR spectroscopic techniques were used. In several cases also the $^{1}H,^{1}H$ COSY homonuclear shift correlation and HSQC heteronuclear single quantum correlation NMR spectra were measured. Structure characterisation of the salicylglycine complexes was studied using UV-vis spectroscopy and dynamic light scattering method.

**New scientific results**

**1. Interaction of Al(III) with Salicylglycine**

(1) The SalGly, a dipeptide analogue proved to be weak Al(III) binder. Various mononuclear 1:1 complexes in different protonation states AlLH, AlL, $AlLH_{-1}$ and $AlLH_{-2}$ are formed in the pH range 2–7. Similarly to Ni(II) and Zn(II) complexes, no indication of deprotonation and coordination of the peptide amide group was observed in the case of Al(III).

(2) According to the UV-vis spectra the phenolic-OH group of SalGly remains protonated in the complexes AlLH and AlL. Deprotonation of the phenolic-OH group starts in the pH range 4–6, where the $AlLH_{-1}$ complex is formed. In this complex SalGly is possible bound to Al(III) in a tridentate way ($COO^{-}$, CO, $O^{-}$) and an $OH^{-}$ is also present in the coordination sphere of the metal ion. Interestingly, increasing the pH the ligand reprotonates in species $AlLH_{-2}$ formed at pH > 5.5. Besides the direct coordination through the $COO^{-}$ donor the phenolic-OH is assumed to be in hydrogen bonding with the metastable hydrolytic product of Al(III). Dynamic light scattering measurement demonstrated that the direct or outer-sphere coordination of the ligand to Al(III) has a great influence on the aggregation behaviour of the $Al(OH)_3$ nanoparticles. Namely, SalGly through the formation of the proposed outer-sphere type complex $AlLH_{-2}$ or more precisely $Al(OH)_3 \cdot HL$ hinders aggregation. The adsorption or outer-sphere binding of the ligand on the surface of $Al(OH)_3$

nanoparticles was pH dependent; the extent of adsorption of SalGly increased with increasing pH.

**2. Interaction of Al(III) with the peptides AspAsp and AspAspAsp**

(3) The ligands AspAsp and AspAspAsp behave similarly in aqueous solutions, as they form mononuclear 1:1 complexes in different protonation states. The greater number of carboxylic groups is in the system, the more stable the complexes that are formed. Accordingly, the Al(III) complexes of AspAspAsp are more stable, than the AspAsp corresponding Al(III) complexes.

(4) The coordination of the AspAsp and AspAspAsp ligands to Al(III) differs mainly in the binding mode, namely the AspAsp ligands prefers a bidentate coordination, while for the AspAspAsp ligand formation of a partial tridentate coordination is more probable. Upon increase of the pH, depending on the metal ion to ligand ratio, precipitation occurs at pH ~ 5 to 6. This indicates that the low level of preorganization of the terminal and side-chain $COO^-$ groups in small peptides, such as AspAsp and AspAspAsp is not sufficient to keep the Al(III) ion in solution and to prevent the precipitation of $Al(OH)_3$ at physiological pH. To achieve this, a more specific arrangement of the side-chain donors is necessary.

**3. Interaction of Al(III) with the heptapeptide AcLysSerProValValGluGly**

(5) The interaction between Al(III) and the AcLysSerProValValGluGly heptapeptide is fairly weak in the acidic pH range. Complex formation becomes predominant only in the neutral pH range when the species $AlLH_{-2}$ is formed. The Al(III)–peptide system could be described with presumption of species 1:1 ligand to metal ratio. NMR measurements confirmed coordination of the peptide to Al(III) through the terminal carboxylate group of Gly and the side chain carboxylic function of Glu, with participation of the central peptide carbonyl group in the weakly acidic pH range.

(6) The Al(III)–AcLysSerProValValGluGly heptapeptide can keep the Al(III) ion in solution and can prevent the precipitation of $Al(OH)_3$ at physiological pH, presumably by outer-sphere coordination. Similarly as in the case of SalGly, peptide molecules

bound to $Al(OH)_3$ nanoparticles will prevent macroscopic occurrence of $Al(OH)_3$ precipitate. Our studies support that Al(III), as inter-chain links between oligopeptides may play even more important role in the aggregation of neuropeptides than can play the intramolecular Al(III) binding and in this way Al(III) may be involved in Alzheimer's disease.

## 4. Interaction of Al(III) with iminodiacetic acid, nitrilotriacetic acid, its mixed carboxylic phosphonic and purely phosphonic derivatives

(7) The phosphonic derivatives of IDA and NTA are very effective binders of Al(III), they can prevent hydrolysis of the metal ion and precipitation of the $Al(OH)_3$ even in equimolar solutions up to pH > 8. Mainly 1:1 ligand to metal ratio complexes are formed with Al(III). Characteristic is the existence of binding isomers with tridentate and tetradentate coordination in the mixed carboxylate-phosphonate systems.

(8) Substitution of $CO_2^-$ by $PO_3^{2-}$ increases the pAl values, which suggest a weaker metal binding capacity of the carboxylic derivatives than the phosphonic ones.

(9) Usually no symmetrical arrangement of the donor atoms can ordinarily be expected with any of these ligands. However, the rate of the intramolecular rearrangement motions within the complex molecules seems to be different for the IDA and the NTA derivatives. According to the $^1H$ NMR spectra of Al(III) complexes with IDA, complex formation results in the inequivalence of the two protons in each $CH_2$ group resulting in formation of complexes with low symmetry and the parallel formation of various binding isomers. At the same time, the $CH_2$ groups of NTA and its derivatives are chemically and magnetically equivalent, resulting in high symmetry complexes. The complex $AlLH_{-1}$ formed in case of NTA3P ligand is fluxional, through the fast intramolecular motions of the methyelenephosphonate arms.

(10) Interestingly the bis complexes are hardly formed due to the large spatial requirement of the ligands and the electrostatic repulsion between the 1:1 complex and the charged second ligand moiety.

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