Physiol. Res. 51: 557-564, 2002

MINIREVIEW

Fluoride Plus Aluminum: Useful Tools in Laboratory Investigations, but Messengers of False Information

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Received March 22, 2001
Accepted May 27, 2002

Summary

Aluminofluoride complexes (AlF_x) form spontaneously in aqueous solutions containing fluoride and traces of aluminum ions and appear to act as phosphate analogs. These complexes have become widely utilized in laboratory investigations of various guanine nucleotide-binding proteins. Reflecting on many laboratory studies, a new mechanism of fluoride and aluminum action on the cellular level is being suggested. The long-term synergistic effects of these ions in living environment and their hidden danger for human health are not yet fully recognized.

Key words

Aluminum • Fluoride • Aluminofluoride complexes • G-protein • Second messenger

Introduction

The transfer of phosphate groups is the basic mechanism in the regulation of the activity of numerous enzymes, energy metabolism, cell signaling, and cell growth. Phosphate is an important component of phospholipids in cell membranes. In view of the ubiquity of phosphate in cell metabolism, a phosphoryl analog might represent a useful tool for laboratory investigations, but also a strong potential danger for living organisms including humans. Such a compound has been already found, described and opted for molecule of the month in March 1997 (Chabre 1990, Wittinghofer 1997). Moreover, the new phosphate analog has been used in experimental work in numerous laboratories (Strunecká and Patočka 1999ab).

Scientists need not buy this powerful compound through any catalog or drug store. Fluoride anions, generally introduced as NaF solutions, have long been known to influence the activity of various enzymes and guanine nucleotide-binding proteins (G-proteins). Sternweis and Gilman (1982) reported that fluoride activation of the purified guanine nucleotide-binding regulatory component of adenylate cyclase depends on the presence of aluminum traces. This fact had at first been ignored because aluminum is a normal component of glass, from which it is etched by a solution with fluoride. Aluminofluoride complexes (AlF_x) form spontaneously in aqueous solutions containing fluoride and traces of aluminum ions. However, the exact structure and the proportions of species such as AlF_3 and AlF_4^{1-} able to simulate PO_4^{3-} group in many biochemical reactions are still disputed.

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AlF_x – analog of a phosphate group

Analogies between the phosphate group and the aluminofluoride complex consist in their atomic and molecular similarities. The fluorine atom has the same size and the same valence orbital as oxygen. Aluminum is close to phosphorus; their valence electrons are in the same shell. An Al-F bond is the same length as the P-O bond in phosphate, i.e. 1.5 to 1.6 Å. Like phosphorus, aluminum has possible coordination numbers of 1 - 6, due to the possible hybridization of its outer shell 3p electrons with the 3d orbital. The complexation state depends on the pH of the solution (Chabre 1990, Schlichting and Reinstein 1999). In aqueous solutions with a pH of less than 5.5, aluminum exists as the octahedral hexahydrate $Al(H_2O)_6^{3+}$, usually abbreviated to Al^{3+}. At pH values above 6.2, $Al(H_2O)_6^{3+}$ undergoes successive deprotonation, becoming tetrahedral aluminate $Al(OH)_4^{1-}$. If fluoride is added, the four equatorial water molecules of the hexahydrate are replaced by fluoride to give $AlF_4(H_2O)_2^{1-}$. At pH values above 6.2, the tetrahedral aluminate species predominates with a varying composition of hydroxyls and fluoride. The theoretical calculation of the aluminum (Al^{3+})-fluoride predominance is demonstrated in Figure 1. We calculated the dependency of the complexation state on pH and fluoride concentration for Al^{3+} 10 μmol.l^{-1}.

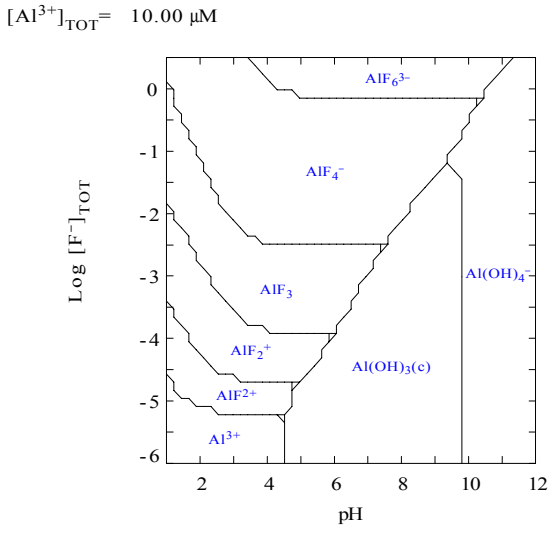

Fig. 1. *Aluminum (Al^{3+})-fluoride predominance diagram. The dependency on pH and fluoride concentration has been calculated for Al^{3+} 10 μM.*

Chabre and co-workers (Bigay *et al.* 1987, Chabre 1990, Antonny and Chabre 1992) suggested that AlF_4^{1-} is probably the active species, which mimics the role of the γ-phosphate. They suggested that the high concentration of fluoride in the solution induces the formation of a soluble tetracoordinated state of aluminum, which has the same geometry, size and coordinance as a phosphate. Their assumption was reinforced by the earlier observation of Sternweis and Gilman (1982). These authors reported that the requirement of fluoride activation of adenylate cyclase for aluminum is highly specific. Of 28 other metals tested, only beryllium could substitute for aluminum. This fact supported the assumption that aluminum acts through its tetrahedral phosphate-like complex AlF_4^{1-}, because all beryllium complexes are tetracoordinated.

In earlier reviews, Martin (1988) had also admitted that the active species might be simply the aluminum ion Al^{3+} and that fluoride complexation would only be needed to allow the penetration of aluminum across the plasma membrane. Once corrected for these effects, Antonny and Chabre (1992) proposed that $AlF_3(OH)^{1-}$ is the main activating species and that the bound form of the complex is tetracoordinated GDP-AlF_3. The studies of the crystal structures of nucleotide binding-proteins complexed with AlF_x indicate that factors other than pH, such as the location of positively charged amino acid of the active site of the phosphoryl-transferring enzyme, may cause deviation from the strict pH dependence of AlF_3 versus AlF_4^{1-} in biological systems. AlF_4^{1-} was determined as the active site species in the case of G-proteins, myosin-S1 and nitrogenase, whereas AlF_3 was bound e.g. in nucleoside diphosphate kinase and uridylate monophosphate kinase (Wittinghofer 1997). It is not clear whether these differences reflect some differences between proteins or are due to technicalities. Schlichting and Reinstein (1999) compared the coordination numbers at different crystallization conditions and suggested that the different coordination numbers originate mainly from the difference in pH at which the enzymes were crystallized. According to these authors AlF_x occurs, as AlF_3 at pH 7.5-8.5 but as AlF_4^{1-} at pH below 7.

Aluminofluoride complexes can bind to proteins by hydrogen bonds to the fluorine atom just as to oxygen atoms of a phosphate ion. AlF_x was found as a good analog of a γ-phosphate for a number of ATP- and GTP-converting enzymes. However, an important functional difference between a phosphate group and the structurally analogous AlF_x exists (Chabre 1990). In the phosphate,

oxygen is covalently bound to the phosphorus and does not exchange with oxygen from the solvent. In AlF_x, ionic bonds are formed between the electropositive aluminum and the highly electronegative fluorine. While the reaction of a bound phosphate compound with orthophosphate is endergonic and slow, the corresponding reaction with AlF_x is rapid and spontaneous. AlF_x bind ionically to the terminal oxygen of GDP β-phosphate. Enzyme-bound GDP or ADP could therefore form a complex with AlF_x that imitates ATP or GTP in its effect on protein conformation. The analogy with beryllium bonding that is strictly tetrahedral led Chabre (1990) to suggest that AlF_x cannot follow this route, since AlF_x causes a structural change that locks the site and prevents the dissociation of the trisphosphate.

The ATPase or GTPase pathway must go through a pentacoordinated transition state for the γ-phosphate. Wittinghofer (1997) described in his article an admirable methodological and intellectual approach, which led to further understanding of the mechanism of phosphoryl transfer reactions using AlF_x and proved that AlF_x acts as the pentacoordinated phosphoryl transfer transition state analog.

Table 1. The effects of fluoride (used as NaF in millimolar concentrations) and aluminum (used as $AlCl_3$ in micromolar concentrations) on levels of the second messenger molecules.

Cell or tissue	Free cytosolic Ca^{2+}	Inositol 1,4,5,P_3	cAMP	References
Hepatocytes	increased	increased		Blackmore *et al.* 1988
Kidney	increased		increased	Zhou *et al.* 1990
Thrombocytes	increased	increased		Deckmyn 1991
Red blood cells		increased		Strunecká *et al.* 1991
Fibroblasts	increased	increased	decreased	Magnaldo *et al*. 1988, Harootunian *et al*. 1991
Osteoclasts	increased	increased	decreased	Moonga *et al.* 1993
Neurons or brain	increased	increased		Nadakavukaren *et al*. 1990, Candura *et al*. 1991
		decreased[a]		[a]Sarri and Claro 1999

AlF_x in laboratory studies

The availability of fluoride and aluminum soluble salts probably contributed to the fact that their use became widely spread in laboratory studies of G-proteins (Wittinghofer 1997, Strunecká and Patočka 1999ab). These studies provided a great deal of knowledge about the involvement of G-proteins in cell signaling. Numerous papers presented evidence that AlF_x influence various functions and biochemical reactions of many cells and tissues of the animal or human organisms. Fluoride in the presence of trace amounts of aluminum affects blood elements, endothelial cells and blood circulation, the function of lymphocytes and cells of the immune system, bone cells, fibroblasts and keratinocytes, ion transport, calcium influx and mobilization, processes of neurotransmission, metabolism of the liver, cell growth and differentiation, protein phosphorylation and cytoskeletal proteins (Strunecká and Patočka 2002). This is not surprising if we consider the role of G-proteins in the cell. Physiological agonists of G-protein-coupled receptors include neurotransmitters and hormones, such as dopamine, epinephrine, norepinephrine, serotonin, acetylcholine, glucagon, vasopressin, melatonin, TSH, neuropeptides, opioids, excitatory amino acids, prostanoids, purines, photons and odorants.

Numerous laboratory studies demonstrated that AlF_x interacts with all known G-protein-activated effector enzymes. Fluorides in the presence of aluminum ions affect the levels of second messenger molecules, including cAMP, inositol phosphates and cytosolic calcium level (Table 1). In the liver, for example, it was concluded that AlF_x mimics the effects of Ca^{2+} mobilizing hormones by activating the G-protein, which couples the hormone receptor to phospholipase C. AlF_x potentiated the effects of submaximal doses of glucagon, vasopressin, angiotensin II and α_1-adrenergic agonists (Blackmore and Exton 1986). Fluoride anions in the presence of aluminum turn the liver metabolism to catabolic

processes such as glycogenolysis, fatty acid oxidation and lipolysis.

The observation that AlF_x complexes activate G-proteins has been useful for studying the mechanism of G-protein activation, for understanding the biochemical mechanism of GTP hydrolysis, and for the elucidation of three-dimensional structures of several GTPases, including the discovery of the GTPase-activating proteins (GAPs). Biochemical evidence showed that GAPs bind with a higher affinity to G·GDP·AlF_x complex than to the triphosphate state of G-protein. GAPs also stabilize the GTPase active conformation of G-proteins. These observations further support the view that AlF_x stabilize the transition state (Wittinghofer 1997).

Moreover, the phosphoryl-transfer analog model of AlF_x may be extended to small G-proteins (for review see Wittinghofer 1997, Strunecká and Patočka 2002). For example, the proto-oncogene product Ras is a component of intracellular signaling pathways involved in cell growth and division. It has a very low intrinsic GTPase reaction rate that is stimulated 105-fold by RasGAPs that downregulate the accumulation of Ras·GTP. The determination of the structure of a complex between RasGAP and Ras·GDP in the presence of aluminum ions and fluoride shows that AlF_3 forms a pentagonal bipyramid, with the fluorides forming the trigonal base with two apical oxygen ligands. Similar studies demonstrated that several classes of small GTPases can stably interact with their respective GAPs in the presence of AlF_3, suggesting that the aluminofluoride complex could bind to a wide variety of GTPases. These observations demonstrate that the GTP hydrolysis mechanism is similar for both small GTPases and Gα- subunits.

The phosphate-analog models of AlF_x action have been accepted for G-proteins but may be extended to all enzymes that bind the phosphate or nucleoside-polyphosphate (Wittinghofer 1997, Schlichting and Reinstein 1999). Regarding the role of phosphoryl transfer reactions in cell metabolism, we can predict hundreds of reactions, which might be influenced. It has been reported, for example, that aluminofluoride complexes impair the polymerization-depolymerization cycle of tubulin (Bigay *et al.* 1987). Changes in shape and disorganization of the spectrin network were observed after addition of 1 mM NaF and 10 μM $AlCl_3$ in human red blood cells (Strunecká *et al.* 1991, 2000). Rapid and dynamic changes of the cytoskeletal network are of vital importance for many cells. AlF_x also binds at phosphate sites of various ATPases and phosphatases. These actions may potentially complicate the interpretation of results regarding modulation of signaling systems by aluminofluoride complexes, particularly when dealing with intact tissue or cell preparations.

The interpretation of laboratory investigations using isolated animal and human cells or tissues in an intact multicellular organism could be discussed. Nevertheless, many ecological and clinical studies brought forth evidence about the detrimental effects of synergistic action of fluoride and aluminum ions in animals and humans.

Fluoride and aluminum in ecosystems

Aluminum, a metal of the earth's lithosphere, is everywhere: in water sources, in nourishment, in different food additives and also in air in the form of dust particles. It has, until relatively recently, existed in forms not generally available to living organisms, and was therefore regarded as non-toxic. Aluminum concentration in fresh waters with the neutral pH is negligible and mostly in the form of insoluble $Al(OH)_3$. With the appearance of acid rains and the use of aluminum in industry, the increase in the amount of uncomplexed aluminum in ecosystems has been observed.

Evidence about the detrimental effects of aluminum on several aquatic species has accumulated. The concentrations of Al^{3+} in the range of 100-800 $\mu g.l^{-1}$ in fresh-water lakes were reported (Jones and Benett, 1985). The reproduction toxicity test using *Daphnia magna* was elaborated. The aluminum concentration of 5 $\mu g.l^{-1}$ caused 50 % mortality of daphnias in the course of 10 h, the concentration of 20 $\mu g.l^{-1}$ caused 100 % mortality in the course of 20 h (Muller 1982). Aluminum ions have been toxic after short-term exposure of juvenile trouts to 75 $\mu g.l^{-1}$ in fresh water. Exley *et al.* (1996) designed a laboratory bioassay to expose fish to kinetically determined differences in aluminum hydroxide solution chemistry. They investigated the hitherto unexpected observations of the acute aluminum toxicity in the fish body at pH 6.5. Supporting experiments have demonstrated that the mechanism of toxicity at this pH was probably asphyxiation brought about by aluminum-induced changes in the rheological and diffusional properties of the mucus lining of the gill epithelium.

Fluoride comes from fluoridated water, from medicines, dental products, pesticides, fertilizers and fuels. About 143 000 tons are pumped yearly into drinking water supplies in the U.S.A. 500 000 tons a year

go into fresh waters and the sea, 155 000 tons of fluoride are released annually into the atmosphere (Hatterslay 1999). Industrial fertilizers and pesticides increase the amount of this element in agricultural products and food sources.

Various physiological ligands, such as citrate, phosphate, and silicic acid, or the low absorption in the gastrointestinal tract, are effective natural barrier systems for aluminum preventing the increased accumulation of this metal under natural conditions. Mullenix *et al.* (1995) demonstrated that the presence of fluoride caused more aluminum to cross the blood-brain barrier and be deposited in the brain of rats. Fluoridation of public water treated with aluminum salts together with the wide use of fluoride and aluminum in medicine, industry and agriculture, increase the loading of living organisms with these ions as never before.

Evidence about the action of AlF_x in humans

Most of the ill effects caused by fluoride were first recognized among workers in aluminum factories, where fluoride and aluminum are present in high concentrations. The levels of fluoride in the serum, urine and hair of these workers are higher than in control subjects. Osteoarthritis and related disorders in such workers have been reported since the 1930´s (McClure 1933). Observation of industrial fluorosis (osteosclerosis) led to the use of fluoride as a treatment to increase bone mass in osteoporosis patients. Psychiatric disturbances were also reported in aluminum smelter workers. The study of persons living near an enamel factory reports a distinct decline in mental activity, poorer memory, and inability to coordinate thoughts and reduced ability to write. Those living further away from the factory were less affected and had a lower urinary fluoride content (Spittle 1994). Fluoride intoxication with multiple nonspecific symptoms has been observed in chronic hemodialysis patients (Arnow *et al.* 1994). In some regions, the water used for the dialysis also contained a high content of aluminum. Some patients used aluminum-containing medications. Moreover, patients with renal failure cannot remove aluminum from the blood. Elevated aluminum levels have also been implicated as the cause of dialysis encephalopathy or dementia (Altman *et al.* 1999). Speech disorders precede dementia and convulsions.

Fluoride has been used in the prevention of tooth decay for over 50 years. Many studies reporting and evaluating the risks and adverse effects of fluoride on the human organism were published during the same period (Waldbott *et al.* 1978, Hatterslay 1999). Some of them demonstrated a positive correlation between the higher intake of fluoride and osteoarthritis, changes in bone structure, and various non-specific symptoms. Lower intelligence of children, various psychiatric symptoms in adults, such as memory impairment, and difficulties with concentration and thinking were reported (Hatterslay 1999, Spittle 2000, Lu *et al.* 2000). Elevated fluoride content was found in embryonic brain tissues obtained from required abortions in areas where fluorosis was prevalent. These studies showed poor differentiation of brain nerve cells and delayed brain development (Hatterslay 1999).

Endocrine glands such as the parathyroid gland, the thyroid, the pituitary gland and the pineal gland are extremely sensitive to fluoride. Of particular importance relating to G-protein activation is the ability of fluorides to clone the role of the thyroid stimulating hormone (TSH). Fluoride is used in laboratory animals specifically to substitute for TSH. The synergistic action of the thyroid on fluoride toxicity has been reported since 1940. Fluoride effects on thyroid hormone synthesis can be observed on many different levels. There is a direct dose-response relationship with iodine: the higher the fluoride intake – the lower the iodine in the system. The major areas of iodine deficiency are identical to endemic fluorosis areas. The functional changes of the hypophysis-thyroid gland system caused by disorders of the regulatory chain and fluorine impact on thyroid hormones metabolism at the level of target cells were reported and the comparison of fluoride toxicity symptoms and symptoms of thyroid disorders has been reviewed (Schuld 2000). Regarding the crucial role of the thyroid in the regulation of growth, development and metabolism of many tissues, AlF_x might influence the proper function of the entire human body.

Chronic exposure of humans to AlF_x begins in the fetus. High fluoride exposure appears to weaken mental functions among children, as well as adults. In respect to the etiology of Alzheimer´s disease, the long-term action of AlF_x also represents a serious and potent risk factor for the development of this new epidemic threat to human civilization (Strunecká 1999, Strunecká and Patočka 1999b). AlF_x may affect all pathological hallmarks of this disease: processes of neuro-transmission, β amyloid generation, plaque formation, metabolism of apolipoprotein E, protein tau phosphorylation, cytoskeletal protein organization,

transport of ions, energy metabolism, and calcium homeostasis Řípová and Strunecká 2001).

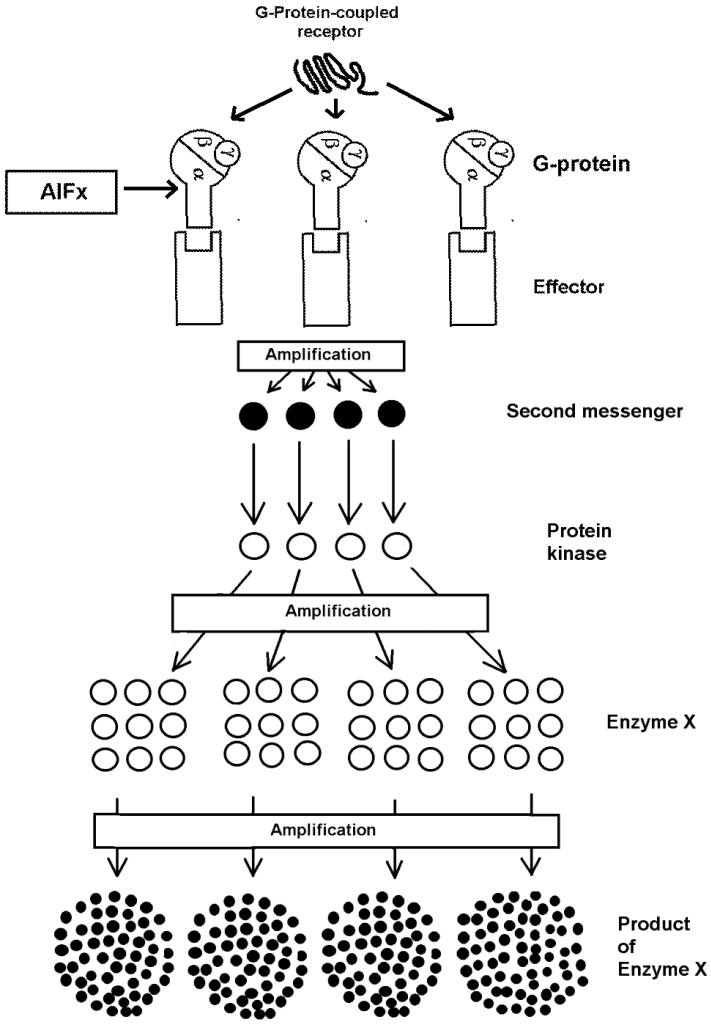

Fig. 2. *AlF_x acts as a messenger of false information. Its message is greatly amplified during the conversion into the functional response of a cell. Effectors are molecules such as cyclases phospholipases C and ion channels. The second messenger molecule could be cAMP, 1,4,5-IP_3, and DAG. Moreover, AlF_x can participate as the analog in the phosphoryl-transfer reactions involved in the signaling cascade.*

Conclusion

The discovery of AlF_x as a new class of phosphate analog has brought numerous demonstrations of their use as the ubiquitous tool in laboratory investigations but also the demonstration of their pharmacological efficacy. It is not surprising with respect to the role of G-proteins in signal transduction. G-proteins take part in numerous biological signaling systems, helping to control almost all important life processes. It has been demonstrated that AlF_x may clone or potentiate the action of numerous extracellular signals. The principle of amplification of the initial signal during its conversion into a functional response has been a widely accepted tenet in cell physiology (Fig. 2). It is evident that AlF_x is a molecule providing false information, which is amplified by processes of signal transmission. Biological signaling pathways interact with one another to form complex networks. Yet, it seems that we shall probably not find any physiological process which is not potentially influenced by AlF_x. These interactions may potentially complicate interpretation of the results.

Understanding the role of phosphate and G-proteins in cell signaling makes it possible to suggest a hypothesis that the synergistic action of fluoride and aluminum in the environment, water and food chains can evoke various and multiple pathological symptoms. AlF_x might induce the alterations of homeostasis, metabolism, growth and differentiation of the living organism. The hidden danger of their long-term action for human health is not yet fully recognized at present.

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