

Aluminium coordination to calmodulin: thermodynamic and kinetic aspects

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Abstract

Calmodulin is an acidic, Ca(II)-binding protein (about 17 000 d) which plays a key role in regulating a broad spectrum of target enzymes in eukaryotic cells. Within each lobe of the dumbbell-shaped molecule there are two adjacent Ca(II)-binding domains, which form a single cooperative unit. Hydrophobic forces are crucial for interfacing the Ca(II)-activated regulatory protein with its target enzyme. Presumably not involving the four specific Ca(II)-binding domains on calmodulin, binding of Al(III) to calmodulin generates considerable dehydration entropies. Al(III)-triggered changes in the α -helix content of calmodulin are involved in the mismatch between calmodulin and its target protein. By altering calmodulin's

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internal dynamics, Al(III) apparently interferes with the proteins capacity to search out conformational substates suitable for proper docking with a specific target protein.

Keywords: Al(III) binding; Al(III)-related dehydration; Al(III) removal; Calmodulin; Calmodulin partners; Protein dynamics

1. Introduction

In eukaryotic cells a small protein, calmodulin, plays a pivotal role in regulating a broad spectrum of target enzymes which in turn are involved in vital cellular processes, such as protein phosphorylation, cyclic nucleotide metabolism, motility, cell division, and sensory perception [1–3]. The regulatory action is accomplished by binding Ca(II) to calmodulin, thereby modulating its conformation in such a manner as to promote specific interactions between the small protein and the respective target. As a cytosolic protein, calmodulin senses changes in intracellular free Ca(II) concentrations, [Ca(II)], ranging from about 10 nM to 1 μ M. Changes in [Ca(II)] are brought about by release of Ca(II) from intracellular stores, or from Ca(II) signals generated in response to the transduction of external input signals across the plasma membrane. Chief among signal transduction pathways is that associated with membrane-bound phosphoinositides [4–6]. Calmodulin is therefore an essential communication link necessary for transforming extracellular information into specific biological responses.

2. Calmodulin

The key to calmodulin's ability to function as a Ca(II)-dependent transducer resides in its primary protein structure. This acidic, globular protein (about 17000 d, 148 amino acid residues, isoelectric point about 4.1) has been highly conserved during the evolution of eukaryotic cells, suggesting the existence of an ancestral gene [7,8]. X-ray diffraction studies demonstrated that calmodulin resembles a dumbbell (length about 6.5 nm) with the two lobes interconnected by an eight-turn α -helix. This central helix (about 2 nm long, amino acid residues 65–92) carries an appreciable net dipole which may play a role in the sequential binding of Ca(II) to the apoprotein [9,10]. Each lobe (diameter about 2 nm) harbours two Ca(II)-binding domains whose helix-loop-helix arrangement (11:12:11 residues respectively) is similar to the structure of Ca(II)-binding units in other Ca(II)-binding proteins [11]. In calmodulin, only the loop regions contain antiparallel β -sheet strands. Coupling antiparallel strands of adjacent Ca(II)-binding loops, hydrogen bonds assist in stabilizing the macromolecule [9,10]. Counting from the amino terminus of calmodulin, the Ca(II)-binding domains are designated as I, II, III and IV respectively. Thermodynamic studies on domain organization in calmodulin indicated that within a lobe the two adjacent Ca(II)-binding domains form a single cooperative block, especially between domains I and II. The two lobes seem to repel each other [12].

The four domains in calmodulin permit specific binding of 4 mol Ca(II) per mol of protein. At approximate vertices of an octahedron, the liganding residues harbour oxygen-containing side chains, such as those of aspartic acid, and the peptidyl carbonyl oxygen. Hydration water is probably part of the ligand sphere [13]. Despite considerable similarities in the primary sequence of the domains' loops, the dissociation constants for Ca(II) vary between 1 μ M and 10 μ M, at physiological conditions. The Ca(II)-binding properties and the sequence of Ca(II)-binding to the protein are critically dependent on the presence of monovalent ions, pH, and divalent cations like Mg(II). Current models indicate that domains III and IV (residing on the C-terminus lobe) are cooperatively binding Ca(II) with high affinity, as compared with the low affinity cooperative binding in domains I and II [2,14]. NMR experiments on calmodulin in solution further corroborate the existence of high and low affinity Ca(II)-binding domains [14]. For the high affinity domains, the rates of Ca(II) dissociation range from 3 to about 10 s⁻¹, while those from the low affinity domains are in the 300 to 550 s⁻¹ range, at room temperature [15,16]. Ca(II) association constants are expected to range from 10⁷ to 10⁸ s⁻¹, since these values are related to the rates of water exchange of hydrated Ca(II) ions [17].

Upon binding of 1 to 4 mol Ca(II) per mol of apoprotein, pronounced structural transformations take place in the protein such as increase of the α -helix content, and enhanced formation of hydrophobic regions on the protein surface. Ca(II)-binding generates a readjustment/formation of the β -sheet within the binding loops of the domains [18]. These helical rearrangements may in part result from coupled movements of a pair of helices [19]. Elicited by coordination reactions [20], especially by those involved in the binding of two Ca(II) in the high affinity domains of the C-terminus lobe, local perturbations in structure are also transmitted to the N-terminus lobe of the flexible macromolecule [21]. Ca(II) binding also effects the protein to elongate by about 7% [22]. Ca(II)-triggered short and long range conformational changes cause an enhanced exposure of hydrophobic regions on the protein surface [23], thus preparing calmodulin for docking with and the activation of the target protein.

The molecular basis for calmodulin's ability to activate numerous target proteins has not been elucidated. Nevertheless, experiments on calmodulin-binding oligopeptides, like the amphiphilic melittin (2845 d, about 1.5 nm long, 26 amino acid residues), demonstrated that hydrophobic forces are crucial for interfacing the Ca(II)-calmodulin with its target [24]. Regarding calmodulin interactions with its partner (molar ratio 1:1), binding constants vary from 10 μ M to 100 pM [25]. The Ca(II)-calmodulin structure apparently collapses around its partner [26], involving hydrophobic and salt bridges [27].

3. Al(III) in biology

3.1. Al(III) in aqueous solution

Since the water content of a typical cell comprises about 80% of the cellular weight, it is essential to understand the behaviour of Al(III) in aqueous solution.

Regarding details of the aqueous chemistry of Al(III) we are referring to informative review articles [28–30]. Briefly, the coordination of the trivalent cation, Al(III), in aqueous solution is generally either tetrahedral, $\text{pH} > 6.2$, like in $\text{Al}(\text{OH})_4^-$, or octahedral, $\text{pH} < 5$, like in $\text{Al}(\text{H}_2\text{O})_6^{3+}$. The ionic radius is 0.054 nm; the hydrated radius has a value of 0.475 nm [31]. As the pH increases, the hexahydrated species hydrolyzes, especially in the range from $5 < \text{pH} < 6.2$. The deprotonation of Al(III) in aqueous solution can be partially or completely inhibited by ligation of Al(III) to ligands, in particular those containing oxygen and fluorine as donor atoms [32]. Given the multitude of ligands in biological fluids, the slow solvent exchange rate of Al(III) is of great import. To illustrate, the formation rate constants of solvated Al(III) with multidentate ligands vary from about 0.1 to $10 \text{ M}^{-1} \text{ s}^{-1}$ [17,32], while those of Ca(II) range from about 10^7 to $10^{10} \text{ M}^{-1} \text{ s}^{-1}$ [17]. In the absence of ligands ($\text{pH} < 7$), formation of polynuclear Al(III) species in aqueous solution is unclear for $[\text{Al(III)}] < 100 \mu\text{M}$ [33,34].

3.2. Al(III) in cells

Formation of polynuclear species is also highly improbable in cells, where numerous Al(III) ligands are available. According to model calculations, the intracellular free Al(III) concentration, in the neutral pH range, is in the subnanomolar range [35]. ATP seems to be a key ligand for Al(III) [36,37], affording subnanomolar concentrations of free [Al(III)] for typical experimental conditions (neutral pH, millimolar ATP, Al(III) doses less than 1 mM) [38]. Concerning the total [Al(III)] in cells, background levels were found to be around 1 μM in neuroblastoma cells, but may reach close to 1 mM in tangle-bearing neurons of patients afflicted with amyotrophic sclerosis [39].

4. Al(III) impacts calmodulin

4.1. Thermodynamic aspects of Al(III) binding to calmodulin

Calmodulin harbours numerous electron pair donor atoms (e.g. carboxyl and carbonyl oxygens, amino and guanidino nitrogens) which are potential ligands capable of participating in metal coordination. In pretransition metals (e.g. Ca(II), Mg(II), Al(III)) the complex formation is critically dependent on the size of the ligand and the nature of the metal ion; electrostatic interactions play a key role [40].

Concerning Al(III) binding to bovine brain calmodulin, equilibrium dialysis ($\text{pH} 6.5$, 10 mM Tris buffer, 5 mol Ca(II) per mol of protein, 5 μM protein) studies demonstrated [41] that the macroscopic dissociation constant is $1.2 \times 10^{-6} \text{ M}$ for the first Al(III) bound to the protein. This reaction is associated with an entropy change of $\Delta S^\circ = 22 \text{ cal deg}^{-1} \text{ mol}^{-1}$. Binding of a second and third mole Al(III) to calmodulin is characterized by an average entropy change of about $\Delta S^\circ = 41 \text{ cal deg}^{-1} \text{ mol}^{-1}$ per each mol Al(III), corresponding to an average dissociation constant of about $0.6 \times 10^{-6} \text{ M}$ [42]. The positive entropy changes observed may be attributed

to the considerable dehydration entropies gained as the solvation structures of the ligand and the metal cation are breaking up upon formation of the complex. In view of spectroscopic evidence [41–43] (see below), that the calmodulin conformation is appreciably perturbed and becomes more randomized upon binding of the second and third Al(III), it is reasonable to expect that these Al(III)-triggered effects are in part associated with conformational entropy changes. These latter changes may thus contribute to the overall entropy changes ($\Delta S^\circ = 41 \text{ cal deg}^{-1} \text{ mol}^{-1}$) observed upon binding of the second and third Al(III) to calmodulin. For comparison, the average entropy change associated with the binding of a mole of Ca(II) per calmodulin is $16.1 \text{ cal deg}^{-1} \text{ mol}^{-1}$, mostly attributable to dehydration processes [44]; the dissociation constants vary in the range from 10^{-5} to 10^{-6} M [45].

The magnitude of the dissociation constants and the extent of dehydration observed suggest that the first three Al(III) ions are binding to localized regions on the protein. Via some form of multidentate chelation, Al(III) ions seem to coordinate directly to a specific set of ligands, because the binding is tighter by several orders of magnitude than expected for Debye–Hückel screening ions [46]. At physiological pH, potential metal coordination sites are carboxyl groups and the imidazolyl moiety of histidine as indicated by the deprotonation pH ranges of amino acids [47]. Important coordination sites are also donor atoms in the lateral groups of amino acid residues (aspartyl, glutamyl), rather prevalent in the acidic calmodulin.

Despite the absence of detailed structural information, it is nevertheless safe to assume that the high-affinity coordination sites of Al(III) do not involve those of the four specific Ca(II)-binding domains on calmodulin. Equilibrium dialysis and atomic absorption studies indicated that Ca(II) remained bound to calmodulin, following addition of Al(III). Sequential addition of Al(III) simultaneously caused an almost complete inhibition of the Ca(II)–calmodulin-dependent cyclic 3':5'-nucleotide of phosphodiesterase activity when the molar ratio of [Al]–[calmodulin] reached a value 4:1 [48]. The observed lack of Al(III)–Ca(II) exchange is not surprising since metal coordination with proteins is generally governed by ionic size and charge [49]. Having similar ionic radii as that of Ca(II) (radius 0.099 nm) [31], lanthanides are able to replace specifically bound Ca(II) in calmodulin which remains enzymatically active. However, with an ionic radius of 0.132 nm, Pb(II) can also replace Ca(II) in specific Ca(II) binding regions [14,50]. Besides the nature of the cation, other factors like stereoselectivity thus also contribute to metal coordination to ligands in calmodulin.

Since Al(III) apparently does not displace Ca(II) from its specific coordination domains, one can only speculate about the location of putative Al(III) binding sites on calmodulin. As a first approximation, Al(III) may bind to sites harbouring Mg(II) which has an ionic radius (0.065 nm) close to that of Al(III). Measuring the infrared absorption maxima in the amide I' region (1644 cm^{-1}) of calmodulin, conformational rearrangements in the α -helical regions were observed following Mg(II) binding [51]. These findings are in accord with those tentatively localizing Mg(II)-binding sites in the N-terminal α -helix of each of the four Ca(II)-binding domains [24], and possibly in the central α -helix [14], connecting the two lobes. The Ca(II) and Mg(II) sites are, however, not identical, and the Mg(II) dissociation constants are in the

millimolar range [24]. As to putative Al(III) binding sites, there is spectroscopic evidence [41] that Al(III) application to calmodulin produces a considerable decrease in α -helix content of the protein. This decrease in helix content may be attributed to Al(III) residing in Mg(II) binding sites mentioned above. Further, these sites harbour aspartyl and glutamyl residues [24] which are favourable for metal coordination. Since the binding affinity of Al(III) is appreciably higher than that of Mg(II), the divalent cation is presumably displaced.

Given the current information, further experiments are necessary to quantify the effect of Al(III) binding to calmodulin, in particular the role of the ionic strength, pH, and the presence of Mg(II). Identifying peptide regions which bind Al(III) with high affinity, Al(III)-chelate affinity chromatography [52] may be applied to purify peptides produced by trypsin digestion of calmodulin. Site-directed mutagenesis [53] can be used to introduce specific amino acids probably involved in interactions between the metal and the protein ligand. Additionally, in studies on calmodulin, efforts should be made to accurately assess the impact of low concentrations of Al(III) by using appropriate metal ion buffers [54]. To better evaluate Al(III)-peptide-protein interactions, data should be generated regarding complex formation of Al(III) and amino acids, in particular aspartic and glutamic acids.

4.2. Kinetics of Al(III) removal by citric acid from calmodulin

The kinetics of Al(III) dissociation from apocalmodulin and Ca(II)-saturated calmodulin was studied by atomic absorption spectroscopy [55]. Following preloading of apocalmodulin with Al(III) (molar ratio 1:3; pH 6.5, 10 mM Pipes buffer, 10 μ M calmodulin, 22 °C), Al(III) was quantitatively removed in the presence of citrate ([citrate]–[Al(III)]=9:1), a strong Al(III) chelator [29]. The kinetics of Al(III) removal could be best described as the sum of two exponential expressions characterized by rate constants: $k_2 = 4.1 \pm 0.7 \text{ min}^{-1}$ and $k_3 = 0.6 \pm 0.08 \text{ min}^{-1}$. The velocity of removal of the first Al(III) exceeded the instrumental deadtime (15 s). In the presence of Ca(II) ([Ca(II)]–[calmodulin]=6:1), the removal of Al(III) was accelerated, as indicated by $k_{2+} = 5.5 \pm 0.5 \text{ min}^{-1}$ and $k_{3+} = 0.5 \pm 0.04 \text{ min}^{-1}$. As judged by the rates of Al(III) removal of the second and third Al(III), the binding sites are heterogeneous and probably noninteracting. The differences in the rates, i.e. k_2 vs. k_{2+} , presumably arise from the accessibility of the chelator to the respective Al(III) binding sites in the flexible apocalmodulin [56], or the more compact Ca(II)-saturated calmodulin [10,57]. Given these observations, one can speculate that the first Al(III) is coordinated to ligands in the central helix region which is readily accessible in the absence or presence of Ca(II). The second and third Al(III) are coordinated to ligands in the vicinity of, or at, the Ca(II)-binding domains, as hypothesized above, where chelator access might be restricted in the more compact Ca(II)-saturated protein compared with the more open apocalmodulin. These types of restriction and the profound denaturation initially produced by Al(III) in the protein [41] may account for the lack of ready reversibility of the Al(III)-calmodulin complex upon application of citrate. It is noteworthy that the rate constants, k_2 and k_3 , correspond to half-lives of about 10 and 70 s respectively. Similar time constants,

ranging from 10 to about 100 s, have been observed in thermal unfolding reactions of ribonuclease A [58], whose molecular weight ($M \approx 13\,700$) is close to that of calmodulin. Citrate-related removal of Al(III) is a considerably slower process than dissociation of Ca(II) from calmodulin. Employing Ca(II)-sensitive fluorescence chelators, the latter rate constants, k_{off} , 550 s^{-1} and about 5 s^{-1} (19°C), correspond to the fast and slow release of two Ca(II) each from the protein [44].

Al(III) application inhibited the Ca(II)- and calmodulin-dependent 3',5'-cyclic nucleotide phosphodiesterase activity by impairing calmodulin rather than the basal activity of the enzyme, examined in the absence of calmodulin [41]. Following exhaustive citrate removal by dialysis, the Al(III)-depleted calmodulin stimulated the Ca(II)-calmodulin-dependent phosphodiesterase activity in a manner identical to a calmodulin which had never seen Al(III) and citrate. Despite such a complete restoration of biological activity, restoration of the α -helix content of calmodulin remained incomplete, irrespective of the presence or absence of Ca(II) [55]. This lack of complete restoration may result from local rearrangements of ligands in the region previously occupied by Al(III).

Clearly, more experiments are necessary to understand mechanisms of chelator-triggered dissociation of Al(III) from calmodulin. Al(III)-sensitive, fluorescent chelators may be used to observe processes related to the removal of the first Al(III). The Al(III)-related pathway of calmodulin refolding should be analysed relative to that operative upon Ca(II) dissociation.

4.3. Al(III) impairs recognition of calmodulin by target proteins

Al(III)-triggered structural changes in calmodulin reportedly led to the inhibition of Ca(II)-calmodulin-dependent enzymes like 3',5'-cyclic nucleotide phosphodiesterase [41], a plant membrane-bound ATPase activity [59], and calpains [60,61]. To get insight into the topography of the enzyme's binding region with Ca(II)-activated calmodulin, a high-affinity, amphiphilic, 26-residue peptide [24,25] from bee venom, melittin, was used [43]. The steady state and time-dependent fluorescence characteristics of melittin's single tryptophanyl residue are sensitive parameters to derive information on the peptide's interface with calmodulin, with Al(III) absent or present. With Al(III) present, the tryptophanyl residue experienced a more polar micro-environment at the interface of melittin with Ca(II)-calmodulin. These findings are probably a consequence of the Al(III)-triggered breakage of α -helices in Ca(II)-calmodulin [41] which in turn would lead to a more open Ca(II)-calmodulin structure compared with the compact Ca(II)-calmodulin structure [62], observed in the absence of Al(III). Al(III)-induced structural changes are coupled with a rearrangement of solvation water, which plays a key role in protein dynamics [63,64] and as a hydrogen bond sink [65]. An open protein structure is also indicated by: (a) a decreased immobilization of a spin probe covalently attached to Al(III)-treated Ca(II)-calmodulin [42]; (b) an improved access of diffusion-controlled, collisional quenching molecules to the fluorophore of melittin, associated (1:1) with calmodulin; (c) an enhanced rotational correlation time of melittin's fluorophore. This faster correlation time (3.5 ns) is indicative of segmental motions in the vicinity of the

tryptophanyl residue compared with restricted motions (8.1 ns) observed in the hydrophobic–hydrogen bonding [65,66] interplay of the compact melittin–calmodulin complex, existing in the absence of Al(III) [43]. Since the basic moiety of melittin apparently associates with acidic amino acid residues [26,67] of the central helix in biochemically active Ca(II)–calmodulin [9,10], it is noteworthy that formation of time-dependent hydrogen bonding patterns by the residues Arg 74, Arg 86, and Arg 90 are crucial in the compaction process during complexation [68]. The central helix has also been suggested to harbour a Mg(II)-binding site [14] which in turn is a putative site for Al(III), as discussed previously. Arg 86 is flanked by three glutamate residues [24] (Glu 82, Glu 83, Glu 84) potentially capable of forming chelate rings through side chain donor atoms [47]. Given the proximity of key residues for the formation of hydrogen bonding patterns and the putative Al(III)-binding site on the central helix, Al(III)-triggered breakage of hydrogen bonds would have profound repercussions on the compaction process during calmodulin's interaction with melittin. Taken together, the presence of Al(III) apparently induces a mismatch between calmodulin and its partner. Such a mismatch may be instrumental in Al(III)-related malfunctions of Ca(II)–calmodulin dependent enzymatic reactions.

Concerning the Al(III)-related lack of recognition between calmodulin and its specific partner, experiments assessing solely the binding constants for the two proteins are obviously insufficient. Detailed structural and dynamic studies are required to ascertain Al(III)-related changes in the complementary surface, crucial for recognizing calmodulin's specific partner protein. Particular attention should be paid to the role of hydration water presumably involved in the Al(III)-disturbed compaction process upon complexation.

4.4. Al(III)-related changes in calmodulin's internal dynamics

Like other globular proteins, calmodulin has a distinct equilibrium structure [10]. Affording calmodulin the capacity to associate with specific partners, other conformations, separated by low energy barriers, and different from the average equilibrium structure, must exist [63,64,69].

Although a clear picture has not yet emerged, the space–time dynamics in calmodulin was illustrated by nanosecond motions of a specific site [70,71], by thermally induced fluctuations modulating dipole–dipole interactions [72], by nanosecond responses elicited via ligand-triggered conformational perturbations [21], and by internal and side chain mobilities in NMR experiments [15,44]. Whether low frequency motions (wave numbers less than 50 cm^{-1}) are important for calmodulin's biological activity has not been elucidated [73].

For the purpose of diagnosing Al(III)-related anomalies in the dynamics of calmodulin, relative to that in the presence of Ca(II), the frictional resistance to local motions of a protein-bound fluorophore was evaluated [74]. The fluorescence anisotropy and lifetime of protein-associated fluorophore are sensitive to global motions of the protein and to local rotations in the fluorophore's microenvironment. From the temperature dependence of these fluorescence parameters the thermal coefficient of the frictional resistance to the fluorophore motions can be extracted [75,76]. As

to calmodulin, Al(III)-generated changes in fluorescence parameters were measured (0.1 M KCl, 10 mM Mops buffer, pH 6.5, 5 mol Ca(II) and 3 mol Al(III) per mol calmodulin, range 10 to 40 °C) through a fluorophore covalently anchored at site 26 of spinach calmodulin where a cysteinyl residue is residing. Both in the presence or absence of Al(III), calmodulin underwent a substate change at a critical temperature of 25 °C [74]. This temperature is appreciably lower than that of the major unfolding of the protein: at 55 °C for apocalmodulin and above 90 °C in the presence of Ca(II) [77]. In the presence of Al(III), the enthalpy, $\Delta H = 8.2 \text{ kcal mol}^{-1}$, was close to that of apocalmodulin ($8.0 \text{ kcal mol}^{-1}$), as opposed to biochemically active Ca(II)-calmodulin ($5.3 \text{ kcal mol}^{-1}$), measured in the absence of Al(III). The lower ΔH value in biochemically active calmodulin apparently reflects the sensitive dynamic aspects essential for the transfer of information within the protein in response to regulatory stimuli. As indicated by a $\Delta H = 8.2 \text{ kcal mol}^{-1}$ and the thermal resistance coefficients, the fluorophore motions appeared to be less disposed to temperature changes when Al(III) was associated (3:1) with calmodulin. Since Al(III) application to calmodulin caused hydrogen bond breakage [41], some of the temperature-dependent changes in ΔH and motional characteristics described above may be attributed to alterations in protein hydration (solvent fluctuations) relative to that seen by biochemically active Ca(II)-calmodulin. The observed restricted fluorophore mobility at site 26, with Al(III) present, may in part result from the increased viscosity in the fluorophore's microenvironment [74], because solvent atoms exert a damping effect on the atoms at the surface of the protein [64]. Taken together, by altering calmodulin's conformational substates, Al(III) apparently interferes with the protein's capacity to search out substates suitable for proper docking with a specific partner protein harbouring melittin-like amino acid sequences.

5. Summary

To fully understand the regulatory properties of multifunctional Ca(II)-calmodulin, the systematic features of the protein's space-time dynamics must be clarified. Links must be established between the critical conformational substate at the onset of ligand binding (e.g. Ca(II) coordination) and the final conformational substate(s) responsible for correct docking between calmodulin and its respective target. By the same token, the temporal and structural gap must be bridged between the initial substate of the Al(III)-coordinated protein and the dysfunctional substate, nonoperative for docking, to understand molecular details of the Al(III)-induced lesion on calmodulin. By employing bioengineered calmodulin with a single intrinsic fluorescence emitter (tryptophan), located at selectively defined sites along the protein backbone, fluctuations of the local state can be monitored at various regions of calmodulin subjected to Al(III) coordination.

Recent studies had indicated that Al(III) is primarily bound to membrane-associated elements of the Ca(II)-phosphoinositide signalling pathway [38,39], rather than to cytosolic calmodulin. However, the possibility cannot be excluded

that Al(III) is also binding to calmodulin-dependent enzymes associated with the membrane [78], or to calmodulin involved in membrane channel functions [79].

Given the considerable amount of information available on structural details (X-ray structure, amino acid sequence), and the availability of genetic tools [53] to design calmodulins differing in amino acid composition, we believe that calmodulin is a unique instrument to derive a microscopic picture on the impact of Al(III) coordination on an important macromolecule.

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