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Interaction of aluminium ions with some amino acids present in human blood

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Summary. The interaction of aluminium with some amino acids present in human blood was studied combining ion-chromatography (IC), atomic absorption spectrometry (AAS) and ultrafiltration (UF) techniques. An IC system for simultaneous determination of ornithine, lysine, glutamic acid, aspartic acid and tyrosine was developed. By adding aluminium to standard solutions of the amino acids and keeping the pH at 6 and 7 it was possible to verify that aluminium caused a reduction on the amino acid chromatographic signals. Similar experiment, carried out for copper showed the same behaviour (with different percentage of signal reductions) and validated the results for aluminium, considering that the interaction Cu-amino acid is well-established. The AAS analysis of sample fractions (500 μl) after the IC separation showed that aluminium (as copper as well) is not present in the fractions in which the amino acid peaks appear in the chromatogram. These approaches carried out with serum samples after UF showed that part of the "free" fraction of serum aluminium is distributed, besides other ligands, among these amino acids. It was found that in serum the affinity for aluminium followed the sequence Lys > Orn > Tyr > Glu ≈ Asp.

Keywords: Aluminium – Serum – HPLC – Metal-binding – Amino acids

Introduction

Aluminium is a non-essential element to which humans are frequently exposed. This exposure can occur orally through food, water and some medicines, and, the worst, parenterally through contaminated pharmaceutical products, mainly solutions for parenteral nutrition and dialysates for hemodialysis.

Although the presence of Al in pharmaceutical products used parenterally is known for at least 18 years (Klein et al., 1982; Klein, 1995), this is still a problem today due to its toxic effect to children and adults with chronic renal failure and to patients on long-term parenteral nutrition (Greger et al., 1997; Alfrey, 1993). Moreover, Al can also be associated to impaired neurologic development in preterm infants, which receive prolonged intravenous feeding (Bishop et al., 1997; Klein, 1998; Koo, 1996; Discroll et al., 1997). In spite of not existing levels considered normal for Al in blood, 1 to 7 μg/l can be found in healthy people. In renal chronic patients, submitted to hemodialysis treatment, Al levels up to 30 μg/l are expected, however, a threshold serum Al level of 60 μg/l is a reliable index for the Al-related bone disease (Daugirdas et al., 1994).

Studies have shown that Al occurs in practically all products for parenteral nutrition (Koo et al., 1986; Berner et al., 1989), including amino acid formulations. In a previous work we observed that glass containers are a possible source of aluminium for solutions for parenteral nutrition (Bohrer et al., 2001), but only when certain species are present in the solution. A more specific study involving only the amino acids used in parenteral formulations revealed that a few of them are able to withdraw aluminium from the glass surface and from an ion-exchange in the Al-form (Bohrer et al., 2002). That study also showed, by comparing aluminium to copper and amino acids to complexing agents for aluminium, that the withdrawing can be related to an affinity of the ligand for the metal. Moreover, the yield of this extraction could be related to the stability of the complexes.

As the amino acids that presented the highest interaction were ornithine, lysine, aspartic acid and glutamic acid, we intended, in this work, to investigate the interaction of these amino acids with aluminium at physiological conditions of the human blood.

The interaction of Al with small-molecule ligands present in the human blood, has been extensively studied

(Berthon, 2002; Rubini et al., 2002) because these ligands could be responsible for the transport of the so-called ''free-fraction'' of Al in the circulatory system. It has been demonstrated that approximately 80% of the Al in blood is bound to proteins as transferrin and albumin and the remaining 20% is free or at least non-protein bound (Sanz-Medel et al., 2002; Harris, 1996). The interaction of Al with phosphate (Atkári et al., 1996) and silicate (Doucet et al., 2001; Exley et al., 2002) and mainly citrate (Öhman et al., 1994; Bantan et al., 1999) has been demonstrated by several authors as well as the possibility of being these anions responsible for Al absorption and excretion in the human body (Berthon, 2002; Berthon, 1996; Yokel, 2000). Amino acids can also be classified as small-molecule ligands because they have at least one amino and one carboxyl group and stability constants for amino acid complexes of several metals are found in the literature (Martell et al., 1998). About 23 amino acids are present in human blood and among them, onithine, lysine, aspartic acid, glutamic acid and tyrosine are found. Their concentration ranges in blood are: Ornithine 30–130 μM, lysine 90–260 μM, aspartic acid 11–54 μM, glutamic acid 0–120 μM and tyrosine 32–87 μM (Henry et al., 1980). As these concentrations are not low and the amino acids seemed to have an affinity for Al, we propose in this work to study their interaction with Al at the physiological conditions of the human blood.

Among the available techniques for the simultaneous determination of amino acids, namely chromatography and electrophoresis, high performance liquid chromatography (HPLC) is the one that offers the largest number of instrumental options (Holme et al., 1998). Different stationary phases as reversed-phase and ion-exchange can satisfactorily be employed. Complexes mixtures of amino acids can be resolved in about one hour by this technique. HPLC also allows the use of different detection systems. Depending on the amino acids, UV and electrochemical detectors can be directly used, and practically all amino acids can simultaneously be determined using derivatising reagents as ninhydrin coupled with photometric detection or ortophthalaldehyde and dansyl chloride with fluorimetric detection (Fekkes, 1996).

In this work a chomatographic system based on ion-exchange separation and fluorimetric detection after derivatisation with OPA was developed for the study of the interaction of Orn, Lys, Tyr, Asp and Glu with Al and Cu. The proposal was to evaluate the interaction through the reduction of the chromatographic signals in the presence of the metals.

Material and methods

The chromatographic equipment consisted of a DX-300 gradient chromatography system (Dionex, Sunnyvale, United States) with a 1100 series fluorescence detector (Hewlett Packard, Waldbronn, Germany) and a GradiFrac fraction collector (Pharmacia, Uppsala Sweden). A C-R6A data processor (Shimadzu, Kyoto, Japan) and a second chromatographic pump LC – IOAS (Shimadzu, Kyoto, Japan) were also used. A SpectrAA-200 atomic absorption spectrometer equipped with a GTA-100 graphite furnace and autosampler (Varian, Melbourne, Australia), a Trox class 100 clean bench (Trox, Curitiba, Brazil), a BSB 939-IR sub-boiling distillation apparatus (Berghof, Eningen, Germany) and a pHmeter D-20 (Digimed, São Paulo, Brazil) were used. A Ultrasart Cell 10 SM 166 66 ultrafiltration system (Sartorius, Göttingen, Germany) was used for serum ultrafiltration.

Reagents

Commercial aluminium and copper standard solutions (1000 mg/l, Merck, Darmstadt, Germany) were used for preparing working solutions of the cations by appropriate dilution. The amino acids ornithine, lysine, aspartic acid, glutamic acid and tyrosine (biochemical grade, 99% pure) were supplied from Sigma (St. Louis, USA). For the chromatographic separation two eluents were necessary. Namely, eluent I (23 mM NaOH + 7 mM $Na_2B_4O_7$) and eluent II (0.4 M CH_3COONa + 1 mM NaOH in 2% (v/v) methanol). All chemicals for eluent preparation were purchased from Merck. The reagent for derivatisation was prepared by adding 2.5 g *o*-phthaldialdehyde (99% pure) (Aldrich, St. Louis, USA) and 20.12 g $Na_2B_4O_7 \cdot 10H_2O$ to a mixture of 800 ml water and 50 ml methanol. The volume was completed to 1 litre with water and the solution was filtered through a cellulose acetate membrane (0.2 μm) (Sartorius). After that, 2.5 ml ethanethiol (99% pure) (Merck) was added to the solution. This solution (after the addition of ethanethiol), OPA-SH solution, was prepared daily.

The water used was distilled, de-ionised and further purified using a Milli-Q high-purity water device (Millipore, Bedford, USA) and the HNO_3 (65%, 1.17 g/ml) from Merck was purified by sub-boiling distillation.

Contamination control

To avoid contamination, only plastic materials were used. All labware (pipette tips, volumetric flasks, etc.) was immersed for at least 48 h in a 10% (v/v) HNO_3/ethanol solution and shortly before being used washed with Milli-Q purified water.

To avoid contamination from the air, all steps in the sample and reagents preparation were carried out in a class 100 clean bench.

Chromatographic system

An Amino Pac PA1 (250 × 4 mm; 9.0 μm) column (Dionex) was used for the separation of the amino acids and the injection volume was 25 μl. The elution gradient starts with 100% eluent I, after three minutes eluent II is introduced and becomes 70% of the mobile phase in the 4th minute. The system keeps this ratio for 13 minutes. During the next minute it changes to 100% eluent II and keeps this mobile phase for the next five minutes. After that eluent I is introduced again and the mobile phase becomes 100% eluent I after one minute. The eluent flow rate was 0.8 ml/min. By means of a three-port valve the column effluent and OPA-SH solution (flow rate 0.2 ml/min) were mixed and carried to the fluorescence detector. The excitation and emission wavelengths were 330 nm and 455 nm respectively.

The influence of the pH of the solution on the amino acid signals was investigated varying the pH of the sample solution (6 μM in each amino acid) from 4 to 8 by adding HNO_3 or $Na_2B_4O_7$ solution to the sample, and carrying the chromatographic analysis.

Table 1. Atomic absorption spectrometer operating conditions

Instrument	Al	Cu
Wavelength (nm)	309.3	324.8
Lamp Current (mA)	10	4.0
Spectral slit width (nm)	0.5	0.5
Background correction	Deuterium lamp	Deuterium lamp
Sample volume (μl)	10	10

Graphite Furnace Pyrolytic coated furnace with L'vov platform and argon as purge gas

Temperature programme

Step	Al			Cu		
	Temperature (°C)	Time (sec)	Gas flow (l/min)	Temperature (°C)	Time (sec)	Gas flow (l/min)
1	85	5	3.0	85	5	3.0
2	95	35	3.0	95	35	3.0
3	120	10	3.0	120	10	3.0
4	1400	20	3.0	300	10	3.0
5	1400	10	3.0	300	15	3.0
6	1400	2	0.0	300	2	0.0
7*	2600	0.7	0.0	1900	0.7	0.0
8*	2600	1	0.0	1900	1.0	0.0
9	2600	1	3.0	2000	1.0	3.0

* Read

Reaction amino acid o-phthaldialdehyde

In order to verify if the amino acid-metal complex does not react with the fluorescence reagent or if the presence of the metal shifts the maximum excitation or emission wavelengths of the fluorescent compound, two sets of solutions were prepared: in the first set the solutions were composed by 1 ml amino acid solution (6 μM), 1 ml water and 1 ml OPA-SH solution. In the second one copper solution (6 μM) was used in the place of water. The spectra of these solutions were collected using a blank made with 2 ml water and 1 ml OPA-SH solution. Additionally, the fluorescence intensity of the solutions containing copper was measured for two hours after their preparation.

Amino acid–metal interaction

A sample containing 6 μM of each amino acid and 60 mM Cu(II) (pH 6) was injected into the chromatographic system and after the separation and detection of the amino acids, the effluent was collected in fractions of 500 μl. All collected fractions were analysed by electrothermal atomic absorption spectrometry (ET AAS). The same procedure was carried out using Al(III) instead of Cu(II) in the same concentration of 60 mM. The AAS operation parameters for both metals are given on Table 1.

The stability of the complexes at the pH values set for the analysis was investigated preparing a 6 μM amino acid solution containing 60 μM Cu(II) or Al(III) and injecting these solutions into the chromatographic system at intervals of 2 h during 8 h.

A third assay was carried out with samples containing amino acids and metal at different molar ratios. The amino acid concentrations were fixed in 6 μM and the metal was added to give molar ratios metal/amino acid of 1:5; 5:5; 10:5 and 20:5 (considering the sum of amino acid concentrations). Two batches were prepared, one had the pH adjusted to 6 and the other to 7, both with $Na_2B_4O_7$ solution. All these samples were injected into the chromatographic system. The experiment was carried out with copper and aluminium.

In order to verify if the amino acid-aluminium complex is not dissociated during the time that it crosses the column, i.e., if the amino acid signal reduction corresponds to the whole fraction of the amino acid bound to aluminium, the chromatographic system was set without the column and with water as eluent. Individual solutions of each amino acid (6 a μM, pH 7) and the same solutions containing aluminium in a rate of 1:5 were injected into the system and the fluorescence signals recorded. Each sample was injected 10 times.

Amino acid detection in serum samples

Before the chromatographic analysis, a pooled serum sample was ultrafiltered (UF) in a ultrafiltration system with a 5,000 Da cellulose acetate membrane. One ml serum was UF and diluted 1:50 with water before being injected into the chromatographic system. This sample was spiked with the amino acids to give concentrations of 6 μM Orn, Lys, Asp, Glu and Tyr for each amino acid. The UF serum sample was also spiked with Al at three different concentration levels: 74, 185 e 370 μM.

Results and discussion

The chromatographic system allowed the detection of the five amino acids in about 25 min. In spite of their different nature the use of two eluents in a gradient arrangement promoted the formation of well-shaped peaks. A calibration curve, obtained with standard solutions containing the five amino acids, presented the parameters depicted

Table 2. Calibration parameters of the amino acid chromatographic separation

Standard (μM)	Amino acid signal (RSD) (μA)				
	Orn	Lys	Glu	Asp	Tyr
3.0	48465 (0.84)	38079 (0.86)	103853 (0.79)	67774 (0.69)	76159 (0.86)
6.0	110777 (0.74)	69235 (0.94)	204244 (0.80)	135009 (0.60)	148856 (0.73)
12.0	231939 (0.70)	141933 (0.69)	387718 (0.63)	283865 (0.58)	311559 (0.73)
$y = ax + b$					
a	19551	11737	32211	23681	25947
b	−4846	692	4846	−2662	−2077
R^2	0.998	0.9992	0.9990	0.9993	0.9994
LOD (μM)*	0.9	1.0	0.2	0.6	0.5
LOQ (μM)**	1.2	1.5	0.4	0.9	0.8

* (3σ); ** (6σ)

in Table 2. LOD and LOQ were calculated from the baseline noise (3σ and 6σ, respectively) according to Massart et al. (1988).

Since the experiments were carried out at different pHs, the influence of the pH of the sample on the amino acids signals was investigated. The results showed that between pH 4 and 8 no significant alteration on the signal intensity was observed.

The post-column derivatisation with OPA-SH can be considered one of the best options for amino acids determination (Fekkes, 1996) because the reagent is stable at room temperature and the reaction proceeds quickly without requiring heat. The reaction is approximately ten times more sensitive than with ninhydrin. It was tried to use a chromatographic system with amperometric detection, however, it presented interferences and even a slight change in the eluent composition promoted such a noise in the basis line that turned inadequate this kind of detection for the proposed investigation. The fluorimetric detection with OPA-SH implied, however, an investigation on the competition between Al and OPA-SH for the amino acid molecules. The first set of experiments included the mixture of amino acids and Al followed by the addition of OPA-SH and the comparison of the fluorescence of these solutions with standards containing no Al. As for that moment, the results for Al could not be reliable, preliminary tests were done with copper because the interaction between copper and amino acids is well-established. Two sets of experiments were carried out, the first was the one already mentioned, mixing the reagents and accompanying the fluorescence intensity of the solutions, and the second one was the collection of the spectra of solutions containing the amino acid, copper and OPA-SH, considering that any change in the maximum of absorption or emission due to the presence of the metal could be misinterpreted as a reduction on the fluorescence by the absence of the free amino acid to react with OPA-SH. Figure 1 shows the Orn-OPA-SH solution spectra, with and without Cu(II) in the solution. It is possible to see that there was a decrease on absorption spectrum at 330 nm when the solution contained Cu(II). The presence of the metal did not change the spectrum, only led to a reduction of intensity. The same behaviour was observed in the solutions of the other amino acids. The fluorescence intensity of the solutions containing copper had a reduction of 13% for Orn, Lys and Tyr, 4% for Glu and 2% Asp. This reduction remained constant for the two hours while the fluorescence was measured.

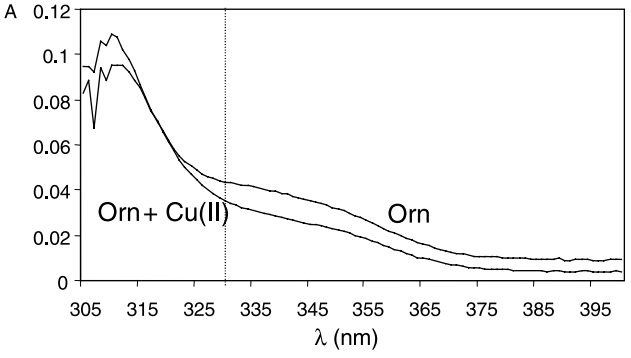

Fig. 1. Absorption spectrum of Orn-OPA-SH solution in the absence and presence of Cu(II)

From these results it can be inferred that, there is a fraction of the amino acids bound to the metal and the addition of OPA-SH to the solution does not alter the amino acid-metal equilibrium in solution, at least in the period of time necessary to carry out the experiment. Therefore, the method can be used to evaluate the extension of the amino acid-metal binding.

Amino acid–metal interaction

The interaction of both Cu(II) and Al(III) with the amino acids was studied at pH 6 and 7. No buffers were used to avoid parallel reactions of the metallic cations, therefore, pH was adjusted with $Na_2B_4O_7$ solution.

After the addition of Cu(II) and Al(III) separately to the amino acid solutions the samples were injected into the chromatograph and fractions of 500 μl of the column effluent were collected during the entire chromatographic run. Cu(II) and Al(III) were measured in these fractions by ET AAS. This is a powerful technique for the determination of very low metal concentrations. Detection limits quoted by the manufacturers of electrothermal atomisers are typically in the range of 10^{-10} to 10^{-12} g or even less (Christian, 1994). This allows the determination of as low concentration as 1 ng/ml Al or Cu in the eluent samples.

Figure 2 shows the overlapping of the metal content of each fraction and the chromatogram of amino acids separation. Copper and aluminium were present in the first 6 fractions only. No metal was found in the fractions corresponding to the amino acid appearance in the chromatograms. Since there was a reduction in the amino acid peak height, it can be concluded that there is no fluorescent compound built from amino acid-metal-OPA-SH and the reduction on the amino acid signal corresponds to the fraction of each amino acid bound to the metal. Moreover, the absence of Al and Cu in the last fractions shows that the complexes are not dissociated during the time they cross the column. This was also confirmed by the injection of samples with and without Al in the chromatograph set without column. The correspondent peaks had the same height in both approaches.

The investigation of the stability of the complexes was carried out injecting a sample containing the amino acids and the metal in the chromatographic system at different time intervals (2 h for 8 h). The results showed that there were no changes in the amino acid signal intensities in the presence of both metals. As there were no changes in the amino acid concentration, it can be concluded that the amino acid fraction bound to the metals did not change during this period.

Figure 3 shows the reduction of the chromatographic signals by increasing the metal/amino acid ratio in solution. Cu(II) and Al(III) showed different behaviour although both have promoted bigger reduction on the basic amino acid signals. The difference on the signal heights in the presence and absence of Al was approximately the same for both approaches (30% Orn, 36% Lys, 5% Asp, 8% Glu and 10% Tyr), showing that the amino acid fraction read with the chromatographic column corresponds to the free amino acid fraction only.

Considering acidic and basic amino acids, the two ligands involved are the amino and carboxyl groups either at the α-carbon or at the side chain. Studies with Cu(II) (Albert, 1952) revealed that, for basic amino acids, when the side chain is small enough to form a five- or six-

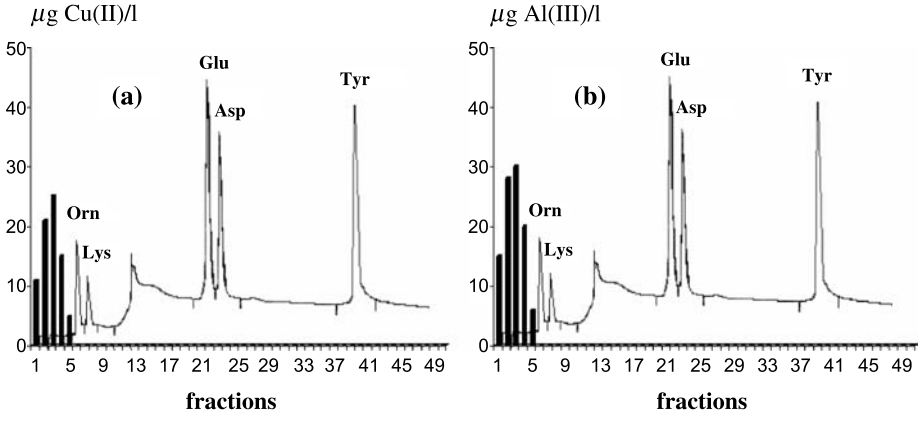

Fig. 2. Copper and aluminium measured in the column effluent collected during the chromatographic separation of the amino acids. Fraction volume: 500 μl. Metal distribution graphic and chromatogram are overlapped according to the time of fraction collection

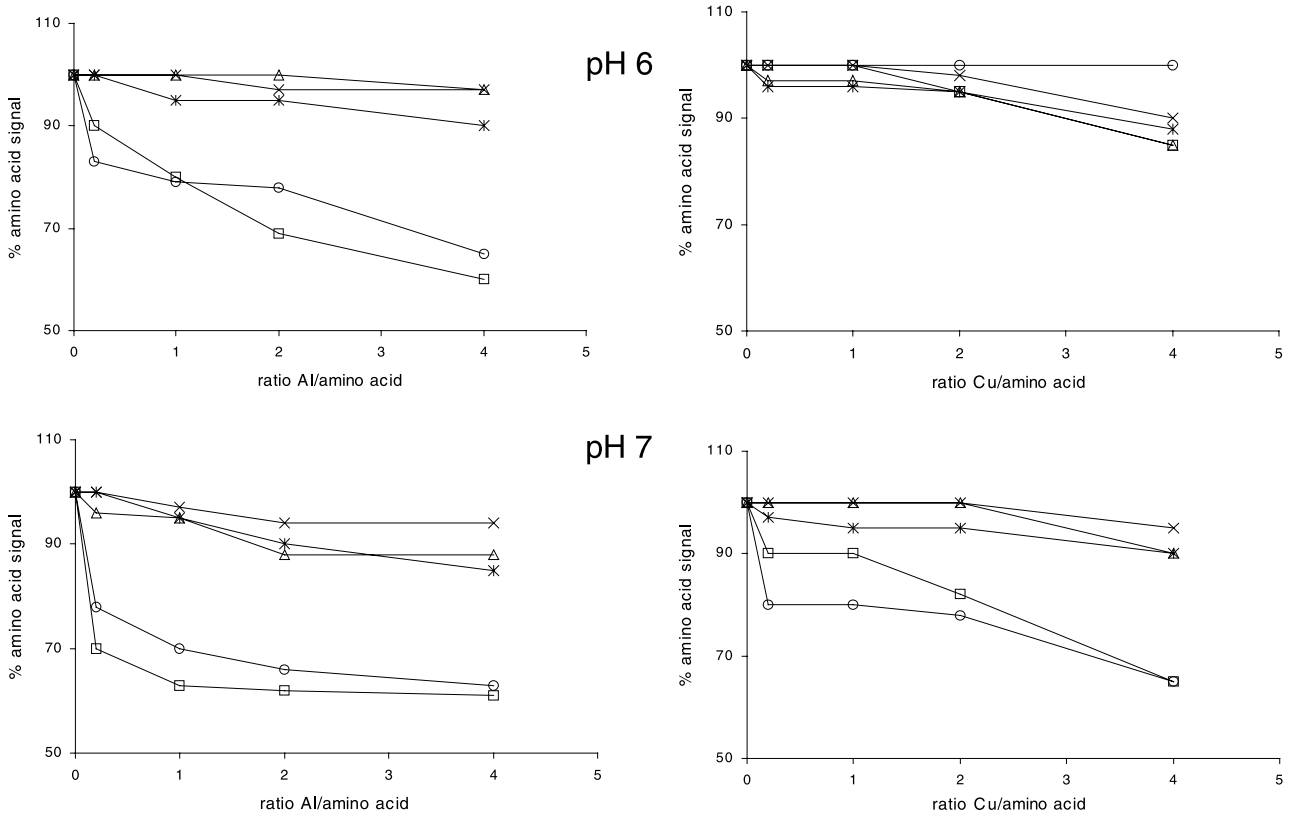

Fig. 3. Percentage of the amino acid signal reduction due to increasing metal-amino acid ratios. (×) Glu, (Δ) Asp, (○) Orn, (□) Lys, (∗) Tyr. Amino acid concentration in the original sample: 6 μM

membered ring, the preference will be for the two amino groups. However, when rings with seven or eight members are formed, the complex includes the α-amino and α-carboxyl groups instead. This way, α,β-diaminopropionic acid and α,χ-diaminobutyric acid form complexes with five and six members involving only the α,β- and α,χ-amino-groups respectively, whereas Orn and Lys, for which the two amino-groups would form 7- or 8-membered ring respectively, build preferentially the 5-membered ring involving the α-amino and α-carboxyl groups. For acidic amino acids, namely Asp and Glu, the proposed Cu(II) complexes involve both the α-amino group and the α-carboxyl group (Albert, 1952; Greentein et al., 1962). According to Bjerrum (1950), in rings of equal size, two carboxyl groups do not bind metals so avidly as a carboxyl and an amino group.

When the results for Al(III) are considered, it would be expected a higher interaction with the acidic amino acids than with the basic ones. It has been considered that the strength of complexation to Al(III) decreases in the sequence dicarboxylic acid > hydroxycarboxylic acid > carboxylic acid > amino acid. The two first members in this series form more stable complexes due to the chelating effect, and the weakness of the last member is attributed to the electrostatic repulsive effect of the NH_3^+-group (Marklund et al., 1990). In fact, some studies have demonstrated a weak interaction between Al(III) and simple bidentate amino acids (Daydé et al., 2002). However, studies including tridentate amino acids with two amino groups are rare, probably because the affinity of Al(III) for N-donors is supposed to be low and it is considered that the involvement of Al(III) with these amino acids is not too likely. Some studies, however, have shown that the amino group participates in the complex formation. Kiss et al. (1997) found the participation of the amino group in the coordination of Asp towards Al(III), attributing the deprotonation of the NH_3^+-group to the proximity of both carboxyl groups. They compared Asp and succinic acid, both containing α and β carboxyl groups, but the amino group was absent in the second. They concluded that the strongest complexation detected with Asp would indicate an involvement of the amino group in the binding mode of the Al-Asp complexes. The authors also suggest that the amino

group can participate in the binding of Al(III) with Glu, where a weaker binding would be expected due to the lower stability of the 7-membered chelate ring. However, a favourable steric arrangement would also be responsible for a tridentate coordination, i.e., involving the amino group. Even in the presence of phosphoryl groups (high affinity for Al) (phosphoserine), they found that the amino group could play a role in chelating Al(III) (Kiss et al., 1998). In addition, the coordination of N-donor groups along with carboxyl groups has been demonstrated by NMR (Iyer et al., 1989) and X-ray crystallography (Valle et al., 1989).

It is important to mention that the involvement of metals with free amino acids cannot be compared with that which occurs when the amino acids are residues in a protein. While for free amino acids chemical reactions are determined by all functional groups they possess, in a protein only the functional group at the side chain may be involved.

Amino acid–aluminium interaction in serum samples

Before the analysis, the pooled serum sample was submitted to a deproteinisation procedure by ultrafiltration to avoid proteins to enter into the chromatographic system. UF also avoids that the Al(III) added to the sample binds proteins instead of amino acids. The UF serum sample was diluted before analysis to match the concentration of the analytes with the calibration curves. Figure 4a shows the amino acid signals of standard sample containing 6 μM of each amino acid and Fig. 4b the chromatogram of a serum sample diluted 1:50. The increase of the amino acid signals of the same sample spiked with the amino acids (Fig. 4c) confirmed their retention times for the serum sample. In order to evaluate the interaction of Al with the amino acids in serum, the diluted serum sample (1:50) was injected into the chromatographic system after being spiked with Al. The addition of Al to the serum did not change the pH of the samples. Comparing

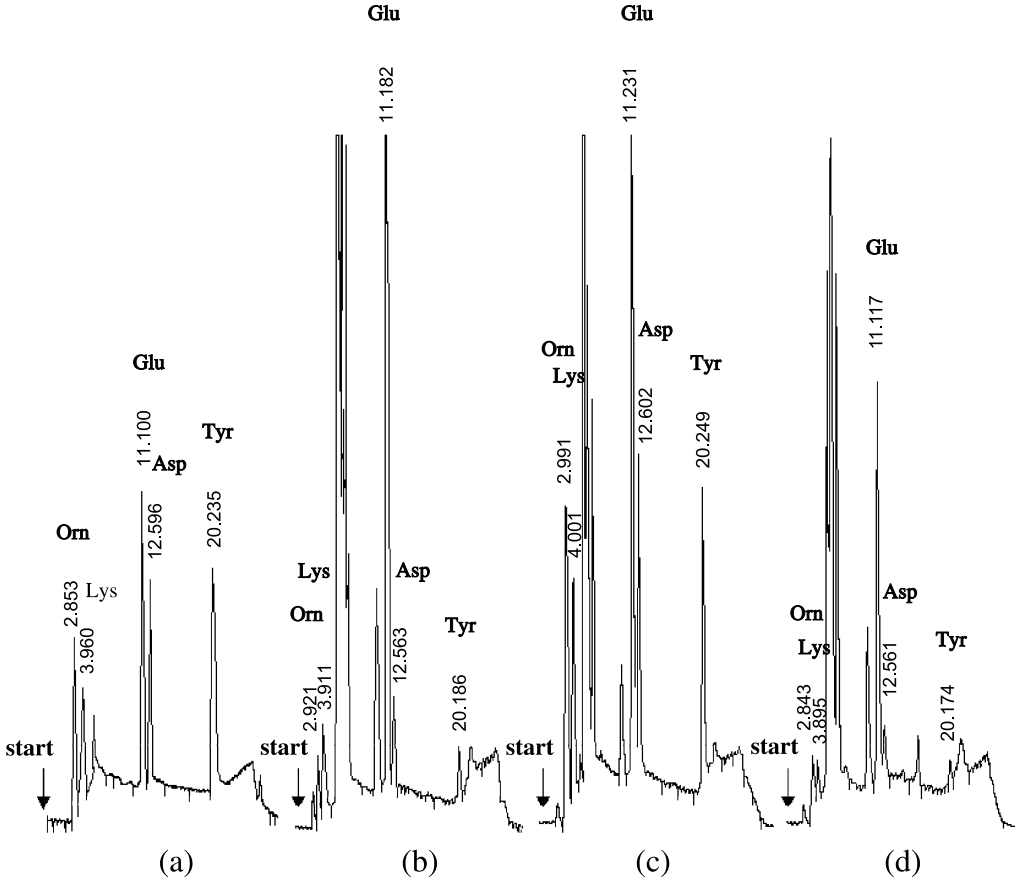

Fig. 4. Chromatograms of amino acid standards and ultrafiltrated serum samples, diluted 1:50. (**a**) Amino acid standards, (**b**) serum sample, (**c**) serum sample spiked with the amino acids Orn, Lys, Asp, Glu and Tyr (6 μM each), (**d**) serum sample spiked with 185 μM Al(III). Eluents: (#1) 0.023 M NaOH + 0.007 M $Na_2B_4O_7$ and (#2) 0.4 M NaOAc + 0.001 M NaOH in 2% methanol

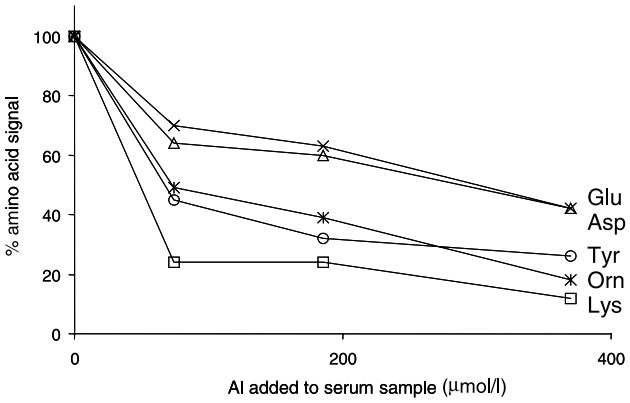

Fig. 5. Percentage of the ultrafiltrated serum amino acid signal reduction due to the presence of increasing Al(III) concentration in the sample

both chromatograms, with and without Al spiking (Fig. 4d and b), it is possible to see that the presence of Al lowered the amino acid signals. Of course these are not the actual conditions in blood because before existing as free species to bind amino acids Al would be attached to proteins as transferrin and albumin (Sanz-Medel et al., 2002). However, the results show that the binding of Al to amino acids under the blood conditions is possible to occur.

The graphic in Fig. 5 shows that the higher the Al concentration the lower the amino acid signals, as it would be expected considering the previous results. The basic amino acids suffered the highest reduction and the acidic ones the smallest one. Tyr, however, that practically did not interact with Al under the standard conditions, showed a relatively high interaction in the serum sample.

Conclusion

The anion-exchange chromatography of amino acids with fluorescence detection can be a useful tool for investigation of the interaction of metals with amino acids. The assay with Cu(II) revealed that the metallic cation promotes a reduction on the amino acid signals. At the studied pH, the signal lowering keeps a relationship to the stability constants, the higher the stability constant of the Cu-amino acid complexes the bigger the signal reduction.

Orn, Lys, Tyr, Asp and Glu were chosen for the study because they are present in the human blood and they showed, in previous works (Bohrer et al., 2001, 2002), the highest affinity for Al when the amino acids, necessary for the human nutrition, were compared to each other. The results found in this work showed that Al also interacts with these amino acids at conditions near the physiological. Even being very different assays, the results found in this and in previous works showed the same tendency, that is, Al is able to interact more strongly with the basic amino acids Orn and Lys than with the acidic Asp and Glu. The experiments carried out with serum samples allowed to conclude that these amino acids can bind Al and consequently they could be responsible, along with other small-molecule ligands, for the transportation of Al in the human body.

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