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Evidence for a hydroxy-aluminium polymer (Al_{13}) in synaptosomes

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The presence of the hydroxy-aluminium polymer (Al_{13}-$(OH)_{24}O_4(H_2O)_{12}^{7+}$) was noticed inside synaptosomes when synaptosomes were incubated with $Al(NO_3)_3$ at neutral pH values. Dysprosium nitrate ($Dy(NO_3)_3$) – a shift reagent – facilitates the identification of the Al_{13} species distinctly inside the synaptosomes. ^{27}Al NMR was used as a tool to detect the Al_{13} complex inside and outside the synaptosomes.

Synaptosome; Dysprosium nitrate; Al_{13} polymer; Aluminium

1. INTRODUCTION

Aluminium (Al) is the most abundant element in the Earth's crust. Aluminium has been suspected to be a causative factor in neurological disorders, but the exact role of Al in these disorders is poorly understood [1,2].

The toxicity of aluminium depends on free Al^{3+}, and its various species are a function of the pH range (3.0–11.0) [3]. Martin [1] indicated that at physiological pH 7.0–7.5, Al predominantly exists as $Al(OH)_3^+$. At lower pH values (<6.0), it exists as Al^{3+}, $Al(H_2O)_6$, $Al(OH)_2^+$ and $Al(OH)^{2+}$, whilst at higher pH values (>8.0), Al exists as $Al(OH)_4^-$. Thus, it is clear that Al^{3+} exists as a polynuclear species. Plee et al. [4] and Bottero et al. [8] showed the presence of the Al_{13} polymer in solutions having neutral and slightly alkaline pH values. Recently, Hunter and Ross [3] have deduced evidence for the presence of an Al_{13} (hydroxy-aluminium polymer) species, which is supposed to be a phytotoxic species of Al, in organic soil horizons. Al_{13} is a polymer with the formula $Al_{13}(OH)_{24}O_4(H_2O)_{12}^{7+}$. The four coordinated aluminium ions are presumed to be located at the centre of a structure, within a symmetrical environment. The tetrahedron of oxygen atoms in the centre of the group contains the four-coordinated Al atoms [8]. It is of interest to know whether such species could be formed in cells, as the pH of the biological systems is understood to be neutral [7]. To investigate this, we have used synaptosomes as a cell system, and ^{27}Al NMR spectroscopy as the monitoring probe. Synaptosomes are subcellular components formed from neural junctions, and are involved in neural transmissions. They resemble intact cells in that they possess a relatively impermeable, external plasma membrane, enclosing a variety of organelles, each in its appropriate medium, and they are capable of maintaining oxidative phosphorylation and an external membrane potential [8].

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2. EXPERIMENTAL

Adult albino rats (Wistar strain) were sacrificed by anesthesia and brain tissue was excised immediately under cold conditions. Synaptosomes were isolated by the method of Dodd et al. [5].

^{27}Al NMR spectra were recorded on a Varian Associate FT-80A NMR spectrometer operating at 20.72 MHz. A spectral width of 4,000 Hz was used, and about 4,000 scans were accumulated to get a reasonable spectrum. A 60–70° pulse was used with a total recycle time of 1 s and samples were referenced from external $Al(NO_3)_3 \cdot 9H_2O$ for ^{27}Al NMR, to within 0.05 ppm. The Al_{13} polymer was identified, based on its chemical shift value of 63.5 ppm with a line width value of 100 Hz [8].

To facilitate the formation of the Al_{13} polymer in solutions, sodium hydroxide was added to reaction volumes to obtain the molar ratio, $r = (NaOH)/Al(NO_3)_3 = (OH)/(Al_T)$. The time required for this addition was fixed at 1 h. The pH of the samples was adjusted back to pH 7.4 from pH 10.0. ^{27}Al NMR spectra were recorded for the following samples.

(A) 0.1 M $Al(NO_3)_3 \cdot 9H_2O$ in mammalian Ringer (pH 7.4)

(B) 0.1 M $Al(NO_3)_3 \cdot 9H_2O$ and synaptosomes (1 mg protein/100 μl)

(C) Synaptosomes were incubated with 0.1 M $Al(NO_3)_3$ for 30 min at 37°C (pH 7.4).

After incubation, synaptosomes were washed thrice with mammalian Ringer containing 0.001 M EDTA and ^{27}Al NMR spectra were recorded in the presence and absence of 5 mg $Dy(NO_3)_3$. Dysprosium nitrate ($Dy(NO_3)_3$), a paramagnetic shift reagent, is used as a probe to differentiate between internal and external concentrations of metal ions in a cellular system [6].

3. RESULTS

^{27}Al NMR spectra of the samples studied showed the presence of the Al_{13} polymer in the neutral-pH buffer and also inside the synaptosomes (Fig. 1). In sample (A) the presence of the Al_{13} polymer was observed in buffer solution with a line width value of 100 Hz and a chemical shift value of 63.5 ppm.

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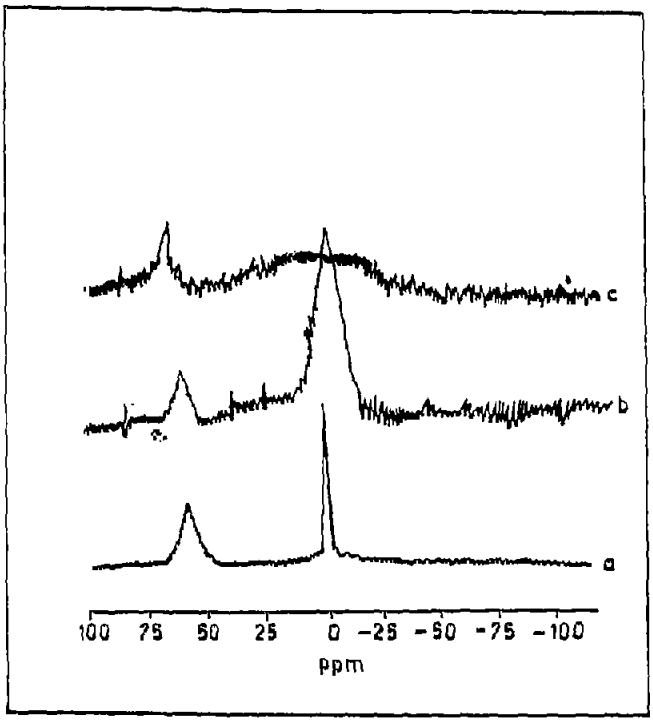

Fig. 1. ^{27}Al NMR spectra of synaptosomes. (a) 0.1 M $Al(NO_3)_3 \cdot 9H_2O$, in buffer; (b) 0.1 M $Al(NO_3)_3 \cdot 9H_2O$, and synaptosomes; (c) 0.1 M $Al(NO_3)_3 \cdot 9H_2O$, synaptosomes and $Dy(NO_3)_3$.

In sample **(B)** when 0.1 M $Al(NO_3)_3$ and synaptosomes were incubated, and the ^{27}Al NMR spectrum was recorded, a signal representing the Al_{13} polymer was identified. It appeared at a chemical shift value of 63.5 ppm with a line width value of 100 Hz.

In sample **(C)** synaptosomes were incubated with 0.1 M $Al(NO_3)_3$ for 30 min, and repeatedly washed with 0.001 M EDTA to remove the externally bound Al. To distinguish the Al_{13} polymer inside and outside synaptosomes, a shift reagent, namely $Dy(NO_3)_3$, was used. When $Dy(NO_3)_3$ was added to EDTA-unwashed synaptosomal samples, the Al_{13} polymer appeared as two peaks at chemical shift values of 63.5 and 67.5 ppm, with line width values of 100 Hz. The chemical shift values at 63.5 and 67.5 ppm were assigned for the Al_{13} polymer in the buffer and inside the synaptosomes, respectively. However, to confirm the presence of the Al_{13} polymer inside the synaptosomes, the above experiment was repeated with EDTA-washed synaptosomal samples, and ^{27}Al NMR spectra showed the presence of the Al_{13} peak at 67.5 ppm, with a line width value of 100 Hz. This indicated the presence of the Al_{13} polymer inside the synaptosomes.

The above results clearly indicate the presence of the Al_{13} polymer, not only in the buffer solution, but also inside the synaptosomes. This is the first report to show the presence of the Al_{13} polymer in a biological system.

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