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Aluminium(III) as a promoter of cellular oxidation

Paolo Zatta [a,*], Tamas Kiss [b], Mario Suwalsky [c], Guy Berthon [d]

[a] *Department of Biology, University of Padova, CNR Center on Metalloproteins, Viale G. Colombo 3, 35121 Padova, Italy*
[b] *Department of Inorganic, Metallorganic and Analytical Chemistry, University of Szeged, Szeged, Hungary*
[c] *Faculty of Chemical Sciences, University of Concepcion, Casilla 160-C, Concepcion, Chile*
[d] *Équipe de Chimie Bioinorganique Médicale, ICMPS-CNRS FR1744, Université Paul Sabatier, Toulouse, France*

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Contents

Abstract

Aluminium has been known as a neurotoxic agent to experimental animals since the last century (Arch. Exp. Pharmacol. 40 (1897) 98). However, great interest arose in it bioinorganic chemistry as well biology when it was demonstrated to be the causative agent in pathologies related to the long-term dialysis treatment of uremic subjects with renal failure (Life Chem. 11 (1994) 197), and as a potential etiopathogenic cofactor for several neurodegenerative diseases. The inorganic biochemistry of aluminium is still largely to be discovered. In this review the pro-oxidative property of aluminium toward biological membrane will be presented and its implications in involvement in human pathology will be discussed in an interdisciplinary frame from the bioinorganic point of view.

Keywords: Aluminium; Free radicals; Metal ions; Neurodegeneration; Plasma membrane; Lipid peroxidation; Antioxidative enzyme; Alzheimer's disease

1. Introduction

In spite of its abundance and ubiquity in the Earth's crust, aluminium has been attributed no role by Nature in living processes. Its poor availability [3] and more probably the unfavorable aspects of its chemistry [4] may have been at the origin of this exclusion. Although formerly characterized as neurotoxic to experimental animals [1] and in a more recent case to man [5], aluminium was long considered innocuous under usual environmental conditions [6]. It was not until the discovery of its role in the so-called *dialysis encephalopathy* (DE) in the mid-seventies [2,7] that attention was called to its toxic properties. In particular, regardless of the large gap between iatrogenic and environmental contamination levels, the aluminium

* Corresponding author. Tel.: +39-049-827-6331; fax: +39-049-827-6330.
E-mail address: zatta@mail.bio.unipd.it (P. Zatta).

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found in the brains of patients deceased from Alzheimer's disease (AD) and Parkinson's disease (PD)—particularly in senile plaques (SP) and neurofibrillary tangles (NFT) for AD [8–11] and *substantia nigra* for PD [12,13]—was suggested to be implicated in the genesis of these neurodegenerative disorders.

Though controversial [14], the most documented case in the above context is AD, with the repeatedly observed correlation between incidence of the sporadic disease (95–98% of the listed cases) and level of the metal in low silica drinking water [15–19]. For coordination chemists aware of the extreme dependence of aluminium complex stoichiometries on aluminium global concentration, it appears that in the same way as the well-defined role of aluminium in DE should be taken as insufficient to predict the implication of the metal in AD, the observation of distinct histological forms for its deposits in DE and AD should also be considered insufficient to rule out its potential contribution to AD genesis. In other words, *different* concentrations of aluminium in *different* brain areas may well result in *different* disorders through *different* chemical processes.

Considering aluminium as a potential risk factor of AD, this possibility requires that at least some of the brain dysregulations associated with the disease may be due to its detrimental influence. Regardless of its concentration, aluminium may *a priori* interfere with a number of metabolic processes. For example, the Al^{3+} ion can directly compete with, and even substitute for, several essential metal ions *in vivo*. Ca^{2+} is its first obvious target in this respect as judged from aluminium-induced osteomalacia [20] and other instances such as gastrointestinal absorption [21] or cell gate regulation [22]. Another essential metal known to be subject to Al^{3+} competition is iron, not only as Fe^{3+} for its substitution in transferrin and ferritin, but even as Fe^{2+} in iron gastrointestinal absorption [23]. Strong competition is also expected between Al^{3+} and Mg^{2+} ions given their close chemical resemblance [24–27]. The influence of aluminium on the metabolism of essential metal ions may be quite indirect, and apart from general discussions reviewing the key effects of the Al^{3+} ion in which semi-quantitative estimations can be used [25], the only effective approach to assess the extent of such interactions requires sophisticated calculations [23]. Even this approach is seriously limited, however, because of the generally poor reliability of aluminium data on which such calculations must be based, and, more conceptually, because of the scarcity of computer programs capable of handling more subtle effects of aluminium. For instance, the Al^{3+} ion is capable of competing with Fe^{3+} for oxygen-containing essential sites to an extent that might be accessible to appropriate calculations, but its indirect influence on the Fe^{2+}/Fe^{3+} redox equilibrium by binding to Fe^{3+} specific ligands (see Ref. [28]) would be more difficult to quantify. In fact, many aluminium interactions *in vivo* are out of reach of quantitative techniques, which explains the frequent lack of certainty about the molecular interpretation of their macroscopic effects. The influence of the Al^{3+} ion on metabolic oxidation processes largely falls within this category, as do its interactions with many of the macromolecular species involved in these processes. The objective of this review is to sum up the present knowledge on these aspects of aluminium biochemistry.

2. Free radicals in aging and pathology

French chemist Lavoisier [29] was the first to explain in 1777 that combustion and respiration are both linked to reactions involving oxygen, a gas that besets us with a curious paradox: while *we cannot live without* (*it*), *we are constantly traumatized by the consequences of oxidative degradation* [30]. Free radical is the general term used to designate *any chemical species capable of independent existence that contains one or more unpaired electrons* [31]. Such species may be extremely reactive and capable of damaging essential biomolecules *in vivo* and trigger the formation of even more reactive free radicals through a cascade of destructive phenomena. The oxidation of biomolecules with oxygen implies the stepwise reduction of molecular oxygen itself into successive intermediates, two of which are themselves free radicals: O_2^- (superoxide radical), H_2O_2 (hydrogen peroxide) and $^{\bullet}OH$ (hydroxyl radical).

$$O_2 \xrightarrow{e^-} O_2^- \xrightarrow{e^-} H_2O_2 \xrightarrow{e^-} {}^{\bullet}OH \xrightarrow{e^-} H_2O$$

The net reaction is:

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$$

Oxidative damage to DNA, lipids, proteins and other macromolecules is an age-dependent phenomenon. It has been postulated that oxidative phenomena are the major, though not the only type of endogenous damage that leads to aging and aging-related pathologies [32]. On the other hand, the immunological system has evolved to produce biological solutions able to regulate and utilize these dangerous unstable radicals to kill invading microorganisms for instance.

Lipid peroxidation in a membrane or free fatty acid is due to the attack of any species that has sufficient reactivity to abstract a hydrogen atom. This leads to the successive production of lipid epoxides and hydroperoxides (ROOH), alkoxyl ($RO^{\bullet}$) and peroxyl ($ROO^{\bullet}$) radicals, the latter two species stimulating the chain reaction by abstracting further hydrogen atoms [32]. Oxidants produced by cells mainly derive from aerobic respiration and mitochondrial oxidative activity. For instance, a mammalian cell can process 10^{12} O_2 molecules daily, with about 2×10^{10} superoxide and peroxide

reactive oxygen species (ROS) leaking out of it [33]. In addition to endogenous sources, exogenous oxidants like NO_x, transition metals; diet derivatives—like natural phenolic compounds from plant food that may generate oxidants by redox cycling—also lead to cell damage [34]. ROS are normally produced in all living organisms via redox reactions and enzymatic processes. Lipids, proteins, carbohydrates and nucleic acids are the major components of the animal body.

Spontaneous reaction of molecular O_2 with organic compounds, however, is not initiated without a catalyst [35]. Given its biradical nature, the O_2 molecule can only accept pairs of electrons with parallel spins. As such pairs are rarely available, electrons must be transferred one at a time. Few compounds have the property to transfer single electrons to oxygen but transition metal ions, which do it readily at low (catalytic) concentrations from a wide variety of reducing agents, notably ascorbate [36]. The destructive reaction of biological membranes by oxidative processes where alkyl radicals capture oxygen is indicated below:

$$\text{Initiation} \quad RH \rightarrow R^{\bullet}$$

$$\text{Propagation} \quad R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$$

$$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$$

$$\text{Termination} \quad R^{\bullet} + R^{\bullet} \rightarrow R\text{–}R$$

$$R^{\bullet} + ROO^{\bullet} \rightarrow ROOR$$

$$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROOR + O_2$$

The central nervous system (CNS) is particularly vulnerable to ROS because of the high concentrations of lipids in neuronal cell membranes (Table 1). Plasma membranes are formed by layers of lipid molecules [37] with double bonds of highly reactive unsaturated fatty acids that are able to combine with hydrogen and other atoms to split at the double bond level. On the contrary, when O_2 combines with $-CH_2-$ groups of saturated fatty acids, the microenvironment increases in polarity, becoming more hydrophilic and also more reactive. Consequently, membranes are compromised in their functioning and eventually destroyed.Plasma membranes are more than a mere passive barrier providing the basic structure, within which proteins are floating, acting as transport molecules, receiving, transmitting and transducing chemical signals, catalysing membrane-associated chemical reactions, and containing specific recognizable receptors. The physiological activity of these proteins is largely determined by the fluidity of their lipid environment [38]. The term membrane fluidity refers to the physical state of fatty acyl chains that constitute the membrane bilayer structure. It is now generally recognized that membrane fluidity and fatty acid unsaturation are related in a rather simple manner. Both unsaturation and acyl chain length affect the physical properties of acyl chains. Acyl chain behavior can also be influenced by other components of the membrane such as cholesterol, proteins and phospholipids [39]. Although generally accepted, however, the concept of membrane fluidity appears not to be adequate enough to describe the complexity of the acyl chain occurrence in membranes.

Table 1
Lipid composition of normal adult human brain (dry weight)

	Gray matter (%)	White matter (%)
Cholesterol	22	27.5
Total phospholipid	69.5	45.9
Phosphatidylserine	8.7	7.9
Galactocerebroside	5.4	19.8
Galactocerebroside sulphate	1.7	5.4

From Agranoff, Hajra, in: G.J. Siegel, B.W. Agaroff, R.W. Albers, P.B. Molinoff (Eds.), Basic Neurochemistry, Raven Press, New York, 1994, pp. 97–116.

Aging processes are closely related to an increased ratio of cholesterol and phospholipids in the membranes of various tissues, particularly the brain. As mentioned above, the activity/functioning of proteins associated with plasma membranes are largely determined by the fluidity of their microenvironment. From an etiopathogenic point of view, this can result in serious consequences in case of iron abnormal accumulation such as in AD [40–43], PD [13] as well as in other neuropathologies. Free radical formation is therefore expected to be a key component in the induction of these diseases [40,44]. During normal cell metabolism, ROS and H_2O_2 are produced in the order of 10^{-9} M or even higher concentrations, depending upon tissue typology. Another potential source of free radicals is the auto-oxidation of neurotransmitters like catecholamines (dopamine, noradrenaline and adrenaline) that are significantly present in the CNS. The auto-oxidation of any of these substances produces O_2^- in the presence of redox metal ions such as iron, enhancing the production of free radicals.

Phospholipid degradation has been proven to be associated with cellular edema and induced by free radicals in animal brain. About 50 years ago, Harmon [45] proposed that free radicals could be involved in aging processes, calling attention to a possible link between free radicals, antioxidant enzymes and a variety of neurological diseases. In fact, membrane lipid peroxidation is now known to be associated with neurodegenerative processes in brain injury as well as in chronic diseases such as AD, PD, amyotrophic lateral sclerosis and other diseases [40,44,46–48]. A large body of data have recently been accumulated demonstrating increased levels of oxidation end products and, therefore, an oxidative burden in the AD brain [47,49,50], with markers of oxidative damage being specifically elevated in the areas pathologically affected [40]. Proteins and

nucleic acid are also oxidized in the presence of metal ions.

3. Metal ions dysregulation and membrane peroxidation

Transition metal ions are effective catalysts in unsaturated lipid peroxidation and are therefore involved in membrane lipid peroxidation through free radical production. In particular, the role of iron in this context has been widely investigated. Fe(II) auto-oxidation results in the formation of O_2^-

$$Fe(II) + O_2 \rightarrow Fe(III) + O_2^-$$

The reverse reaction is also possible. Fe(II) is also oxidized in the presence of H_2O_2

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^-$$

(Fenton Reaction)

$$Fe(II) + H_2O_2 \rightarrow Fe(III)OH + OH^-$$

Both $^{\bullet}OH$ and Fe(III)OH species are extremely reactive towards an overwhelming number of biological molecules including lipids, proteins and nucleic acids. Other reactions can occur in the biological environment, such as the so-called Haber Weiss reaction:

$$O_2^- + H_2O_2 \rightarrow O_2 + {}^{\bullet}OH + OH^-$$

which requires the catalytic contribution of redox metal ions in the two reactions below (including the Fenton reaction referred to above)

$$Fe(III) + O_2^- \rightarrow Fe(II) + O_2$$

$$Fe(II) + H_2O_2 \rightarrow Fe(III) + {}^{\bullet}OH + OH^-$$

It has been controversially proposed that a critical Fe^{2+}/Fe^{3+} 1:1 ratio is needed to maximise lipid peroxidation through the Fe^{2+}–Fe^{3+}–O_2 complex [51–53]. More recently, it has been advanced that the principal route to iron mediated lipid peroxidation is via Fe^{2+}–O_2 complexes rather than the Fenton reaction [54]. Addition of H_2O_2 to a culture medium of L1210 cells, used as a model for brain tissue, actually decreased the formation of oxidation products [55], corroborating results on model lipid systems in which the auto-oxidation of Fe^{2+} was shown to initiate lipid peroxidation [56].

Whereas transition metal ions can initiate the formation of ROS *in vivo*, main group essential metal ions may limit oxidative damage through direct or indirect competition with these. It is in particular the case of zinc [57] and magnesium [58]. In this connection, it has recently been suggested that the vulnerability of neurons to iron-dependent oxidative injury is an inverse function of the extracellular magnesium concentration, the direct inhibition of lipid peroxidation by high concentrations of magnesium being presumably due to its competition with iron for phospholipid binding sites [59].

3.1. Metal ions accumulation in human neurodegenerative diseases

The first case of 'aluminosis' was reported by Lapresle et al. [60] in an alcoholic subject with mental disturbances and progressive neurological deteriorations. Later, several papers reported an abnormal accumulation of metal ions, especially iron and aluminium, in human subjects affected by certain neurological diseases. Brain aluminium concentrations were found to be significantly higher in patients dying with DE [61]. The grey matter contained about three times as much aluminium as the white matter. Also, brain aluminium levels remained elevated for up to 4 years following restoration of normal renal function through transplantation. Moreover, uremic patients administered $Al(OH)_3$ as a phosphate binder to contrast hyperphosphatemia had a higher mean aluminium concentration in grey matter and whole brain [61]. While acute aluminium-induced DE may not consistently result in AD-type lesions (see Section 1), immature plaques and amyloid-β (Aβ, a peptide considered to initiate SP formation in AD—see Section 7, paragraph 2 below), were found in chronic dialysis patients [62]. Interestingly, increased gastrointestinal absorption of aluminium in the presence of citrate was observed in AD subjects (<77 years) compared with age- and sex-matched controls [63]. Also, patients with probable AD have been shown to have statistically significant higher serum aluminium levels than patients with other types of senile dementias (alcoholic, vascular, multi-infarct) and an age-matched control group, whereas no significant differences were found between serum aluminium of patients with non-AD senile dementias and the age-matched controls [64].

The aluminium detected in SP and NFT is contained in lipofuscin granules [65], where lipofuscin granules are a degenerated form of lysosomes whose main function is to degrade unnecessary substances in the cell. Inorganic materials like aluminium cannot be degraded and may thus be accumulated in lysosomes, gradually leading to lysosomes dysfunction, leak of catabolic enzymes and eventually brain cell degeneration. Brains with AD contain more lipofuscin granules than brains without dementia [65].

In the last 25 years many studies demonstrated the capability of aluminium to interfere with a variety of neurological processes associated with several pathologies (Table 2). Abnormal accumulation of aluminium has been reported in histopathological hallmarks of AD such as SP and NFT [8–11]. The human brain accumulates aluminium at very low concentration, between 1.9 and 2.2 μg g^{-1} dry weight [61]. Yasui et al. [13] described an abnormal accumulation of iron and aluminium also in PD. The colocalization of these two metals in the brain may induce synergistic effects in neurodegenerative processes [66]: whereas increased

Table 2
Involvement of aluminium in different pathological conditions [11]

Alzheimer's disease
Anemia
Bone disease
Cancer
Cardiotoxicity
Dialysis encephalopathy
Gastrointestinal toxicity
Renal osteodistrophy

levels of aluminium in NFT-laden neurons are expected to stimulate iron-induced lipid peroxidation [67]. Lipid peroxidation may facilitate in turn aluminium accumulation as was shown in rat brain synaptosomes [68].

The abnormal distribution of iron in AD brain might result from alterations in iron regulatory proteins (IRP) such as IRP-1 and -2, the main control elements of cellular iron homeostasis. While IRP-1 was observed to be present at similar levels in both AD and control brain tissue, IRP-2 showed striking differences and was associated with intraneuronal lesions, including NFT, SP, neurites and neuropil threads. Since IRP-2 colocalizes with redox-active iron, alterations in IRP-2 might be directly linked to impaired iron homeostasis in AD [69]. In a histochemical study of iron, transferrin and ferritin in AD brains, both proteins and iron were found to be predominantly present in oligodendrocytes as in normal brain, but the distribution of all of these was altered in the vicinity of SP [41]. Transferrin was homogenously distributed around the SP extracellularly, in astrocytes in the cerebral cortical white matter of the AD tissue rather than in oligodendrocytes as in normal tissue. A strong ferritin immunoreaction was shown to accompany SP and many blood vessels in the AD brain tissue. Most of the ferritin-containing cells associated with SP and blood vessels were microglia. Iron could also be demonstrated in SP, both diffusely in proximity of the plaques and in cells associated with plaques. These data strongly suggest a disruption in brain iron homeostasis in AD [41].

A significant decrease in copper and significant increase in zinc and iron have been found in AD hippocampus and amygdala, areas showing severe histopathologic alterations in AD. None of these elements were significantly imbalanced in the cerebellum in AD [70]. High levels of ceruloplasmin can inhibit iron lipid and protein peroxidation by oxidizing Fe^{2+} to Fe^{3+} and lead to the incorporation of Fe into ferritin [71]. However, low levels of ceruloplasmin increase lipid peroxidation by partially oxidizing the Fe^{2+} pool, resulting in a Fe^{2+} to Fe^{3+} ratio of ca. 1:1, which is ideal for redox chemistry [72]. Because the iron pool is greater in AD, lower levels of ceruloplasmin may have an even greater effect on free radical generation, the Fe/Cu ratio, statistically constant in the normal brain, being imbalanced in pathology-rich regions of the AD brain [70]. A statistically significant elevation of iron and zinc was independently observed in multiple regions of AD brain [73].

Transport mechanisms pertaining to iron and manganese have recently been reviewed in the context of AD, PD and Huntington's disease, manganese accumulation in the brain appearing to target the same regions as iron. Emphasis was put on the necessity of a stringent regulation of the concentration of these metals in the brain [74]. The recent *in vivo* evaluation of brain iron in AD using magnetic resonance imaging [43], suggests that basal ganglia ferritin iron levels are increased in AD, and this non-invasive technique may be considered as a promising approach to new neurotoxicity prevention strategies.

4. Aluminium as a cell membrane destabilizer

As mentioned above, a common feature of the AD brain is the occurrence of increased levels of iron and aluminium, two metals involved in membrane lipid peroxidation. At least part of aluminium neurotoxicity is likely to be due to aluminium stimulation of iron-induced oxidative damage to neurons [40]. The properties of the two metals make them likely to act synergistically in the peroxidation process: whereas the binding of aluminium to the neuronal membrane is expected to facilitate its attack by iron-induced free radicals [67], the resulting oxidation of the membrane will in turn increase its binding to aluminium, thus aggravating oxidation [68]. Thus, the pro-oxidant effect of aluminium on the lipid peroxidation of brain homogenates of aluminium intoxicated mice as observed by some authors was interpreted in terms of direct interaction of the Al^{3+} with the cell membrane [75] (see also Section 4.2).

4.1. Aluminium phosphate interactions

As typical hard metal ions, Fe^{3+} and Al^{3+} display a strong affinity for oxygen donors, especially those carrying negative charges. Inorganic phosphate as well as the numerous phosphate-containing biomolecules are therefore privileged ligands for both of these. It is not the case, however, for the borderline Fe^{2+} ion. Solubilities of the neutral monophosphate salts of the three metal ions, whose constants at 25 °C at zero ionic strength [76] are $K_{so}(FePO_4) = [Fe^{3+}][PO_4^{3-}] = 3.7 \times 10^{-27}$, $K_{so}(AlPO_4) = [Al^{3+}][PO_4^{3-}] = 8.5 \times 10^{-23}$ and $K_{so}(Fe_3(PO_4)_2) = [Fe^{2+}]^3[PO_4^{3-}]^2 = 1.0 \times 10^{-36}$, clearly reflect this. Although allowing no direct comparison because of their different units ($mol^2\ dm^{-6}$ for the first two, $mol^5\ dm^{-15}$ for the third), these constants can

be used in simple calculations based on the ionic product of water and phosphate protonation constants (12.38, 19.59, 21.71 for the overall logarithmic values under the above conditions [76] to obtain hypothetical free concentrations of the metal ions. The oversimplification and significance of such concentrations usually transcribed into pM values have recently been discussed in the general context of computer-aided speciation [77]. For a concentration of metal and phosphate of 10^{-6} M used as a reference example [78], the free concentrations are calculated with a modified version of the SPE program [79] as 2.416×10^{-11} (pM = 10.62), 3.657×10^{-9} (8.44) and 10^{-6} M (pM = 6) for Fe^{3+}, Al^{3+} and Fe^{2+}, respectively—the value relative to the Fe^{2+} ion simply indicating that its above phosphate salt does not precipitate under these conditions.

In general, metal–phosphate interactions in solution are difficult to characterize because poorly soluble neutral complexes may precipitate in the course of relevant titrations [28,80]. However, formation constants for soluble monohydrogenphosphates can usually be calculated in the acidic pH range. Thus, the logarithmic constants for the equilibrium $M^{n+} + HPO_4^{2-} \Leftrightarrow [MHPO_4]^{(n-2)+}$ under the above conditions (9.30 for $[FeHPO_4]^+$, 6.10 for $[AlHPO_4]^+$ and 3.6 for $[FeHPO_4]^o$ [76] confirm the $Fe^{3+} > Al^{3+} > Fe^{2+}$ order of affinities for the phosphate group.

A comparison of stability constants for organomonophosphato complexes of these metal ions would be even more relevant to analyze the metal binding capacity of membrane phospholipids. Unfortunately, no data for these three metal ions with the same ligand can be found in the literature [76]. As, however, virtually no difference was found between formation constants of Al^{3+} complexes with (C–O–P containing) organophosphates and (C–P containing) organophosphonates [80], a comparison of methylphosphonate (MP) complex stabilities can be used. The available data for Fe^{3+} ($\log K_{ML} = 9.05$) and Al^{3+} ($\log K_{ML} = 6.48$) [76] confirm the stability sequence of hydrogenphosphates.

Summarizing the above formation constant comparisons, the following sequence may be proposed for the interactions of membrane phosphate end groups with iron and aluminium ions: $Fe^{3+} \ggg Al^{3+} \gg Fe^{2+}$, with each inequality sign indicating a decrease of about one logarithmic unit in stability constants, from 9 for Fe(III) to 4 for Fe(II).

4.2. Aluminium binding to membranes

There are many reports indicating that Al(III) interacts with cell membranes (see reviews in [81,82], which constitute the primary targets of this trivalent cation to induce membrane structural and functional perturbations [83,84]. Thus, the interaction of Al(III) with red cell membranes for instance resulted in the production of osmotic fragility [85], anisocytosis, poikilocytosis and shape changes [86–88], alterations in membrane dynamics [89], and decrease in membrane fluidity [86,90] (Fig. 1). Other cellular models include neurons and synapses of invertebrate and vertebrate [91], brain endothelial cells [92], dorsal root ganglion neurons [93], and the blood-brain barrier [94]. Reported functional perturbation include changes in resting membrane potential and input resistance [91,95], voltage-activated ionic channels [81,93], transmitter secretion [81], Ca^{2+}-ATPase activity [92], alkaline band formation [84], transmembrane potential difference and short-circuit current [87,88]. In addition, aluminium displaces membrane-associated ions of physiological significance [96], altering the physiological functionality of the plasma membrane.

These and other alterations of cell membrane activities might be: (i) direct interaction of Al(III) with proteins forming ion channels, receptors and enzymes; (ii) induction of structural alterations in the lipid matrix; or (iii) action on the lipid | protein interfaces. To elucidate among these alternatives, and given the structural complexity of native cell membranes, lipid bilayers have been widely used as cell membrane models. Aluminium tris-acetylacetonate ($Al(acac)_3$) and aluminium maltolate ($Al(malt)_3$) complexes react with DL-α-dipalmitoylphosphatidylcholine (DPPC) under a 1:1 molar ratio in $CDCl_3$ at 37 °C as shown by the release of ligands from the metal coordination sphere (^{1}H-NMR), changes in ^{1}H-NMR spectrum of DPPC and

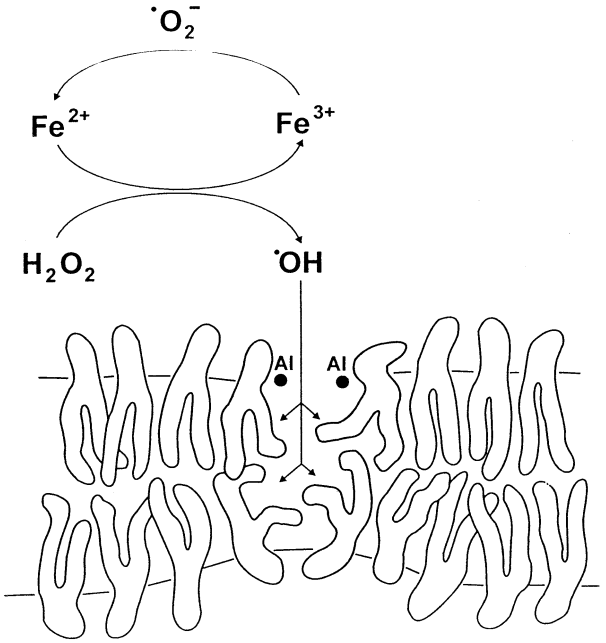

Fig. 1. The peroxidation of membrane lipids: effects of aluminium and iron. (Modified from van Rensburg et al., in: P. Zatta, M. Nicolini (Eds.), Non-neuronal Cells in Alzheimer's disease, World Scientific, Singapore, 1995.)

appearance of a ^{31}P-NMR signal due to the metal-coordinated DPPC. ^{31}P-NMR in fact revealed that both $Al(acac)_3$ and $Al(malt)_3$ react with DPPC [86]. DPPC, which represents a major component of plasma membranes lipid bilayer, reacts both with $Al(acac)_3$ and $Al(malt)_3$ with the release of β-enolate ligands as established by the parallel development of ^{31}P signals due to metal-coordinated DPPC and the coexistence of two different sets of ^{1}H signals due to DPPC in equimolar Al(III)–DPPC complex through phosphate binding sites (Figs. 2 and 3). The formation of Al(III)–phosphatidic acid groups has been proposed by other authors [97–101]. X-ray studies on the interaction of four aluminium compounds ($Al(acac)_3$, $AlCl_3$, AlF_3 and the aluminium citrate complex $K_5[Al(C_6H_4O_7)_2]$) with lipid bilayers built-up of dimyristoylphosphatidylcholine (DMPC) and dimyristoylphosphatidylethanolamine (DMPE) have also been carried out [88]. The results showed that they interact with and produce different structural and functional effects on the model membranes. In fact, these studies revealed that $AlCl_3$-induced the most damaging effect 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. Lipid structure alteration resulting from the binding of Al(III) to membrane polar heads can induce important modifications in membrane biophysical properties and dynamics, which may result in injurious consequences for biological transport processes and cellular metabolism [89].

Al^{3+} ion also affects vesicle fusions [102] and alters membrane permeability [103,104]. According to Jones and Kochian [83], plasma membrane—not enzymatic binding domains—is the most likely site of aluminium interaction and, therefore, the site of toxic effects. Exposure of rabbit erythrocytes to Al^{3+} ions causes a marked decrease in membrane fluidity [86] without an appreciable modification of the conformation of some strategic proteins such as spectrin and band 3, demonstrating that membranes are the strategic site where aluminium exerts its toxicity (Figs. 3–6).

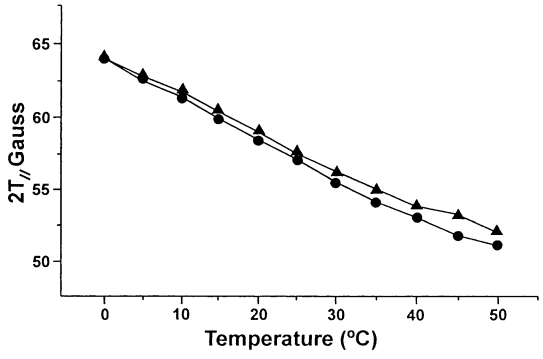

Fig. 2. Effect of Al(III) on $2T_{\parallel}$ of rabbit erythrocytes ghost after labeling with 5-NSA. $Al(acac)_3$ = triangle; control = circle. (Modified from Zatta et al. [86].)

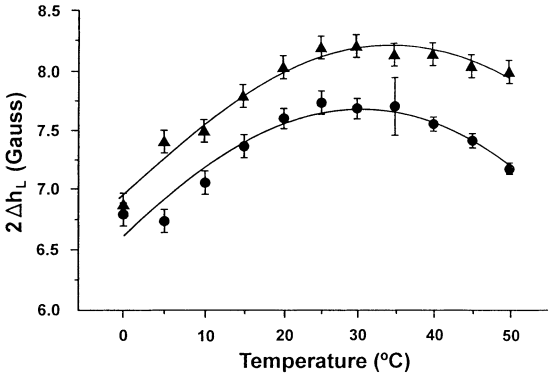

Fig. 3. Effect of $Al(acac)_3$ on Δh_L of rabbit erythrocyte ghost after labeling with 5-NSA. $Al(acac)_3$ = circle; control = triangle. Bars = S.D. (From Zatta et al. [86].)

4.3. Aluminium potentiation of membrane oxidation

The first mention of the capacity of Al^{3+} ions to enhance membrane oxidative damage dates back to 1985 [67]. Aluminium salts were shown to accelerate peroxidation of membrane lipids induced by Fe(II) salts at acidic pH values. It was suggested that Al^{3+} ions produced a subtle rearrangement in the membrane structure that facilitated the oxidative action of iron, a relative lack of effect being observed on already *disorganized* micelles [67]. The later report of the increased peroxidizability of brain homogenates from aluminium intoxicated mice [75] was interpreted as a possible consequence of a higher rate of production of oxidative reactions *in vivo*, this being probably due to a direct interaction of aluminium with cell components

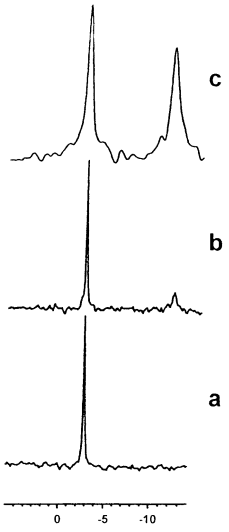

Fig. 4. ^{31}P-NMR spectra in chloroform-*d* at 24 °C of (a) DPPC (10^{-3} M); (b) DPPC (10^{-3} M)+$Al(acac)_3$ (10^{-2} M); (c) DPPC (10^{-3} M)+$Al(malt)_3$ (10^{-2} M).

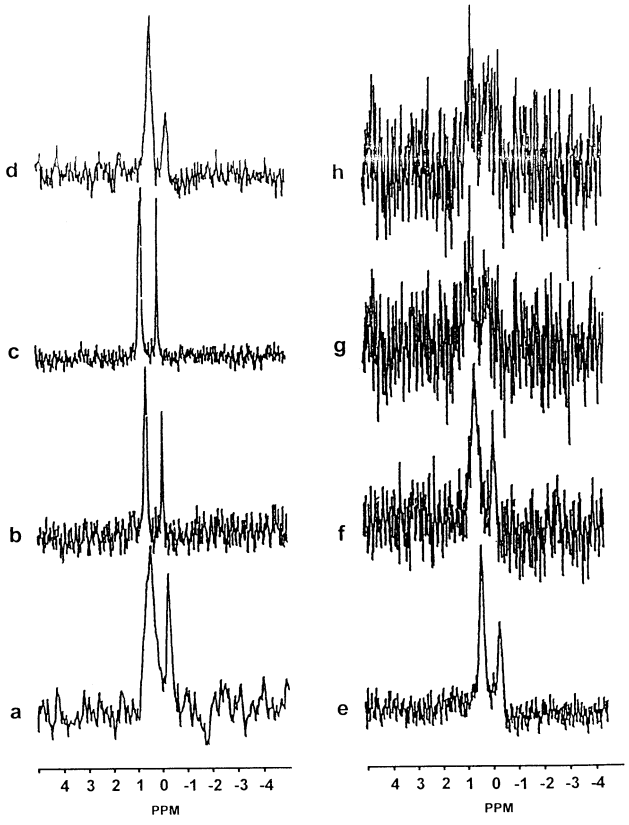

Fig. 5. ^{31}P-NMR spectra of rabliterythrocytes ghost 1% Triton X-100 Tris buffered (pH 7.4) aqueous solutions at 24 °C (a), 37 °C (b), 50 °C (c) and back to 24 °C (d) compared with those of the ghosts reacted with an equal volume of 10^{-1} M Al(lact)$_3$ in 1% triton X-100 Tris-buffered solutions at 24 °C (e), 37 °C (f), 50 °C (g) and back to 24 °C (h).

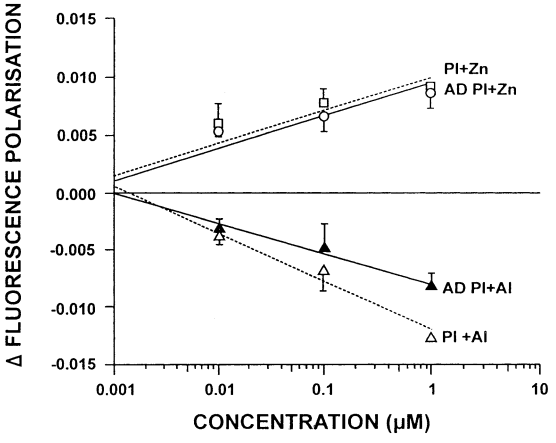

Fig. 6. Changes in microviscosity of platelet membranes obtained from normal, healthy controls or AD patients, upon addition of increasing concentrations of aluminium or zinc. (□) Normal platelets and zinc; (○) AD platelets+zinc; (△) normal platelets and aluminium; (▲) AD platelets and aluminium.

(aluminium being by nature unable to participate in redox reactions). A higher binding of aluminium to the membrane was predicted to cause a greater rearrangement of the membrane phospholipids, rendering these more accessible to the attack of free radicals. Interestingly, the direct addition of aluminium to brain homogenates from control animals resulted in the dual effect: now antioxidant-like in the absence of added iron, now pro-oxidant in the presence of added iron [75]. Further investigations on mouse brain membranes by the same group provided evidence that Al^{3+} was the species involved in the promotion of Fe^{2+}-induced lipid peroxidation [105] and that membrane integrity was necessary for the manifestation of the aluminium stimulatory effect.

The mechanism of action of aluminium in the above process was investigated on model membranes. The addition of Al^{3+} ions to a suspension of liposomes resulted in a marked stimulation of Fe^{2+}-induced liposomal peroxidation at pH 7.4, depending on the concentration of these ions [106]. Also, the lag phase of the reaction was shortened by the addition of aluminium in a dose-dependent manner. In a parallel study, Oteiza [107] showed that the aluminium pro-oxidant effect increased with decreasing pH and with increasing the negative charge density of the liposomes, and was significantly correlated with its capacity to promote liposome aggregation, permeability and fusion. In addition, aluminium was demonstrated to cause fatty acid chain packing, thus facilitating the propagation of lipid peroxidation [107]. Other non-redox metals (Sc, Ga, In, Y, La, and Be) also caused liposome aggregation and fusion, and stimulated Fe^{2+}-induced lipid peroxidation in a dose- and time-dependent manner under similar experimental conditions [108].

In a study on rat brain dialysates, Xie et al. [109] proposed that the aluminium facilitation of Fe^{2+}-mediated oxidative injury should be due to mechanisms other than increased extracellular OH generation. Using bovine brain extracts and pure phospholipids [110], the same authors then demonstrated considerable peroxidation of a mixture of phospholipids possibly found in cellular membranes in the presence of intracellular aluminium and iron concentrations. They also ruled out the previously hypothesized formation of a Fe^{3+}–O_2–Al^{3+} complex as well as an aluminium alteration of metabolic activity to generate ROS. Finally, they confirmed that lipid peroxidation occurred to a greater extent at pH 5.5 than at 7.4—aluminium activity being thus expected to be favored in acidic sub-cellular environments such as lysosomes—and was largely dependent on the phospholipid structure, the pH, and aluminium and iron concentrations. The variation of the aluminium effect with different factors was also investigated on liposomes, the maximum stimulation being observed around pH 6.0 and shown to be a function of the proportion of phosphatidylserine in the phospholipid composition [111]. The promotion of iron-induced generation of ROS by aluminium was also confirmed on

rat crude synaptosomes [112] as well as in cultured rat hippocampal neurons [113].

More recent developments have provided complementary information on the aluminium facilitation of the iron-mediated lipid peroxidation process. In particular, Laurdan fluorescence excitation and emission spectra and generalized polarization measurements have been used to investigate the effects of Fe^{2+} and Al^{3+} ions on phase components of phospholipid model membranes [114]. An increase in the generalized polarization of oxidized liposomes has been observed, revealing the presence of a less polar environment around the probe [114]. Fluorescence spectra confirmed the aluminium facilitation of iron-mediated lipid peroxidation, but no quantitative influence was calculated relative to general polarization and derived phase state determinations, suggesting a less significant influence of aluminium on membrane structures than originally expected [114]. In parallel, Oteiza and coworkers using brain membranes *in vitro* and *in vivo* established that, because of its high relative lipid/protein ratio, myelin was a preferential target of aluminium-mediated oxidative damage, supporting the hypothesis that ions without redox capacity can stimulate *in vitro* and *in vivo* lipid oxidation by promoting phase separation and membrane rigidification [115]. The effect of aluminium on lipid peroxidation was recently confirmed to depend on metal concentration, pH, and membrane structure in liposomes, microsomes and whole homogenates of rat brains [116]. It was also confirmed in primary neuronal cultures using the fluorescent probe dichlorofluorescin [22]. A more effective stimulation of Fe^{2+}-initiated lipid peroxidation in phospholipid liposomes was observed with aluminium acetylacetonate relative to aluminium chloride [117]. Fluorescence anisotropy measurements suggested that treatment of the liposomes with the aluminium complex caused a decrease in their lipid fluidity. Furthermore, the aluminium effect would be markedly enhanced by incorporation of the complex into the liposomal membranes through an acceleration of Fe^{2+} oxidation due to a strengthened packing between the acyl chains in the lipid layer [117].

The effect of aluminium on iron-induced lipid peroxidation has recently been reported in mice brain homogenate in a concentration- and time-dependent manner, with protein oxidative modifications being enhanced at high, but suppressed at low, concentrations of Al^{3+} ions [118], which is reminiscent of the dual effect reported above [75]. A significantly higher content of brain myelin galactolipids was observed in aluminium intoxicated mice following pre-natal and early post-natal exposure with respect to controls, a significant correlation being found between concentration of myelin galactolipids and lipid peroxidation. This synergistic effect of aluminium with galactolipids was confirmed on liposomes, where it induced higher phase separation and membrane rigidification [119]. Aluminium was also shown to enhance lipid peroxidation of microsomes from rat liver under acidic conditions and to attenuate the antioxidant action of flavonoids under neutral conditions [120]. With reference to humans, aluminium has been shown to induce lipid peroxidation and aggregation of blood platelets, there being a correlation between the two phenomena through stimulation of the lipogenase pathway [121].

5. Aluminium alters the activity of antioxidative enzymes (catalase, SOD, glutathione peroxidase)

Aerobic cells have to cope with a difficult challenge. They require redox-active metals like iron and copper to carry out essential respiratory reactions under conditions that may provoke the formation of ROS [30]. To counter this threat, aerobic organisms have elaborated specific metalloenzymes whose function is to protect the intracellular medium by degrading the products of cellular oxidation (glutathione peroxidase, GPX) and disproportionating the superoxide anion and hydrogen peroxide

$$2O_2^- + 2H^+ \rightarrow O_2 + H_2O_2$$

(Superoxide dismutase, SOD)

$$2H_2O_2 \rightarrow O_2 + 2H_2O \quad \text{(Catalase)}$$

$$H_2O_2 + \text{cofactor} \rightarrow \text{cofactor oxidation} + H_2O$$

(Peroxidases, PX)

By contrast, in extracellular fluids, where redox metal ions play no basic functional role, protection is mainly afforded by appropriate ligands that limit their free concentrations to a minimum [122]. Low molecular mass antioxidants like vitamins C and E also participate in the detoxification. An increased level of lipid peroxidation has been found in brain tissues following ingestion of aluminium [123]. Decreased levels of vitamin E and zinc have also been detected in the serum and brain of AD patients [124].

Aluminium has been controversially reported to alter SOD activity, by inhibiting in various experimental models [125–128], as well as in uremic subjects [129] in experimental animals [130], or activating enzyme activity as reported in the case of 45 patients poisoned by aluminium phosphide in north India in 1992 [131].

In erythrocytes from patients with chronic renal failure, catalase has been shown to be inhibited by Al^{3+} [132], and a 25% inhibition in rat catalase activity was observed when liver supernatant was incubated with 5 mM $AlCl_3$ [133]. Finally, high serum aluminium levels observed in uremic subjects on dialysis as well as following transplantation were shown to be associated with lower GPX activity [134].

6. Other potential mechanisms for aluminium-induced brain oxidation

In addition to the above direct effects, aluminium may also exert an indirect influence on iron-mediated oxidation and its consequences. The examples below are not exhaustive.

6.1. Aluminium competition for iron ligands

As developed in previous paragraphs, it is now a well-accepted notion that iron is the most probable agent responsible for lipid peroxidative damage in the brain [135] and that aluminium can aggravate its action. The effect of aluminium, however, largely depends on the respective concentrations of the two metals through their competition for negatively charged oxygen groups of membranes [75,110]. This competition also occurs for a variety of other oxygen-containing ligands that occur in brain tissues and fluids. It is particularly the case for the large number of carboxylic acids that are ubiquitous *in vivo*, whether exogenously absorbed as nutrients or endogenously produced in the Krebs cycle. The competition between Al^{3+} and Fe^{3+} ions for the anionic forms of these acids may locally, but significantly, influence both the global availability of Fe^{3+} ions to membrane binding sites and the redox potential of the Fe(II)/Fe(III) couple, these two effects resulting in increased membrane oxidation. The more Al^{3+} in the cerebrospinal fluid (CSF), the less acid anions available to bind Fe^{3+}, so not only the more Fe^{3+} likely to bind surrounding membranes but also the higher the redox potential of the Fe(II)/Fe(III) couple, hence the more oxidation. This has recently been demonstrated by one of us with citrate taken as an example [28].

Citrate is an appreciably strong ligand of Al^{3+}, Fe^{3+}, and even Fe^{2+} ions, and its concentration in the CSF is double that in blood plasma whereas that of transferrin—the main plasma carrier of Al^{3+} and Fe^{3+}—is 250 times lower than in plasma [136]. Thus, citrate is expected to be critical to the distribution of these ions in the CSF, especially in the context of their competition for the binding sites of neuronal membranes. This potential effect has been tested *in vitro* with suspensions of liposomes in which iron-induced peroxidation was measured at different aluminium and citrate concentrations. Fig. 7 shows the concentrations of MDA detected with 25 μM $FeSO_4$ as a function of citrate with and without 50 μM $Al_2(SO_4)_3$ at pH 5.5. Clearly, the presence of aluminium enhances iron oxidative activity at most citrate concentrations tested, with a maximum effect near 50 μM. Beyond this limit, citrate becomes concentrated enough to satisfy both metal ions, and peroxidation decreases.

Similar but less marked effects have been observed at pH 7.4. This pH dependence is of particular interest if one considers the high concentrations of iron and aluminium that occur in the acidic lysosomes (see Section 4 above; see also Ref. [110]). An aluminium-induced malfunction of these organelles may indeed lead to electron leakage and ROS formation [110].

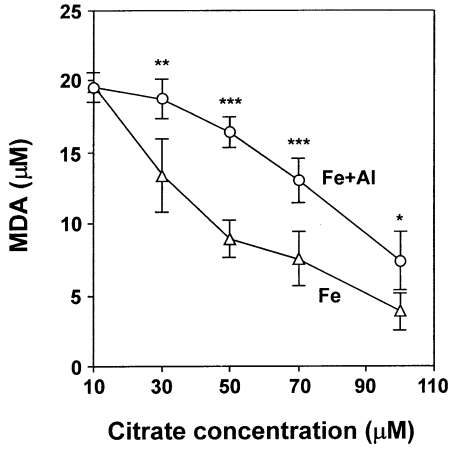

Fig. 7. Iron-induced peroxidation of phosphatidylcholine/phosphatidylserine, 60:40, liposomes in the absence and presence of aluminium. Individual points shown represent means of four measurements and vertical bars denote ±S.D. (*$P < 0.02$; **$P < 0.01$; ***$P < 0.001$). (From Berthon and Dousset [28].)

6.2. Aluminium and the fate of the amyloid β peptide

The amyloid peptide (Aβ) is considered to play a key role in AD. Its sequence of up to 40–43 residues is constitutively produced by normal cells [137] from a transmembrane glycoprotein known as the amyloid protein precursor (APP). Neurotrophic in its original soluble state, Aβ becomes neurotoxic following aggregation and precipitation [138]. *In vitro* aggregation of human Aβ has been obtained through interaction with metal ions—notably aluminium [139–142]—as well as via metal-catalyzed oxidation, this process mainly affecting His and Tyr residues [143]. (Rodent Aβ, which differs from human Aβ by the three substitutions Arg→###Gly, Tyr→Phe and His→Arg at respective positions 5, 10 and 13, aggregates differently, which is in line with the fact that rodents rarely develop AD [144]. On the other hand, Aβ reversible aggregation properties would contribute to maintain regional structural integrity [145] at local injury sites where Aβ deposits rapidly appear following injury [146]. The inverse correlation found in AD patients between CSF Aβ levels and dementia severity [147] would also suggest some Aβ protective function.

As already mentioned, oxidative stress and inflammation are a determining component of AD pathogenesis, and amyloid β and oxidative damage are inextricably linked *in vivo* [148]. In addition to metal-mediated Aβ aggregation and amyloidogenicity through oxidation [143], various oxidative processes are involved in the

expression of Aβ neurotoxicity [149]. The $A\beta_{25-35}$ sequence appears to be determining in this respect *in vitro* [150]. This, however, must be put in perspective with the predominance of $A\beta_{1-42}$ over $A\beta_{1-40}$ as a major determinant for early onset of AD [151]. (Notably, plasma $A\beta_{1-42}/A\beta_{1-40}$ ratios in persons with Down syndrome (DS) are higher than normal [152].) Another specific parameter may be taken as the prime criterion for the neurotoxicity of human Aβ in the absolute: the very nature of its sequence, as opposed to that of rodents that are not subject to AD [149]. Observations relative to Aβ deposition onto AD brain tissue *in vitro* [149,153] have clearly shown that a function exerted by some (or all) of the three residues present in human $A\beta_{1-40}$ but absent from rodent $A\beta_{1-40}$ overrides the influence of the peptide length and the role played by the $A\beta_{25-35}$ sequence. It was then demonstrated that Aβ deposition depends on amino acid oxidation and protein cross-linking [143] and that His and Tyr residues (both being particularly sensitive to modification by hydroxyl radicals are responsible for Aβ aggregational behavior [144]. Furthermore, only human Aβ showed a strong tendency to aggregate and become highly amyloidogenic in the presence of radical generating systems, wherefrom it was suggested that the difference in the aggregational behavior of human and rodent sequences was due to their different content of His and Tyr residues. Metal-mediated amino acid oxidation would therefore be required *in vitro* and in AD to initiate Aβ aggregation [144]. After generation of a core of cross-linked amyloidogenic fragments, further fragments would extend the deposition without further involvement of metal-mediated oxidation processes [143,144,153].

More recently, human $A\beta_{1-40}$ was shown to aggregate in the presence of Cu^{2+} ions at normal serum level as the pH was lowered from 7.4 to 6.6 (acidotic pH limit anticipated within the metabolically diseased brain parenchyma) [145], but neither rat $A\beta_{1-40}$ nor histidine-modified human $A\beta_{1-40}$ could do so, indicating that Cu^{2+}–histidine coordination is required for $A\beta_{1-40}$ aggregation [145]. The specific composition of the hydrophilic sequence of human Aβ ($A\beta_{1-28}$) therefore appears as the prime determining factor for peptide neurotoxicity—which does not negate the auto-oxidizing role suggested for the Met35 residue in $\beta A4_{25-35}$ or $\beta A4_{31-35}$ sequences [154,155], but tends to relativise it given the ubiquitous occurrence of redox metal ions *in vivo* [149].

Inflammation is known to induce local acidification of the physiological *milieu* [149]. It is therefore no surprise that AD brains are more acidic than controls [156]. Inflammatory conditions are also known to be associated with elevated copper levels. Blood plasma copper increases with the onset and persistence of active inflammatory disease but returns to normal with remission [157]. Again it is no surprise that copper in the CSF of AD patients has been found to be 2.2 times more elevated than in controls [148] while affected tissues appear to be copper depleted (see Section 4 above) [75]. Several factors encountered in AD therefore concur to suggest a possible interaction between Aβ and Cu^{2+} ions *in vivo*. Recently, the notably high reduction potential of the Cu^{2+}–$A\beta_{1-42}$ together with the simultaneous Aβ binding to Cu^{2+} and Zn^{2+} led to the suggestion that Aβ may function as a SOD-mimetic [158]. Copper definitely catalyses the oxidation of Aβ *in vivo* [159]. In a comparison of modifications of human amyloid-derived Aβ with those induced by metal-catalyzed oxidation systems *in vitro*, a decrease in the content of histidine residues could be replicated *in vitro* by incubating synthetic Aβ with H_2O_2 and copper, but not iron [159]. Evidence has also been provided that Aβ plaques of AD represent the redox-silencing and entombment of Aβ by zinc [160], substitution of either His13 or His14, but not His16 eliminating the zinc-mediated effects [161].

Another hypothesis has also recently been advanced of a possible protective function of Aβ in association with copper in the CSF [149]. In the extracellular medium where anti-oxidant enzymes are virtually non-existent, free iron and copper ions are maintained at extremely low levels so that they cannot trigger Fenton chemistry (see above). Extracellular production of $^{\bullet}OH$ radicals thus critically depends on the ligands predominantly engaged in copper and iron complexed fractions [102]. By comparison with iron, copper-driven Fenton type reactions are much faster [163] and highly site specific, $^{\bullet}OH$ radicals reacting immediately with the ligand bound to the metal center [164]. Copper-mediated Fenton type reactions may thus be deleterious or protective to essential biomolecules in their immediate vicinity, depending on the nature of the ligand bound to the Cu^{2+} ion at the outset of the Haber–Weiss reaction [162]. According to this hypothesis, Aβ would act as an $^{\bullet}OH$-inactivating ligand [162], undergoing oxidation in the place of neuronal membranes before being normally proteolyzed [165]. In this scenario, the unexpected deposition of oxidized Aβ into the irreversible forms of the observed plaques implies that some chemical factor prevents its structure from being recognized by its specific protease, presumably by changing its conformation. The first possibility is the binding of the oxygen donors of the Aβ oxidized form to (a) suitable metal ion(s). Among several candidates including Zn^{2+}, [142], Al^{3+} is the most likely to play this role [149]. To conclude on this point, it is worth reiterating Atwood et al. recent assertion [166]: *Although Al(III) may not be able to precipitate Aβ directly, the possibility that it may destabilize Aβ conformation thereby promoting its aggregation warrants further investigation*.

7. Conclusion

All possible ways of aluminium interference with *in vivo* redox regulations likely to facilitate peroxidation reactions considered a general cause of aging and age-related diseases (in particular neurodegenerative) have been examined in this review. The most important well-established fact is the definite evidence that Al^{3+} ion can effectively aggravate Fe^{2+}-initiated lipid peroxidation. This effect is largely due to the high polarising power of the Al^{3+} ion, which causes fatty acid chain packing by interacting with membrane phospholipid moieties. However, its extent *in vivo* may be modulated as a function of the relative concentration of aluminium with respect to essential metals at the target site. For example, while aluminium acts as an effective pro-oxidant through direct interaction with membranes, it may also appear antioxidant-like by displacement of membrane-bound iron, although its competition with iron for the same oxygen donating ligands may shift the Fe^{2+}/Fe^{3+} redox equilibrium towards more oxidative damage for essential biomolecules. Similarly, given the chemical resemblance between the two ions, Al^{3+} may also thwart Mg^{2+} well-known antioxidant activity. Most of these effects are species dependent, aluminium membrane destabilization being higher for the free Al^{3+} ion than for stable neutral complexes, which, by contrast, induce more efficient aluminium diffusion into tissues.

The capacity of Al^{3+} to promote oxidative damage to, for instance, brain membrane is not only based on the presence of polyunsaturated fatty acids and phospholipid components, but also that of molecules with a high content of lipid/protein ratio (like e.g. myelin) supporting the fact that ions without redox capacity can stimulate lipid peroxidation by promoting phase separation and membrane rigidification.

In the context of the potential involvement of aluminium to the genesis of AD, it has been hypothesized that the possible binding of Al^{3+} ions to the OH groups resulting from the copper-mediated oxidation of the Aβ peptide under inflammatory conditions may prevent proteolysis of the oxidized form of the peptide and favor Aβ further oxidation and deposition.

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