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# Aluminium in Biological Environments: A Computational Approach

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**Abstract:** The increased availability of aluminium in biological environments, due to human intervention in the last century, raises concerns on the effects that this so far "excluded from biology" metal might have on living organisms. Consequently, the bioinorganic chemistry of aluminium has emerged as a very active field of research. This review will focus on our contributions to this field, based on computational studies that can yield an understanding of the aluminum biochemistry at a molecular level. Aluminium can interact and be stabilized in biological environments by complexing with both low molecular mass chelants and high molecular mass peptides. The speciation of the metal is, nonetheless, dictated by the hydrolytic species dominant in each case and which vary according to the pH condition of the medium. In blood, citrate and serum transferrin are identified as the main low molecular mass and high molecular mass molecules interacting with aluminium. The complexation of aluminium to citrate and the subsequent changes exerted on the deprotonation pathways of its tritable groups will be discussed along with the mechanisms for the intake and release of aluminium in serum transferrin at two pH conditions, physiological neutral and endosomatic acidic. Aluminium can substitute other metals, in particular magnesium, in protein buried sites and trigger conformational disorder and alteration of the protonation states of the protein's sidechains. A detailed account of the interaction of aluminium with proteic sidechains will be given. Finally, it will be described how alumnium can exert oxidative stress by stabilizing superoxide radicals either as mononuclear aluminium or clustered in boehmite. The possibility of promotion of Fenton reaction, and production of hydroxyl radicals will also be discussed.

Mini Review Article

## I. INTRODUCTION

Aluminium is the most abundant metal element on the Earth crust, however, biological systems have evolved in the absence of this abundant metal. This apparent paradox can be understood in terms of the effective geo-chemical control of aluminium by means of its interaction with silicic acid [1]. Other metal ions such as Mg(II), Fe(II)/Fe(III), Ca(II), Zn(II) etc, have been biologically available, and biological systems have evolved in the presence of these metals, coordinated to phosphate, carboxylate, hydroxyl and other ligands. However, in the last century, human intervention has made aluminium, sparingly soluble, so available for biological systems that one can say that we have started to live in the aluminium age. However, little is still known on the effects of the human exposure to this element, although one could suspect that its effects should be important due to the highly charged nature of aluminium. In fact, in the last years, there are increasing evidences that aluminium could be behind of a variety of toxic effects in biological systems [2–4], with significant risks for human health. Therefore, the open of the geochemical pandora-box of aluminium into biological systems is unlikely to be without consequences.

The aluminium speciation problem, that is the characterization of the type of aluminium complexes likely to be formed in biological medium, is a complex problem, due in part to the vast variety and complexity of aluminium hydrolytic species [5], their low solubility and their spectroscopic silence. In this sense, computational methods have become a fundamental tool to understand aluminium speciation in biological systems and determine the characteristics of aluminium interaction with molecules of biological interest. With no claim of being complete, we can list four connected areas in which computation can help to unveil specific details of aluminium-ligand interactions:

i) Characterization of aluminium interaction with biomolecular building blocks: amino acids, phosphates, etc, so that fundamental understanding of the intrinsic affinity of aluminium for functional groups representing the building block motifs of biomolecules can contribute to the elucidation of aluminium binding sites in biological systems. In addition, comparison of these affinities with those of essential biometals can help in understanding the propensity for displacement of a given metal by aluminium.

ii) Determination of the various aluminium hydrolytic species that could be formed in aqueous solution as a function of pH implies the study of various protonation states, tautomers and oligomers that aluminium can form in solution [6–9].



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iii) Interaction of aluminium with high molecular weight (HMW) ligands such as proteins, is central for the determination of aluminium speciation in blood. In this sense, serum transferrin is one of the most important blood aluminium carriers. Besides, interaction with β-amyloids have also been identified, and it could be behind the controversial role of aluminium in neurodegenerative diseases.

iv) Interaction of aluminium with low molecular weight (LMW) species commonly present in biological media, could play a role in its transport and fixation in solution. These molecules normally contain various carboxylate-type functional groups in the same unit. Oxalate and citrate are examples of this type of molecules. Besides, interaction with LMW ligands could also be behind some of its most relevant toxic effects. Namely, it has been recently pointed out that aluminium can be involved in the stabilization of superoxide complexes [10] that trigger the Fenton reaction [11].

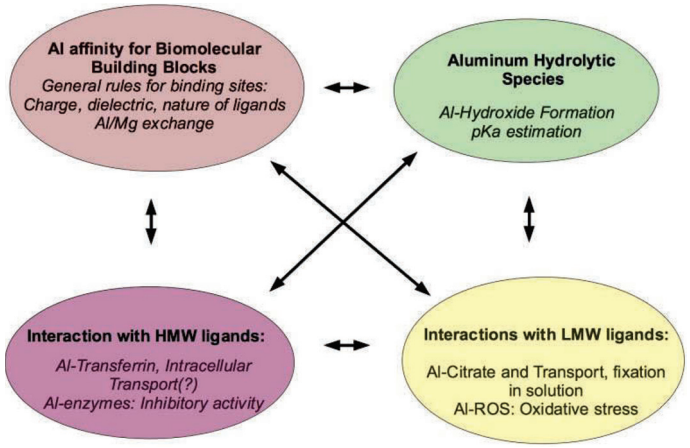


**Figure 1.** Understanding the problem of aluminium speciation in biology requires the interplay between different areas.

All these areas are interconnected, for instance to characterize the mode of interaction of aluminium with proteins (Section IV B), it is important to understand first the interaction with amino acid sidechains (Section II), and how aluminium affects the $pK_a$ of these amino acids (Section III). The characterization of the protonation/deprotonation equilibria is fundamental to understand how aluminium chelates low molecular weight ligands (Section IV A) and high molecular weight proteins (Section IV B). In addition, the analysis of aluminium hydrolytic species is key to understand changes in affinities of aluminium with respect to ligands (Section V A), and some of these interactions could be behind the toxic effects of aluminium (Section V). In the present review, we give examples of how computational studies can assist in each of these areas.

The calculations can shed light on the type of aluminium species that one could find in aqueous environment, and the affinity of aluminium species towards common biomolecules. In addition, calculations can also shed light on the effect that a highly charged metal such as Al(III) could have in the structure of biomolecules bound to this metal. Herewith, we give a number of selected examples of how computational methods can be used to unveil some of the essential characteristics of aluminium interaction with biological systems, and in this sense, help in the understanding of the hazards that living in the aluminium-age could have for biology.

## II. ALUMINIUM INTERACTION WITH BIOMOLECULAR BUILDING BLOCKS: PROTEIN ENVIRONMENTS

Understanding the interaction of aluminium with biological building blocks is essential for the determination of the effect of aluminium in biological systems. The most interesting building blocks with respect to aluminium interaction are amino acid side chains commonly present in metal-ion binding sites, and phosphates ubiquitously present in DNA, RNA, ATP, etc [12]. A first step towards this goal in the group was carried out by Mercero [13–18] and then Rezabal [19–21], who analyzed a series of clusters in which aluminium interacts with various amino acid sidechains in a proteic environment. The protein environments were modeled with the so-called cluster-continuum approach [22, 23]. In this approach, we consider different molecules representing the amino acid sidechains (acetate as a representative model for glutamate and aspartate, methyl-thiol/thiolate for cysteine, methylthioethane for methionine, acetamide for asparagine and glutamine, methanol for serine and threonine, methylimidazole for histidine, and toluene and methylbenzenol for phenylalanine and tyrosine respectively) chelating the metal, and the rest of the octahedral first-coordination shell around aluminium is filled with water molecules. The chosen ligands do not only represent the metal binding site in a protein, but also other organic molecules present in the biological systems, taking part in aluminium metabolism. The whole cluster, considering various combinations and different number (1 to 3) of ligands, is then surrounded by a continuum dielectric to represent different proteic environments, from protein buried sites (small dielectric values $\varepsilon = 2$, 4, …) to fully solvent exposed areas (high dielectric values $\varepsilon = 80$). The results were compared to analogous Mg(II) clusters.

### *A. Metal binding Affinity*

The metal binding affinity was evaluated by calculating the energy of the following reaction:

$$L_m{}^q + X(H_2O)_6{}^{ch} \rightarrow XL_m(H_2O)_{(6\text{-}m\text{-}n)}{}^{(ch+q)} + (m+n)H_2O$$

where ch and q are the charge of the metal cation and the sum of the charges of the m ligands, respectively, n corresponds to the number of ligands (acetates) bound bidentately, and X stands either for the Al(III) or the Mg(II) cations. The reaction defines the metal binding affinity as the water/ligand substitution from the first hydration shell of the metal, where all the exchanges occur simultaneously. It was observed that both Al(III) and Mg(II) share ligand preferences, favoring binding to oxygen and nitrogen groups, in particular negatively charged oxygens. Therefore, the negatively charged acetate and the neutral methylimidazole, followed by formamide and methanol were seen to be preferred for binding Al(III). The monodentate binding mode of acetate was stabilized as compared to the bidentate mode, due to the interaction of the metal-free carboxylate oxygen atoms with the metal-bound water molecules. The binding of the metals to the bioligands was found to be mainly dictated by the favorable Coulomb interactions between the positively charged cation and the negatively charged or neutral ligands, and the solvation free energies of the products and reactants in the dielectric environment considered. Al(III), due mainly to its high charge, has a strong tendency of binding these bioligands, but its desolvation free energy is also very high. The delicate balance between the charge and number of ligands and the dielectric environment regulates the affinity of the metal for the binding sites.

Therefore, we stablish that aluminium will prefer to bind proteins (low dielectric environment) rather than small low weight molecules,