ISSN: 0975-8585

Research Journal of Pharmaceutical, Biological and Chemical Sciences

Studies on Complexation of Aluminium by some Natural Amino Acids.

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ABSTRACT

Aluminium complexes of some natural amino acids viz., glycine, α-alanine, L-aspartic acid and L-glutamic acid have been synthesized in aqueous medium at room temperature. Analytical results suggest the complexes to be of formulae, $[Al(gly)_3]$, $[Al(aln)_3]$, $[Al_2(asp)_3]$ and $[Al_2(glu)_3]$, where, gly, aln, asp, and glu are glycinate, α-alaninate, L-aspartate and L-glutamate respectively. In the infrared structure, the stretching and bending modes of NH_2 group of amino acids have been found to split into two or shift from their position upon complexation. This suggests a coordinated nature of amino group in the complexes. Evidence for additional H-bonding in the complexes is also observed in the infrared spectra. The ^1H NMR spectra of the ligands and complexes also suggest coordination of NH_2 group of amino acids in the complexes. Utility of the results in aluminium toxicology has been discussed.

Keywords: Aluminium complexes, Amino acid complexes, Aluminium toxicity, Aluminiumbiotoxicology.

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ISSN: 0975-8585

INTRODUCTION

Aluminium is the most abundant metal in the earth's crust. Despite abundance, aluminium does not have any useful biological function. Rather, it causes potential toxicity and health hazard when ingested in excess [1]. Aluminium ingestion has been implicated in diseases like osteomalacia [2], dialysis encephalopathy [3, 4] and Alzheimer's disease [5, 6]. Aluminium may enter into the body through food, water or air borne dust particles. Alfrey*et al*. [7, 8] have proved the detrimental effect of aluminium neurotoxicity. In Alzheimer disease abnormal accumulation of aluminium in the brain has been shown [9, 10]. It has also been recently hypothesized that aluminium could be involved in binding to the hyperphosphorylated tau proteins in this disease [11]. Studies in aluminium biotoxicology has recently been receiving much attention [12-24]. The neurotoxicity of aluminium might involve its complexation with bio-compounds.

As such, an investigation into the coordination chemistry of aluminium in the vicinity of natural compounds would reveal more about the mechanism of its biotoxicity. Such studies would also be helpful in designing drugs for aluminium detoxification.

With the above views in mind, we have presently studied the complexation of aluminium by some natural amino acids viz., glycine, α-alanine, L- aspartic acid and L-glutamic acid.

MATERIALS AND METHODS

All chemicals used were of A.R. (Analytical Reagent) grade.

The complexes were prepared as follows:

Complexes of glycine and α-alanine

0.015 mole of amino acid (glycine or α-alanine) was suspended in 25 ml of distilled water. Dilute NaOH solution was added drop wise, with constant stirring to neutralize the solution (pH-7). The amino acid got dissolved and a clear solution was obtained. Next, 0.005 mole of aluminium acetate was added to the solution. The reaction mixture was stirred at room temperature for 4h, on a magnetic stirrer. The pH was maintained at 7 during the reaction. At the end, the reaction mixtures were clear. The complexes were separated from the reaction mixture by crystallization. The crystals were filtered, washed with small quantity of distilled water and dried at 110º C in an air oven and preserved over anhydrous calcium chloride.

Complexes of L-aspartic acid and L-glutamic acid

0.015 mole of amino acid (L-aspartic acid or L-glutamic acid) was suspended in 25 ml of distilled water. Dilute NaOH solution was added drop wise, with constant stirring to neutralize the solution (pH-7). The amino acid got dissolved and a clear solution was obtained. Next, 0.005

ISSN: 0975-8585

mole of aluminium acetate was added to the solution. The reaction mixture was stirred at room temperature for 4h, on a magnetic stirrer. The pH was maintained at 7 during the reaction. At the end, the compounds were filtered and washed well with distilled water. The compounds were dried at 110º C in an air oven and preserved over anhydrous calcium chloride.

Analysis of Complexes

A known weight of complex was decomposed by 10 ml of conc. HNO_3 by heating and evaporating to dryness. The dry residue was extracted with distilled water to a known volume (100 ml) into a measuring flask. Aluminium in the solution was estimated spectrophotometrically using Eriochrom cyanine-R reagent [25]. C, H & N were estimated microanalytically.

Infrared Spectra

FTIR spectra of ligands and complexes were recorded in KBr phase in the range of 4000-450 cm^{-1}, on a Excalibur HE3600 infrared spectrophotometer.

NMR spectra

^1H NMR spectra of ligands and complexes were recorded in DMSO- d_6, on a BrukerAvance II 400 NMR Spectrometer.

RESULTS AND DISCUSSION

Compounds were found to be stable when stored under dry condition. Analytical data of the compounds are recorded in Table-1. Analytical results suggest a Metal : Ligand mole ratio of 1:3 in case of glycine and α-alanine complexes, and 2:3 in case of L-aspartic acid and L-glutamic acid complexes.

Table 1: Analytical data of compounds

Compound	Analysis of % found (calculated)			
	Al	C	H	N
$[Al(gly)_3]$	10.78 (10.97)	30.73 (29.26)	4.36 (4.87)	15.48 (17.07)
$[Al(aln)_3]$	9.15 (9.27)	36.75 (37.11)	6.53 (6.18)	13.46 (14.43)
$[Al_2(asp)_3]$	11.86 (12.08)	32.87 (32.21)	3.82 (3.35)	8.92 (9.39)
$[Al_2(glu)_3]$	10.80 (11.04)	35.32 (36.80)	3.91 (4.29)	8.24 (8.58)

Where, gly, aln, asp and glu are glycinate, α-alaninate, L-aspartate and L-glutamate respectively.

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Infrared Studies

FTIR spectra of ligands and complexes were recorded in KBr phase in the range of 4000 – 450 cm^{-1}. In the infrared spectra of glycine a broad band at 3414 cm^{-1} may be assigned to N-H stretching vibration. This band may actually be due to the zwitterionized NH_2 group. Upon complexation with aluminium, this band shifts down by about 11 cm^{-1} and appears at 3403. This shift shows coordination of NH_2 of glycine to Al. The bending mode of NH_2 of glycine shows at 1624 as a sharp band. This band, has been found to split into two, showing at 1636 and 1525 as weak bands. The split of NH bending suggests further a coordinated NH_2 in the complex. It is difficult to spot carboxylate bands and differentiate between the free amino acid and of the complex, because in free amino acid the COOH will be zwitterionized and will not be free. In the complex also the COOH would be deprotonated. More or less the position of COOH will be the same as that in the ligand and complex. The υ_{NH} of α-alanine showed at 3404 cm^{-1}. In the aluminium complex this band shifts-up to 3424. It seems the zwitterionized NH_2 of α-alanine gets involved in coordination to Al in the complex. The bending mode of NH_2 showed at 1626 in α- alanine. This band has been found to split into two, at 1630 and 1563 cm^{-1}. This split further suggests coordination of NH_2 to the metal in the complex.

In L- aspartic acid, the NH_2 stretching vibration is spotted at 3402 cm^{-1} as a broad band. This upon complexation splits into two in the region 3500-3000 cm^{-1}. This splits show the coordination of NH_2 to Al upon complexation. The bending mode of NH shows at 1626 in the ligand (L-aspartic acid). This band has been found to shift upward by 11 cm^{-1} in the complex, suggesting further the coordination of NH_2 in the complex.

In L-glutamic acid, the υ_{NH} occurred at 3405 cm^{-1}as a broad band. This upon complexation with aluminium splits into two, occurring at 3402 and 3020. This suggests the coordination of NH_2 to the metal in the complex. The NH bending mode was spotted at 1626 as a sharp band in the spectra of L-glutamic acid. This shifts upward to 1637 in the complex, suggesting further, coordination of NH_2 in the complex. Appearance, mostly, of an additional band at 2400 cm^{-1} in the spectra of ligands and complexes suggest a state of H-bonding both in the ligands and complexes.

NMR Studies

^1H NMR spectra of ligands and complexes were recorded in DMSO- d_6. In the ^1H NMR spectra of glycine, three signals are located at 2.5, 3.3 and 8.1 ppm. The COOH group of glycine would mostly remain in zwitterionized form and is likely to show as a low intensity signal. The signal at 8.1, as such, with a very low intensity might be assigned to OH proton of COOH. The intense signal at δ 3.3 might be due to CH_2 protons. The amino protons are shown as a multiplet (medium intensity) at δ 2.5. In the aluminium complex of glycine, it is expected that the zwitterionization will break and COOH would be deprotonated. In this process the amino group would also be disturbed. In the ^1H NMR spectra of complex, the NH_2 signal has been found to split into two occurring rather down field at δ 2.6 and δ 2.5. This split shows a coordinated mode of NH_2 group in the complex. The CH_2 proton signal more or less, is not

ISSN: 0975-8585

disturbed and remains at δ 3.3. Strong signal (doublet) at δ 7.9 suggests that some of NH protons are involved in some additional association, probably H-bonding (intra molecularly) with the neighbouring suitable groups.

Figure 1: [Al (gly)$_3$]

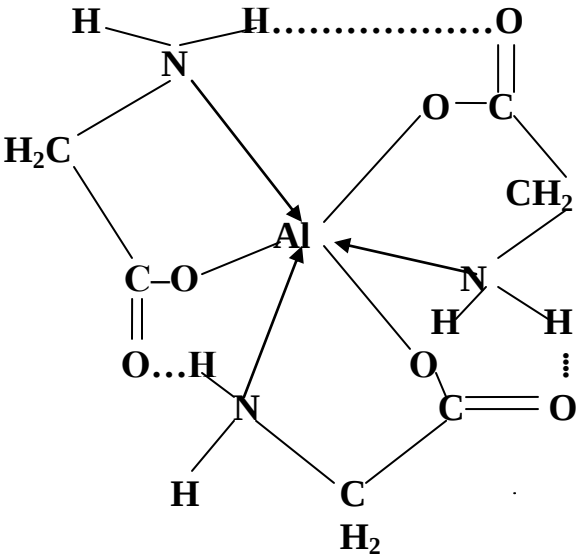
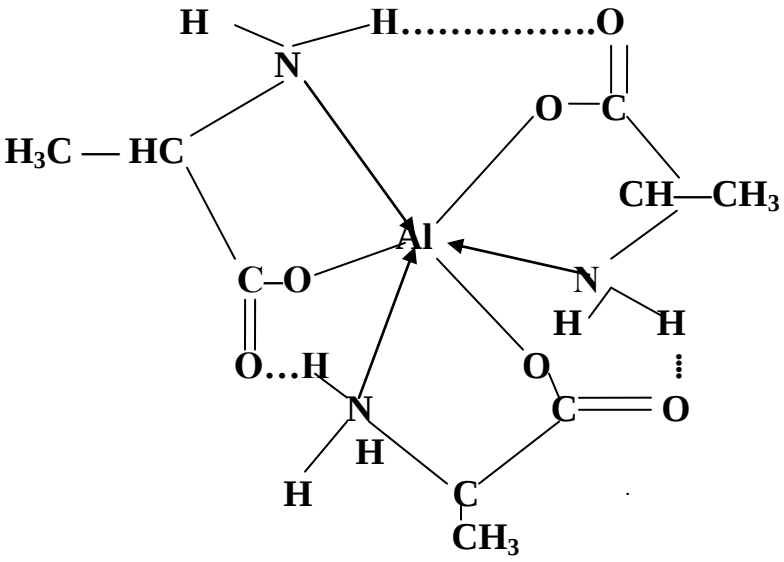


Figure 2: [Al (aln)$_3$]



^{1}H NHR spectra of α-alanine shows signal due to CH_3 protons at 3.3 ppm. The NH_2 protons may be assigned to a medium signal at δ 2.5. The zwitterionized proton is shown at δ 8.1. Upon complexation, the signal due to amino proton has been found to split into two at δ 2.5 and 1.8. Suggesting the coordinated nature of amino groups and also probably involvement of NH protons in H-bonding. The signal at δ 8.1 shifts down to δ 8.0. Suggesting further the involvement of NH protons in some association, probably H-bonding.

ISSN: 0975-8585

The L-aspartic acid shows a signal at δ 8.1 in its PMR spectra. It may be zwitterionized proton. The CH proton is shown at δ 3.3. The amino protons are shown at δ 2.5 with medium intensity. Upon complexation this signal due to amino protons weakens largely in intensity.

Figure 3: $[Al_2(asp)_3]$

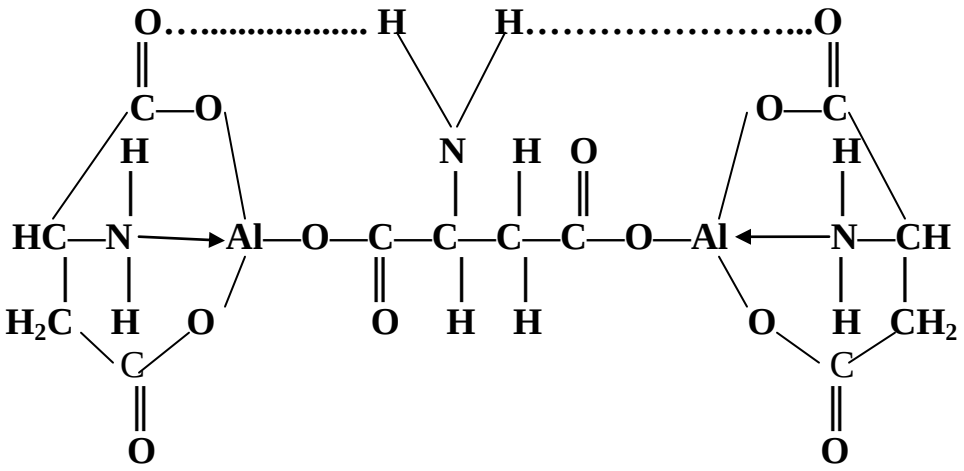
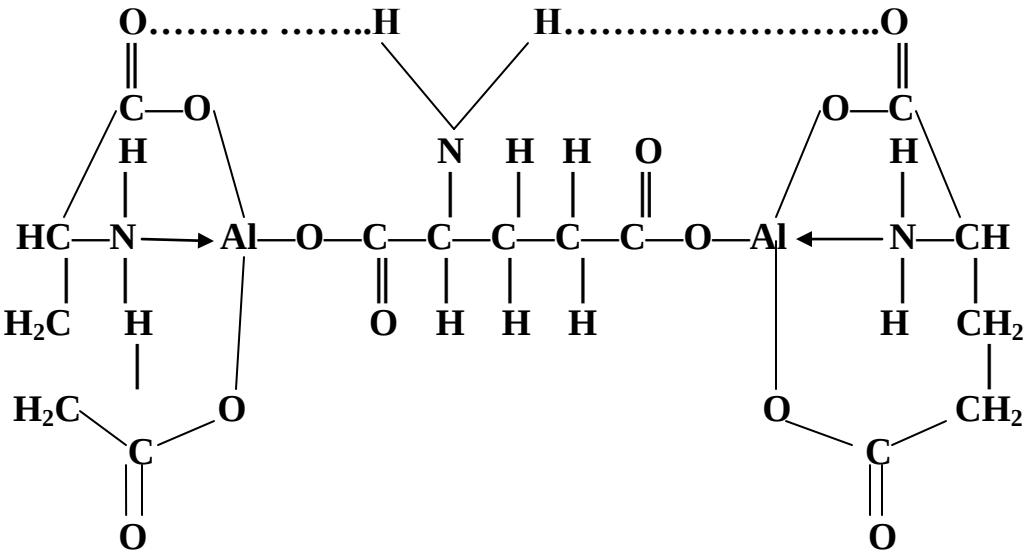


Figure 4: $[Al_2(glu)_3]$



The PMR spectra of L-glutamic acid shows CH_3 protons at δ 3.3. The amino protons are shown at 2.5. The signal at δ 8 might be zwitterionized proton. Upon complexation the signal due to amino protons splits into two, at δ 2.5 and 1.9. Suggesting coordinated nature of amino groups. On the basis of analytical and spectral studies, the structure and bonding of the complexes may tentatively be proposed as shown in Figs.1 to 4. Formation of aluminium complexes by the natural and physiologically important amino acids suggests their probable role in the chemical mechanism of aluminium toxicology.

ISSN: 0975-8585

CONCLUSION

The amino acids that we have presently worked with are biologically important ones. The brain has a good amino acid pool and the amino acid (particularly glutamate) serves in many detoxification reactions. The basis of neurotoxicity of aluminium has been suggested to be due to its accumulation in the brain. Role of complexation of aluminium (by hyperphosphorylated tau proteins) in its expression of toxicity has also been hypothesized [11]. Complexation of aluminium by the amino acids might offer a good protection from Al-toxicity. As such, these amino acids could be explored for aluminium detoxification. However, a possibility also exists that complexation of aluminium by the brain amino acids (in case of Al-toxicity) may lead to depletion of brain amino acid pool, leading to adverse affects. As such, aluminiumcomplexation by the natural amino acids should be probed *in-vivo*, in animal experiments, for a better understanding of the role of complexation in aluminium toxicology.

ACKNOWLEDGEMENTS

Authors express gratefulness to the SAIF, Panjab University, Chandigarh for ^{1}H NMR spectral facility, and to the SAIF, CDRI, Lucknow for FTIR spectral and microanalytical facilities.

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