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Effects of Al(III) speciation on cell membranes and molecular models

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Contents

Abstract

Aluminum, a very abundant metal, might play an important role in several pathologies which could be related to its interactions with cell membranes. Although the effects of Al(III) on biological membranes have been extensively described, direct information concerning the molecular basis of its biological activity is rather scarce. One reason for this lack of molecular information is the ill-defined chemical speciation of the metal compounds utilized in toxicological experimental protocols. Another is the complex molecular structure of cell membranes. For this reason, molecular models consisting in phospholipid bilayers are commonly used. In this review the interaction of four Al(III) compounds with phospholipid bilayers built-up of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) and their effects on ion channels present in isolated toad skins are discussed. The aluminum compounds are $Al(acac)_3$, $AlCl_3$, AlF_3 and the Al–citrate complex $[K_5Al(C_6H_4O_7)_2]$. It is concluded that they interact with and produce different structural and functional effects on the model and biological membranes. X-ray diffraction revealed that $AlCl_3$ (1 mM) induced the most damaging effects to both DMPC and DMPE bilayers whereas the Al–citrate complex caused only slight perturbation, the effects of $Al(acac)_3$ and AlF_3 being intermediate. The inhibitory effects on the isolated skin, in descending order, were (100 μM): $AlCl_3$, possibly by indirect and direct inactivation of Na^+ channels and/or perturbation of an ATPase; AlF_3, by direct inactivation of the Na^+ channel and mild ATPase inhibition; Al–citrate, by decrease of Na^+ permeability, and lastly, $Al(acac)_3$, which decreased Na^+ transport only at far higher concentration (250 μM).

Keywords: Aluminum; Membrane; Phospholipid bilayer; Ion channel

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1. Introduction

Aluminum is the most abundant metal and the third most common element. However, despite its abundance, no useful biological function has been discovered. On the contrary, it is recognized as a toxic metal. In fact, compelling evidence has shown that abnormally high Al(III) levels are linked to pathologies such as dialysis dementia, iron-adequate microcytic anemia, osteomalacia and possibly Alzheimer's disease [1]. The goal of understanding the cellular and eventually the molecular basis of Al toxicity has stimulated enormous efforts to develop animal, cellular and molecular models.

The cell membrane is a diffusion barrier, which protects the cell interior. Therefore, its structure and functions are susceptible to alterations as a consequence of interactions with metal ions. In general, they can influence the viscosity of cell membranes both in vitro and in vivo. When membrane suspensions in vitro are incubated with metal ions their change in rigidity will depend on the type of membrane, the pH, the nature and concentration of the metal. Corain et al. [2] found that 0.34–8 mM Al(III) caused an increase in the microviscosity of rabbit erythrocytes; Van Rensburg et al. [3] reported that 0.01–10 μM (pH 5.5) increased the microviscosity of erythrocyte membranes, but decreased that of platelet membranes. On the other hand, Al(III) salts injected in vivo into the brains of rabbits resulted in fragmentation of the endoplasmic reticulum and in the formation of monofilamentous neurofibrillary tangles in the brains of cats; in renal dialysis patients suffering from aluminum encephalopathy, post mortem examination showed immature plaques. While none of these conformed with the paired helical filamentous neurofibrillary tangle or classical senile plaque found in Alzheimer's disease patients, Al was responsible for these neuropathological aberrations [3]. High Al and Mn concentrations in the drinking water in the Guam islands in the Western Pacific together with low dietary Ca and Mg have been implicated in the etiology of the so-called Parkinson-dementia complex of Guam [4]. Additional effects of Al(III) on membrane structure and function have been summarized by Banks and Kastin [5]. Thus, whereas the effects of Al(III) on biological membranes have been extensively described, direct information concerning the molecular basis of its biological activity is rather scanty. The reason for this lack of molecular information is mainly due to the ill-defined chemical speciation of the metal compounds utilized in toxicological experimental protocols.

In aqueous solutions aluminum solubility is highly pH dependent. Under acidic or alkaline conditions, or in the presence of appropriate ligands, soluble species are formed, but in the range of physiological pH values (between 6 and 8) Al(III) is generally insoluble. At low pH values ($pH < 5$), the main species is $Al[(H_2O)_6]^{3+}$. However, as the pH increases, $Al(OH)^{2+}$ and $Al(OH)_2^{+}$ are gradually formed and at neutral pH amorphous $Al(OH)_3$ precipitates; at basic pH this precipitate dissolves to form $Al(OH)_4^{-}$ [6]. The concentration distribution of the hydrolysis products of Al(III) as a function of pH has been reported elsewhere [7,8]. For this reason we have used as a chemical model aluminum acetylacetonate ($Al(acac)_3$), a neutral, chemically well defined, hydrolytically stable and lipophilic compound, on cell and model membranes [7,9]. As either Al(III), or its hydrated form $[Al(H_2O)_6]^{3+}$, is believed to be the principal reactive form in biology [10,11], we thought it of interest to carry out, by means of aqueous solutions of $AlCl_3$ and AlF_3, studies similar to those performed with $Al(acac)_3$ in order to detect the structural and functional effects that the metal ion might induce in cell membranes. In particular, we took into account that Al(III) salts are commonly used in water treatment plants [10,12,13] and the reports that AlF_3 induces widespread neurotoxicity in rats [14,15]. We have also included in these studies an Al complex with citric acid $[K_5Al(C_6H_4O_7)_2]$, as this carboxylate ligand is the predominant small molecule Al(III) binder facilitating its incorporation into mammals [16].

With this aim, various concentrations of aqueous solutions of these four salts were incubated with molecular models of biomembranes. These consisted of multilayers of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE). They represent phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively [17]. The capacity of these four Al(III) compounds to perturb the structures of these phospholipid multilayers was determined by X-ray diffraction. On the other hand, the best known biological model for ion transport across tight epithelia is the isolated toad skin, which has been extensively used to obtain an insight into the mechanism of action of numerous compounds. Thus, this work examines the effects of the four aluminum compounds on the amphibian skin. These systems and methods have also been used to determine the interaction with and perturbing effects on membranes by other metal ions such as Zn(II) [18], Cu(II) [19] and Hg(II) [20,21].

2. X-ray studies on the interaction of aluminum species with phospholipid multilayers

Synthetic DMPC (lot 80H-8371 A grade MW 677.9), DMPE (lot 68F-8350 A grade MW 635.9) from Sigma, aluminum acetylacetonate ($Al(acac)_3$, acac = 2,4-pentanedionate), prepared as described elsewhere [22], $AlCl_3$ (Titrisol) from Merck, AlF_3 from Aldrich and the

aluminum–citrate complex [$K_5Al(C_6H_4O_7)_2$], prepared as described elsewhere [23], were used without further purification. About 1 mg of each phospholipid was introduced into 1 mm diameter special glass capillaries, which were then filled with 200 μl of (a) distilled water, aqueous solutions of (b) $Al(acac)_3$, (c) $AlCl_3$, (d) AlF_3, and (e) aluminum–citrate in a wide range of concentrations. The specimens were X-ray diffracted 2 days after preparation in flat plate cameras provided with rotating devices. Specimen-to-film distances were 8 and 14 cm, standardized by sprinkling calcite powder on the capillary surface. Ni-filtered Cu–K_α radiation from a Philips PW 1140 X-ray generator was used. The relative reflection intensities on films were measured by peak-integration using a Bio-Rad GS-700 microdensitometer and Bio-Rad Molecular Analyst/PC image software; no correction factors were applied. The experiments were performed at 17 ± 2 °C, which is below the main phase transition temperature of both DMPC and DMPE. Each experiment was repeated three times and in case of doubts additional experiments were carried out.

2.1. $Al(acac)_3$

The graphs in Fig. 1(a) exhibit the results obtained by incubating DMPC with water and aqueous solutions of $Al(acac)_3$ and the Hacac ligand. As expected, water altered the structure of DMPC: its bilayer width increased from about 55 Å in its dry crystalline form [24,25] to 64.4 Å when immersed in water, and its low-angle reflections were reduced to only the first two orders of the bilayer width. On the other hand, a new and strong reflection of 4.2 Å showed up in the high-angle region, whose appearance was indicative of the fluid state reached by DMPC bilayers and corresponded to the average distance between the fully extended acyl chains organized with rotational disorder in hexagonal packing. Addition of 10^{-3} mM $Al(acac)_3$ produced a marked decrease in the phospholipid reflection intensities. In fact, the low- and high-angle reflections showed intensities considerable lower than those of Al-free DMPC. These results imply that this low concentration induced molecular disorder of the DMPC bilayer. It can also be observed that concentrations up to 1 mM $Al(acac)_3$ caused a slight reordering of DMPC molecules. Fig. 1(a) also shows that the perturbing effect of the Hacac ligand was less substantial than that induced by $Al(acac)_3$.

Fig. 1(b) illustrates the results of the X-ray diffraction analysis of DMPE incubated with water, $Al(acac)_3$ and Hacac. As reported elsewhere [26], water did not significantly affect the bilayer structure of DMPE. However, $Al(acac)_3$ in increasing concentrations (up to 0.1 mM) progressively decreased the low- and high-angle reflection intensities of DMPE. The findings indicate that $Al(acac)_3$ perturbed the lipid bilayer structure in a concentration-dependent manner. The effect was reversed at 1 mM $Al(acac)_3$, as the DMPE X-ray pattern showed a significant increase in the low- and high-angle reflection intensities. These events suggest that 1 mM $Al(acac)_3$ induced a molecular reordering of DMPE, particularly in the polar head region, an effect that was not observed with its ligand Hacac.

2.2. $AlCl_3$

Fig. 2(a) compares the diffraction pattern of DMPC alone to that of DMPC incubated with $AlCl_3$ in a 10^{-3}–

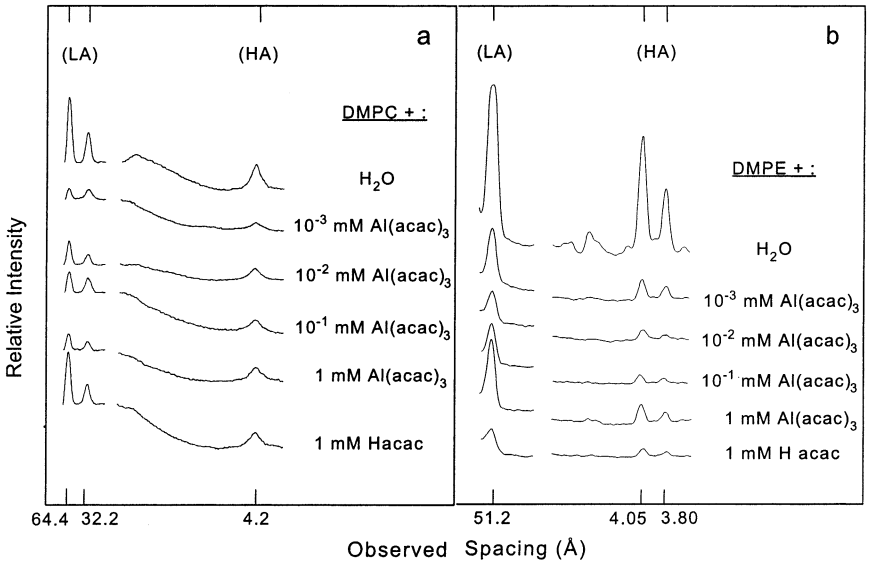

Fig. 1. Microdensitograms from X-ray diffraction patterns of (a) DMPC and (b) DMPE in water and aqueous solutions of $Al(acac)_3$. LA and HA correspond to low- and high-angle reflections.

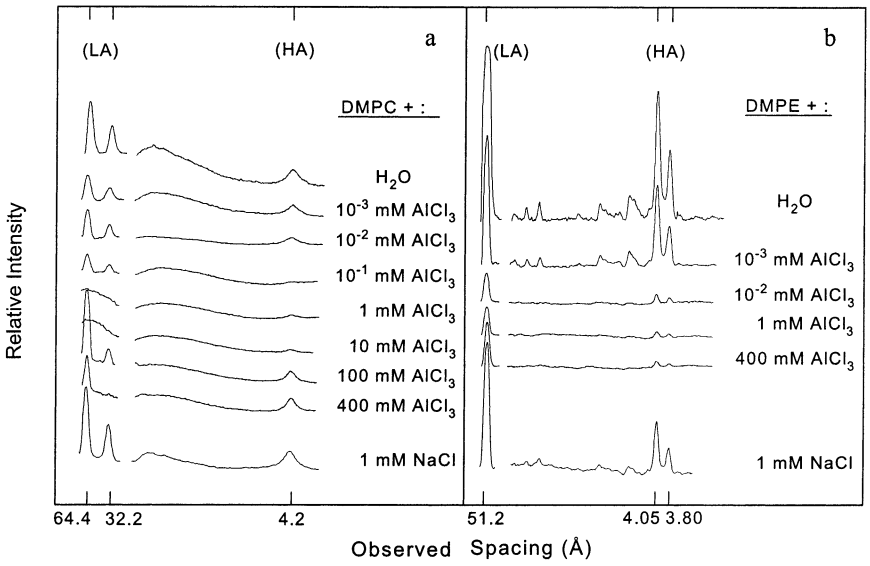

Fig. 2. Microdensitograms from X-ray diffraction patterns of (a) DMPC and (b) DMPE in water and aqueous solutions of $AlCl_3$. LA and HA correspond to low- and high-angle reflections.

10^2 mM concentration range. The response consisted of a gradual decrease in the phospholipid reflection intensities, until the 4.2 Å and the low-angle reflections practically disappeared after exposure to 10^{-1} and 1 mM $AlCl_3$, respectively. However, these and some additional reflections showed up again at 100 mM. These results imply that $AlCl_3$ induced a stronger molecular disorder of the DMPC bilayer than $Al(acac)_3$, an effect that was also reversed but at an $AlCl_3$ concentration 100 times higher. Fig. 2(b) shows that 10^{-3} mM $AlCl_3$ induced a very slight effect on the low- and high-angle reflections of DMPE. However, 10^{-2} mM produced a considerable weakening of all these reflection intensities which remained unchanged up to 400 mM, concentration under which the first low-angle reflection considerably increased its intensity.

2.3. AlF_3

Fig. 3(a) reveals the effects of AlF_3 interactions with DMPC in the 1–400 μM (the upper limit that could be attained) concentration range. DMPC reflections were present in all the assayed concentrations; however, as the concentration increased (up to 5×10^{-2} mM there

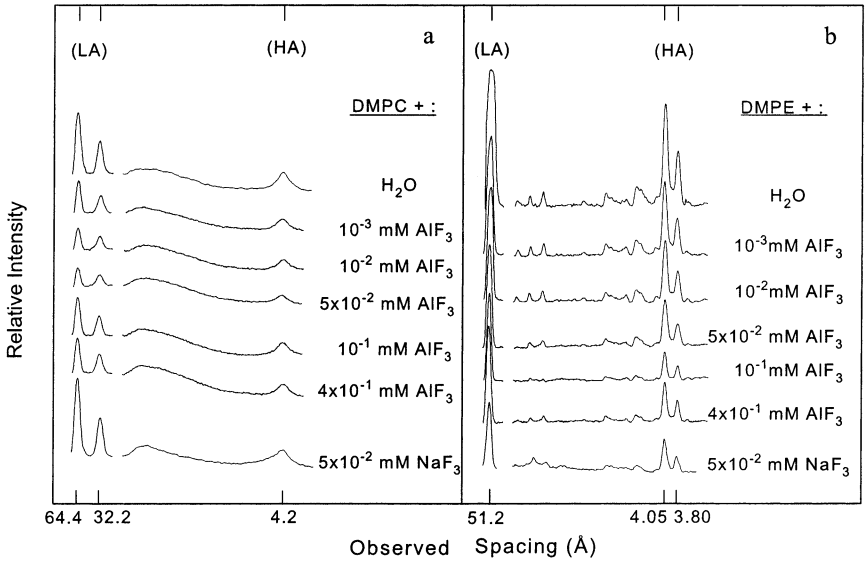

Fig. 3. Microdensitograms from X-ray diffraction patterns of (a) DMPC and (b) DMPE in water and aqueous solutions of AlF_3. LA and HA correspond to low- and high-angle reflections.

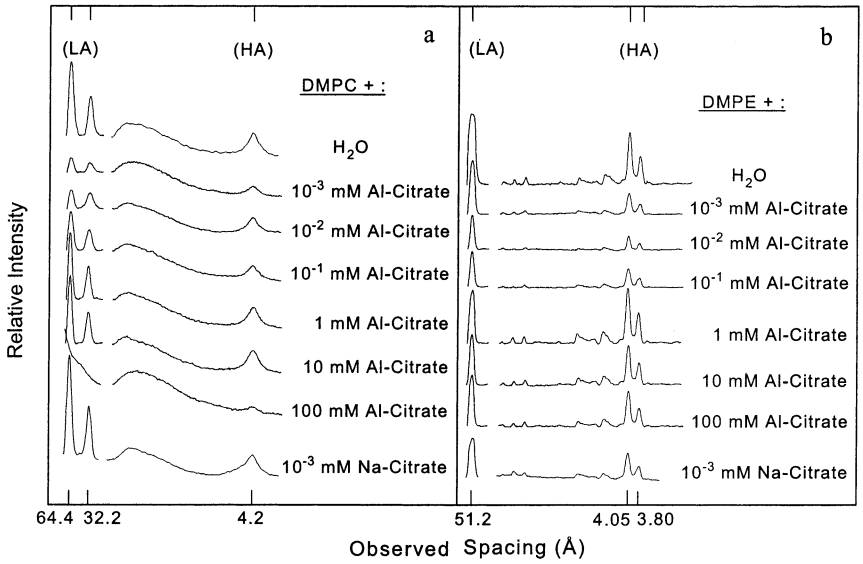

Fig. 4. Microdensitograms from X-ray diffraction patterns of (a) DMPC and (b) DMPE in water and aqueous solutions of the Al–citrate complex. LA and HA correspond to low- and high-angle reflections.

was a gradual weakening of their intensities. Higher concentrations caused an increase in all the reflection intensities, with the resultant pattern somewhat similar to that obtained with 10^{-3} mM AlF3. Fig. 3(b) shows that increasing concentrations of AlF_3 caused a slight but gradual weakening of all DMPE reflection intensities; however, none of them was absent in any of the assayed concentrations.

2.4. Al–citrate complex

Fig. 4(a) presents the outcome of the Al–citrate complex–DMPC interaction. Al–citrate (10^{-3} mM) considerably reduced the low- and high-angle reflection intensities. The increase of the Al(III) salt concentration induced a gradual reordering of DMPC molecules up to 10 mM, concentration under which its X-ray pattern became similar to that observed with Al(III)-free DMPC. However, 100 mM Al–citrate completely disrupted the organized structure of DMPC as no reflections were present. In the case of DMPE bilayers the situation was somewhat different as illustrated in Fig. 4(b). In fact, Al–citrate (10^{-3} up to 10^{-1} mM) produced a weakening of both the low- and high-angle reflection intensities; however, with higher concentrations they increased to values similar to those found in Al(III)-free DMPE, even at a 10^{2} mM concentration.

From these results it can be concluded that the four Al(III) salts produced different degrees of structural perturbations to DMPC and DMPE bilayers. While $AlCl_3$ induced the highest disorder in both lipid molecular arrangements, AlF_3 produced the lowest disturbance. In general, however, the extent of these effects was less pronounced in DMPE than in DMPC.

2.5. Discussion

Despite a well-recognized toxic role of Al(III) in experimental toxicology, the molecular bases that explain the metal ion toxicity are far from being fully understood. This is mainly due to the ill-defined nature of the Al(III) in aqueous solutions and the complexity of cell membranes. In order to overcome these difficulties, we used four different Al(III) compounds to achieve interaction with simple cell membrane molecular models consisting of DMPC and DMPE bilayers. They represent phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively [17]. These phospholipids differ only in their terminal amino groups, these being $^{+}N(CH_3)_3$ in DMPC and $^{+}NH_3$ in DMPE. Moreover, both molecular conformations are very similar in their dry crystalline phases [24,26] with the hydrocarbon chains mostly parallel and extended, and the polar groups lying perpendicularly to them. In both cases the molecules pack laterally to form monolayers. Bilayers are formed through molecular interactions at the ends of their acyl chains in such a way that the monolayers are related by twofold rotation axes perpendicular to the plane. The three-dimensional arrangement is achieved by the location of one bilayer on top of the other. The bilayer structure is stabilized by hydrophobic interactions between the acyl chains and electrostatic interactions between the negatively charged phosphates and positively charged amino groups of their polar heads. However, DMPC bilayers present highly polar interbilayer spaces. In an aqueous medium, water molecules occupy these spaces which, when filled, produce a gradual separation of the bilayers increasing their width from 54.5 Å when dry up to about 64 Å when it is fully

hydrated. This phenomenon allows the incorporation of Al(III) into DMPC bilayers: subsequently the ion may interact with the lipid phosphate groups [16]. Consequently, the stabilizing inter-head group interactions are disrupted producing structural perturbations. On the other hand, DMPE molecules pack tighter than those of DMPC due to their smaller polar group and higher effective charge, resulting in a very stable bilayer system that is neither significantly affected by water [25,26] nor by a number of compounds [27–29]. However, this organization did not prevent low concentrations of three out of the four Al(III) salts assayed from interacting with and perturbing its structure, effects that were nevertheless considerable milder than those observed in DMPC bilayers.

At relatively high Al(III) concentrations reordering effects took place in both phospholipid bilayers. Although these ordering–disordering effects might look surprising, we have reported similar effects with other cations such as Zn(II) [18], Cu(II) [19], and Hg(II) [20]. The structural fluctuations can be explained as follows: at low concentrations Al(III) ions bind electrostatically to a few lipid polar groups disrupting the stabilizing inter-head group interactions; in consequence, some head groups and acyl chains change their orientations. This type of structural perturbation results in the weakening of the phospholipid reflection intensities. However, as the concentration increases, more Al(III) ions link to neighboring polar groups leading to a cooperative ordering of the lipid molecules.

These differences in the capacity of the four Al(III) salts to perturb both DMPC and DMPE bilayer structures, besides their speciation characteristics, can be related to two other main parameters: their dissociation constants and lipophilicity. The first parameter means that the higher its value more Al(III) ions will concentrate in the water | lipid interface and will interact with phosphates of the polar head groups disrupting their arrangement and consequently the whole of the lipid bilayer structure. On the other hand, the more lipophilic salts will preferentially locate their hydrophobic moieties into the acyl chain region altering this region as well as the whole molecular packing arrangement. The reported pK values of the four salts are the following: $AlCl_3$ −3.5 [30], Al–citrate 8.1 [16], AlF_3 15 [30] and $Al(acac)_3$ 21.4 [31]. On the other hand, the organic nature of $Al(acac)_3$ and Al–citrate ligands confers a lipophilic character [32] on them. These considerations can explain why Al(III) in its $AlCl_3$ form induced the most prominent structural perturbation to both DMPC and DMPE followed by $Al(acac)_3$ and the Al–citrate complex.

The structural changes observed in these two phospholipid bilayers are not due to the four salt ligands or to the pH of their corresponding compound solutions. As may be noticed in Figs. 1–4, the ligand effects on DMPC and DMPE are negligible when compared with those induced by their Al(III) compounds. On the other hand, these phospholipids did not show any change when exposed to the pH of each of the Al(III) compound solutions which caused maximum structural perturbation to DMPC and DMPE.

3. Effects of aluminum species on the electrical properties of a Na^+ transporting membrane

Heavy metal ions such as aluminum bind to several cellular enzymes and their toxicity on other biological targets, including cell membranes, has been extensively described [3,5,33]. The plasma membrane, as well as a defensive barrier, is a platform for dynamic processes, principally metabolite exchange, signal transfer and assembly of multiprotein complexes [34] and each process is susceptible to the action of the different metal compounds present in food, beverages and pharmaceutical products used in everyday life. The pathogenetic mechanism of metal intoxication is probably multiple and is influenced by factors such as the compound involved, the species and duration of exposure. An improved understanding of the role of the aluminum species appears to be one of the most important goals to be attained, to better define the molecular mechanisms that might explain the influence of this metal ion on cell membranes. Al(III) has been found to induce single-channel Na^+ current in neuroblastoma cells, i.e. activation of metal-ion channel conductance [35]. Over the past 10–15 years, extensive investigation has yielded impressive structure–function data for cell membranes and their communication links between the extracellular and intracellular milieus. Although there has been a dramatic expansion in the knowledge of the systems for signal transduction, the following possibilities for alterations of membrane activity by aluminum might be considered: (a) disruption of the lipid bilayer; (b) changes in lipid structure and protein dysfunction; (c) direct interaction with proteins forming ion channels, receptors or enzymes; and (d) damage to channels or enzymes facing the cytoplasm.

The present review will now focus on the effects of the four aluminum species on the electrical properties of the Na^+ transporting membrane found in the skin of the toad *Pleurodema thaul*. The toad skin epithelium is the classical model tissue for the study of the mechanisms involved in transepithelial NaCl absorption. Na^+, followed passively by Cl^-, enters the cells via apical (outer surface) Na^+ channels and is extruded across the basolateral (inner surface) membrane by the Na^+–K^+–ATPase [36], a process measured by the short-circuit current (I_{sc}) and the potential difference (PD) across the skin. Sections of abdominal skin were dissected from pithed *Pleurodema thaul* toads (10–20 g) kept in tap

water 24 h prior to sacrifice. Skins were mounted between two halves of a Ussing chamber: a circular area of 1.0 cm^2 was exposed to 3.0 ml toad Ringer's solution on each side, which was oxygenated with an aerator. The I_{sc} was monitored with non-polarizable Ag | AgCl electrodes placed at 15 mm distance from the epithelium and connected to a voltage-clamp circuit set to keep the PD across the skin at 0 mV. The PD was measured at intervals of 2 min for 4 s. Both parameters were displayed on a 2-channel Cole–Parmer recorder. Thirty minutes after steady readings had been obtained, the aluminum compound was applied in the solution bathing either the outer or the inner surface of the skin in the final concentrations specified in the text. Results are expressed as means ± S.E.M. Student's paired *t*-test was used for statistical analysis.

3.1. *Al(acac)₃*

The use of $Al(acac)_3$ on the study of Na^+ transport in the toad skin epithelium was prompted by the finding that Al^{3+} reversibly affected Na^+ current [33]. We found that $Al(acac)_3$ applied to either surface of the skin caused a concentration-dependent and reversible decrease in the electrical parameters, which for the maximal concentration used (320 μM) was a 50% decrease in I_{sc} and a 40% decrease in PD (Figs. 5(a) and 6(a): skin resistance did not change significantly. The minimal concentration used (160 μM) caused a nonsignificant (less than 2%) decrease in both parameters.

3.2. *$AlCl_3$*

Electrophysiological measurements of the toad skin were made before and after exposure of both surfaces of the skin to $AlCl_3$. The response to a maximal concentration of 100 μM $AlCl_3$ applied to the outer surface was a 32% partially reversible decrease in I_{sc} (Fig. 5(b)) and a significant increase in resistance; at the inner surface, the decrease in I_{sc} was only 17% (Fig. 6(b)). In 70% of the experiments, an initial transient increase (15%) occurred.

3.3. *AlF_3*

Fluoride irreversibly inhibits the Na^+–K^+–ATPase, an action speeded by $AlCl_3$, consistent with AlF_4^- being the active species [37]. AlF_3 has been shown to enhance the effect of $AlCl_3$ on interconnections between aggre-

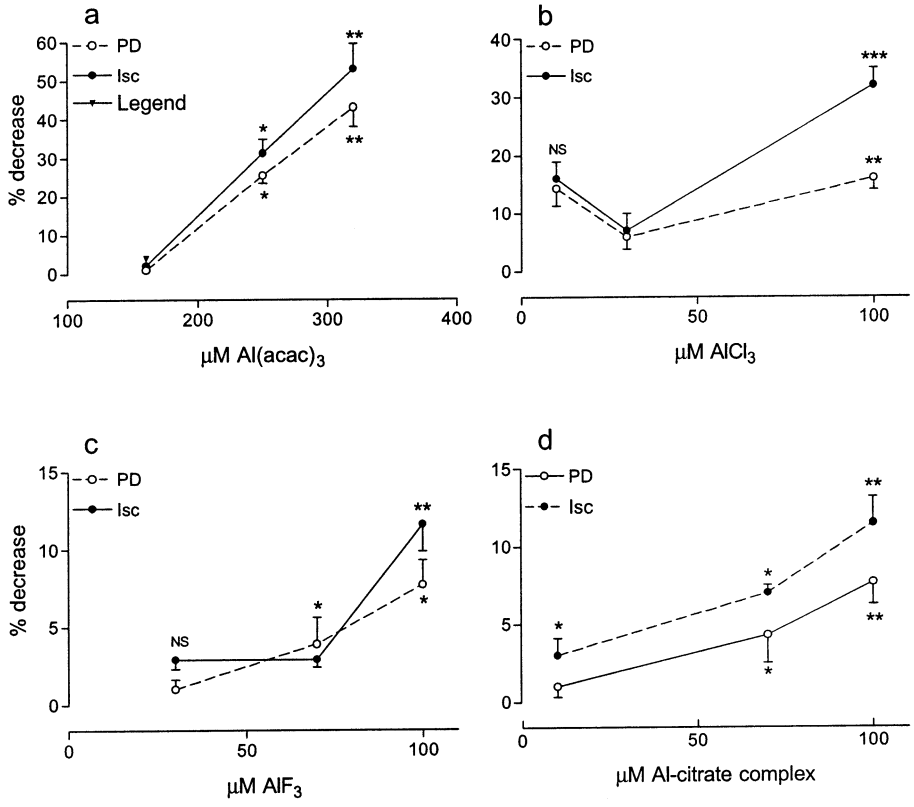

Fig. 5. Effects of the four aluminum (Al) species (increasing concentrations, outer surface): (a) $Al(acac)_3$; (b) $AlCl_3$; (c) AlF_3; and (d) the Al–citrate complex on the electrical properties of the isolated toad skin. Results are expressed as percentage increase in control values. Each point represents means ± S.E.M. of seven experiments. PD, potential difference; I_{sc}, short-circuit current. Values for untreated skins were: PD, 34.7 ± 3.1 mV; I_{sc}, 45.2 ± 3.9 μA cm^{-2}. In contrast with control values, $^*P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$: NS = not significant (Student's paired *t*-test).

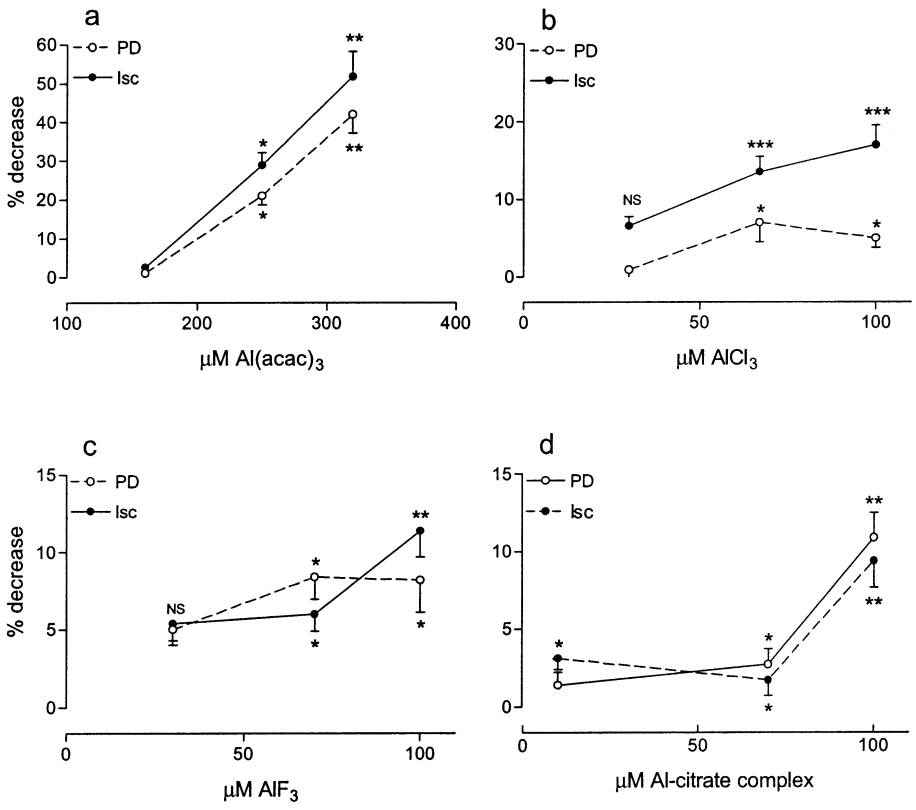

Fig. 6. Effects of the four aluminum (Al) species (increasing concentrations, inner surface): (a) $Al(acac)_3$; (b) $AlCl_3$; (c) AlF_3; and (d) the Al–citrate complex on the electrical properties of the isolated toad skin. Results are expressed as percentage increase in control values. Each point represents means ± S.E.M. of seven experiments. PD, potential difference; I_{sc}, short-circuit current. Values for untreated skins were: PD, 33.8 ± 3.1 mV; Isc, 44.7 ± 3.7 μA cm^{-2}. In contrast with control values, $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$: NS = not significant (Student's paired t-test).

gates of hippocampal neurons [15]. These considerations led us to the current study of the effect of AlF_3 on the ion-transporting cells of the toad skin. In 90% of the experiments a concentration-dependent biphasic change in the electrical parameters was obtained. The maximal concentration used (100 μM, either surface) was followed by a 25% initial transient increase followed by a 12% decrease (Figs. 5(c) and 6(c)) which was only partially reversible. Skin resistance did not change significantly during the responses.

3.4. Aluminum–citrate complex

Citrate was found to prevent aluminum lipid binding, which explains the importance of this organic acid in a detoxification mechanism in plants [38]. Effects of $AlCl_3$ on phospholipase A (a metal-dependent enzyme) could be negated with citrate. On the other hand, citrate was found to enhance mucosal permeation of aluminum hydroxide in rat intestine [39]. The effects of aluminum citrate on the electrical parameters of the isolated toad skin were therefore examined as a contribution to the investigation of its role in aluminum toxicity. The maximal concentration (100 μM) caused a biphasic response in 58% of the experiments: an initial increase (9%) was followed by a 10% decrease in the electrical parameters, changes which, although significant, were less than with $AlCl_3$ and AlF_3 (Figs. 5(d) and 6(d)). In the remaining experiments the effects were solely inhibitory and a significant increase in resistance was evinced.

3.5. Discussion

The differential effects of the four aluminum species on the isolated toad skin may be examined in the light of the magnitude of their perturbation of the lipid bilayers and the impressive advances in the knowledge of the actions of aluminum ions on membrane molecular processes. In accordance with the four mechanisms proposed in Section 3, the following biochemical alterations may be postulated for $Al(acac)_3$: mechanism (a) is ruled out because the destruction of the bilayer implies decreased resistance across the skin, whereas exposure to $Al(acac)_3$ increased the resistance. Mechanisms (b) and (c) are possible because the perturbation of the phospholipid bilayer leads to membrane protein changes which could involve direct interaction with apical ion channels [40]. Novel functions have been described for the sodium channel, which may be affected

by aluminum (see review by Isom [41]). A sodium channel signaling complex is proposed, involving protein channel modulators and cell adhesion molecules which interact with the extra and intracellular matrix, processes which may be altered by the metal ion. The DMPE perturbation by $Al(acac)_3$, indicative of damage to the inner leaflet of the lipid bilayer, could affect transport enzymes at the inner facing membrane of the skin, e.g. Na^+–K^+–ATPase (mechanism d); Gandolfi et al. [42] found that the activity of Ca^{2+}–ATPase was almost suppressed by Al in the inter-endothelial cleft where junctional complexes are formed in the blood–brain barrier.

$AlCl_3$ was less active at the inner surface of the skin, suggesting that the main site of interaction is the outward facing epithelial membrane. It has been shown that one of the primary targets of aluminum is the plasma membrane, which is affected by the metal from the cell exterior [43]. Note that 150 μM $Al(acac)_3$ induced a 2% decrease in the electrical parameters whereas 100 μM $AlCl_3$ was followed by an initial transient increase and then a 32% decrease in I_{sc}. The effects of this aluminum species on the skin were interpreted as reflecting a slight and transient stimulation followed by prolonged inhibition of the active transport of ions, and were far more pronounced with $AlCl_3$ than with $Al(acac)_3$. The results are in accordance with a time-dependent, biphasic modulation of ion transport in response to changes in the molecular structure of the membrane lipid bilayer, which interfere with channel proteins and/or membrane enzymes or other proteins. Mechanism (a) cannot explain these effects of $AlCl_3$ since the resistance increased; mechanism (b) may be borne in mind because the molecular disorder of DMPC alters protein activity, thereby affecting apical Na^+ channels; and it has been found that micromolar concentrations of $AlCl_3$ decreased the conductance of single outer membrane channels from rat brain mitochondria [44]. Apparently, the toxic mode of Al is not through an interaction with enzymatic catalytic binding sites but through interaction with specific membrane lipids; Jones and Kochian [38] found that $AlCl_3$ had minimal effects on metal-dependent enzymes except for with phospholipase A where an interaction with $AlCl_3$ occurred. In accordance with mechanism (d), aluminum chloride inhibits different ATPases [45]. The initial stimulatory response to $AlCl_3$ could be due to activation of the Na^+–K^+–2Cl cotransporter and apical secretion of Cl^-. Paulais and Turner [46] found that the cotransporter is under tight regulatory control via several protein kinase/phosphatase systems in rat parotid acinar cells; alternatively, activation of the apical sodium channels may be a possibility, as $AlCl_3$ treatment increased the peak amplitude of sodium currents in snail neurons [47].

Comparison of the effects produced by AlF_3 showed that for a similar concentration (100 μM) the initial increase in the response was 25% and only 15% for $AlCl_3$, and the decline in the electrical parameters was about 2% for $Al(acac)_3$, 32% for $AlCl_3$ and 12% for AlF_3. These findings point to different mechanisms of action for the three aluminum species. It has been mentioned (Section 2.5) that AlF_3 was one of the compounds which caused least perturbation in the lipid bilayer, interacting with phosphates of the polar head group, an effect which can alter several membrane biochemical operations. The initial rise in the electrical parameters might be referred to various processes. In agreement with mechanisms (c) and (d), the receptor-bound AlF_3 could activate a phospholipid C (a cytoplasm-facing lipid) cascade, increasing intracellular Ca^{2+} which stimulates Cl^- secretion and increases I_{sc} [48]. Data indicating a tight regulatory control of the Na^+–K^+–2Cl cotransporter via multiple protein kinase stimulation, suggest that the cotransport stimulation might be due to the aluminofluoride ion AlF_4^- [45]. In addition, AlF_4^- was found to increase I_{sc} in T_{84} epithelial cells by activating Cl^- secretion via a Ca^{2+}-dependent mechanism [49]. In canine kidney cells, AlF_3 responses stimulated apical transport probably by activation of protein kinase A (mechanism d) [50]. The mild decrease (in accordance with the slight effects on the lipid bilayers) in the baseline activity following the initial stimulation of the electrical parameters could be due to progressive inhibition of second messengers that regulate the Na^+–K^+–2Cl cotransporter [51]. It had been previously observed that fluoroaluminate complexes (AlF_4^-) inhibit 'P' type cation-transport ATPases [37,45]. These mechanisms depend on the binding of AlF_3 to enzymes facing the cytoplasm. The inhibition may also be due to interaction with specific membrane lipids. The change in resistance was not significant; therefore lipid bilayer integrity was not affected and mechanism (a) is not feasible. Jones and Kochian [38] found that toxicity of Al may be through binding to a lipoprotein (mechanism b).

Al–citrate showed very mild modulation of the electrical parameters of the skin. The initial stimulatory response might be due to mechanism (b): direct activation of a receptor-mediated cyclic AMP/protein kinase A cascade which increases the number of active apical Na^+ channels [52]. Johnson and Jope [53] found that 1 μM aluminum citrate elevated levels of cyclic AMP in rat brain cortex. The compound also decreased uptake of Ca^{2+} into bone in embryonic chicks [54]; if uptake in cell calcisomes is also reduced, the increase in Ca^{2+} could stimulate apical Cl^- secretion and increase the electrical parameters of the skin. The slight although significant decline in the electrical parameters is not easy to explain as contradictory experimental results have been reported. Apparently the absorption of aluminum

citrate in rat intestine mucosa was consistent with passive paracellular permeation and little tissue transport [39], a mechanism which may reflect the mild effects of the compound in the toad skin. Mechanism (b) could be involved; aluminum citrate elevated cyclic GMP levels in rat hippocampus [53], and a cGMP-dependent protein kinase could decrease apical sodium permeability.

4. Conclusions

1) Al(III) bound to four different ligands (acac, Cl^-, F^- and citrate) induces different types and extent of structural perturbation to a cell membrane model constituted by DMC and DMPE multilayers. They represent phospholipid classes located in the outer and inner monolayers of the human erythrocyte membrane, respectively. The capacity of these four Al(III) compounds to perturb the structures of these phospholipid multilayers was determined by X-ray diffraction.
2) While $AlCl_3$ induced the most prominent disorder to both lipid molecular arrangements, AlF_3 was least effective. These results are due to the different speciation, dissociation constants and lipophilicity of the four salts. In general, however, the extent of these effects was less pronounced in DMPE than in DMPC given the more open packing arrangement of the latter.
3) The inhibitory effects of the four Al(III) compounds on the Na^+ transport in biological membranes was determined in the toad skin by measuring the bioelectric parameters.
4) The experimental results indicate that the rank order of potency of equal concentrations (100 μM) of the four compounds was: $AlCl_3 > AlF_3 >$ Al–citrate complex $> Al(acac)_3$.
5) The four compounds alter the Na^+ transport through different molecular mechanisms: $AlCl_3$ possibly by indirect and direct inactivation of Na^+ channels and/or perturbation of an ATPase; AlF_3 by direct inactivation of the Na^+ channel and mild ATPase inhibition; the Al–citrate complex by decrease of Na^+ permeability, and $Al(acac)_3$ by inactivating Na^+ transport but at a far higher concentration (250 μM). However, the membrane lipid bilayer integrity was not altered even at the maximal concentrations used.

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