The Metal Ions Absorption – and Transportation Properties of L-Aspartic Acid in the Body

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Abstract: The intra molecular interactions in complexes of Aspartic acid with divalent metal ions (Mg, Ca & Zn) M(Asp)[1] were investigated in this work. The acidity constants of Asp and stability constants of M(Asp) are determined and reported as well. The effect of intra molecular interactions can be seen by comparison of these stability constants. Mg and Ca ions form weaker bonds to Asp than Zn ion. Zn(Asp) complex undergoes an intra molecular interaction and builds a chelate in two forms (an open – isomer $M(Asp)_{op}$ and a closed – isomer $M(Asp)_{cl}$). The concentration independent intra molecular interaction constant for two isomers has been calculated and reported.

The obtained results demonstrate that for some M(Asp) complexes the stability constant is largely affected by the affinity of metal ions for amine group. This shows a kind of selectivity of metal ions and transfer them *via* complex – building with the aspartate. Also all three metal ions complexes could be considered as mineral carriers. These complexes can release the minerals in human body in certain conditions (e,g. pH – range).

Keywords: Divalent metal ions, potentiometric titration, acidity and stability constants, mineral absorption, minerals in body.

INTRODUCTION

It is known that metal ions are important for numerous biochemical reactions. For example, enzymes work only in the presence of such metal ions. The metal ion complexes of many amino acids have been investigated [1-7]. Ion exchange, chelating, and electrostatic interactions form the basis of metal sorption. The behavior of various materials functionalized with polypeptides and other molecules is a topic of interest because of its applications in affinity separations, biosensors, and other uses including site-specific interactions [8]. An example of the latter involves the removal of heavy metals from aqueous solutions [9-12]. These sorbents are made of a variety of materials containing many different functional groups. The advantage of affinity separations is that they may be tailored for the desired selectivity and capacity. The functionalization of materials is of vital importance for the production of new materials with specific properties. The characterization of these new materials is also critical.

A study of the evaluation of poly-L -aspartic acid and poly-L -histidine as binding agents to enhance micro dialysis recovery of metal ions is presented. Investigations were carried out to compare micro dialysis recovery for Cr, Cu, Ni, and Pb using water as the perfusion liquid as well as applying various concentrations of poly-L-aspartic acid and poly-L-histidine in the perfusion liquid [13]. This is aimed at understanding the mechanism of the selectivity of such reactions with aspartic acid (Fig. **1**).

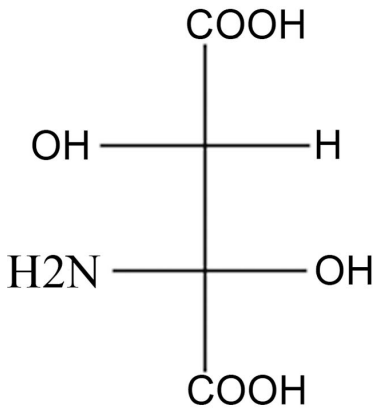

Fig. (1). Chemical formula of L-aspartic acid.

EXPERIMENTAL

Materials

The L-Aspartic acid (extra pure) was purchased from Merck, Darmstadt, Germany. The nitrate salt of Na^+, Ca^{2+}, Mg^{2+}, and Zn^{2+} (all pro analysis) were from Merck. All the starting materials were of reagent grade and used without further purification. Potassium hydrogen phthalate and standard solutions of sodium hydroxide (titrasol), nitric acid, EDTA and of the buffer solutions of pH 4.0, 7.0 and 9.0 were all from Merck. All solutions were prepared with de-ionized water. Water was purified by Milil-Q water purification system, de-ionized and distillated.

pH Titrations

Reagents

Carbonate-free sodium hydroxide 0.03 M was prepared and standardized against sodium hydrogen phthalate and a

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[1]M: Mg^{2+}, Ca^{2+}, Zn^{2+}; Asp: L-Aspartic Acid.

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standard solution of nitric acid 0.5 mM. M(II) nitrate solution (0.03 M) was prepared by dissolving the above substance in water and was standardized with standard solution of EDTA 0.1 M (triplex).

Apparatus

All pH titrations were performed using a Metrohm 794 basic automatic titrator (Titrino), coupled with a thermo-stating bath Hero at 25°C (±0.1°C) and a Metrohm combined glass electrode (Ag/AgCl). The pH meter was calibrated with Merck standard buffer solutions (4.0, 7.0 and 9.0).

Procedure

For the determination of acid dissociation constants of the ligand L, an aqueous solution (0.03 mM) of the protonated ligand was titrated with 0.03 M NaOH at 25°C under nitrogen atmosphere and ionic strength of 0.1 M, $NaNO_3$. For the determination of binary (a ligand and M^{2+}) system, the ratios used were 1:1, M^{2+} : Ligand and 1:1, M^{2+} : L, 0.3 mM. This solution was titrated with 0.03 M NaOH under the same conditions mentioned above. Each titration was repeated seven times in order to check the reproducibility of the data.

Calculation

The acid dissociation constants, $K^{H}_{H_2(Asp)}$ and $K^{H}_{H(Asp)}$ for $H_2(L)$ were calculated by an algebraic method. The equilibrium involved in the formation of 1:1 complex of L and a divalent metal ion may be expressed as equations (4) & (5).

RESULTS AND DISCUSSION

The potentiometric pH-titrations (25°C, 0.1 M, $NaNO_3$) were carried out to obtain the acidity and stability constants which are summarized in Table **1**.

Acidity Constants

Asp (L^{2-}), $^{-}O_2CCH_2CH(NH_2)CO_2^{-}$, is a two-basic species, and thus it can accept two protons, given $H_2(L)$, for which the following de-protonation equilibrium are hold:

$$H_2(L) \rightleftharpoons H^+ + H(L)^- \quad (1a)$$

$$K^{H}_{H_2(Asp)} = [H(L)^-][H^+]/[H_2(L)] \quad (1b)$$

$$H(L)^- \rightleftharpoons H^+ + L^{2-} \quad (2a)$$

$$K^{H}_{H(Asp)} = [L^{2-}][H^+]/[H(L)^-] \quad (2b)$$

The two proton in $H_2(L)$ are certainly bound at the terminal acetate and amino groups, i.e., it is released from $HO_2CCH_2CH(NH_3^+)CO_2^-$ according to equilibrium (1) & (2). It is known as zwitter-ion. It is also closed to the de-protonation of acetate groups which occurs at the terminal acetate groups of aspartic acid [6, 14]. L^{2-} can release one more proton from the terminal acetate group. Hence, here due addition to equilibrium (3) should be considered, which takes place above a pH ≈ 2.

$$H_3(L)^+ \rightleftharpoons H^+ + H_2(L) \quad (3a)$$

$$K^{H}_{Asp} = [H_2(L)][H^+]/[H_3(L)^+] \quad (3b)$$

Here, the aforementioned reaction is not considered further.

Stability of Binary Complexes

If we abbreviate for simplicity associating with Ca^{2+}, Mg^{2+}, and Zn^{2+} with M^{2+}, then one may write the following two equlibria of (4) & (5):

$$M^{2+} + H(L)^- \rightleftharpoons M(H;L)^+ \quad (4a)$$

$$K^{M}_{M(H;Asp)} = [M(H;L)^+]/[M^{2+}][H(L)^-] \quad (4b)$$

$$M^{2+} + (L)^{2-} \rightleftharpoons M(L) \quad (5a)$$

$$K^{M}_{M(Asp)} = [M(L)]/[M^{2+}][L^{2-}] \quad (5b)$$

The experimental data of the potentiometric pH titrations may be completed by considering the above-mentioned equilibrium (1) through (5), if the evaluation thereof is not carried into the pH range, where hyrdoxo complex formation occurs.

The schematic illustration of equilibrium between different protonated species is shown in Fig. (**2**). Based on this point we can define following equilibrium:

$$M^{2+} + (L)^{2-} \rightleftharpoons M(L) \rightleftharpoons M(L)_{op} \rightleftharpoons M(L)_{cl} \quad (6a)$$

$$K^{M}_{M(Asp)} = ([M(L)_{op}] + [M(L)_{cl}])/[M^{2+}][L^{2-}] \quad (6b)$$

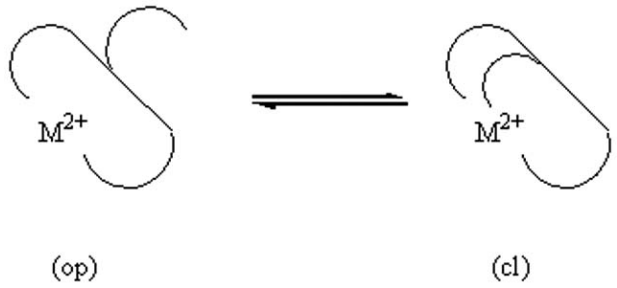

Fig. (2). Schematic equilibrium between an "open" isomer $M(Asp)_{op}$ and a "closed" species, $M(Asp)_{cl,}$ see eq. (6).

Approximately TTA[2] can represent the open form.

Potentiometric Analyses

Now we are able to compare the stability constants of two species M(TTA) and M(Asp). It could easily distinguish that those constants of M(Asp) is generally larger than those of the corresponding M(TTA) species. This increased stability of the difference between the stability constants as defined in eq. (6) [15-18]:

$$\Delta\log K = \log K_{(Asp)} - \log K_{(TTA)} \quad (7)$$

Positive amount of $\Delta\log K$ indicates the back – binding of the metal ion, with other words the building of chelate (see Fig. **2**).

According Fig. (**2**) we can define the dimensionless equilibrium constant K_I as follows:

[2]TTA: tartaric acid.

$$K_I = [M(Asp)_{cl}]/[M(Asp)_{op}] \quad (8)$$

The equilibrium constant may be deduced [19-22] from the experimentally accessible overall stability constant, $K^M_{M(Asp)}$ using eq. (9),

$$K_I = (K^M_{M(Asp)} / K^M_{M(Asp)}) - 1 \quad (9)$$

Now we can combine eq. (7) and (9) to receive eq.(10),

$$K_I = 10^{\Delta \log K} - 1 \quad (10)$$

The percentage amount of closed species in eq. (11), which represent the intra molecular interactions i.e. chelating can be calculated *via* eq. (11) as follows,

$$\% M(Asp)_{cl} = 100(K_I/1+ K_I) \quad (11)$$

This results are summarized in Table **1**. As we can see from these results, the stability constants of the binary complexes, such as M(L) were refined separately using the titration data of this system in a 1:1, ligand:M^{2+} ratio in the same conditions of temperature and ionic strength (according eq.(5)), as they were in good agreement with reported value [6, 23]. We didn't received reasonable results for $K^M_{M(H;Asp)}$. The stability constants of Table **1** show the following trends. The obtained order for TTA and Asp is Ca^{2+}< Mg^{2+}< Zn^{2+}. The last observed stability order for aspartate follows the Irving-Williams sequence [24].

Aspartic chelates metal ions weakly *via* the amino nitrogen and carbonyl oxygen. A stronger chelating occurs upon an amide nitrogen bound hydrogen by some metal ions such as Zn^{2+}. This reaction occurs in neutral pH conditions (pH≈ 7) with Zn^{2+}. A crystal structure of M^{2+} chelate with a structure analogous has been studied [25].

If we now consider the two isomers which occur in equilibrium Fig. (**2**) as M $(Asp)_{op}$ and M $(Asp)_{cl}$, we can define the chelate equilibrium constants K_I by eq. (9).

In the last column of Table **1** are summarized the results of the difference between stability constants of TTA and Asp eq.(7). The amount of Δlog K for Ca^2+ and Mg^{2+} is not significant, but in the case of Zn^{2+} the difference are remarkable. The increased amount of stability constants in the case of Asp shows that the formation of chelate is taken place. This means the additional complex stability is a degree of the affinity of the metal ions for Asp. Hence, if both these chelate isomers do exist, the percentages calculated for $M(Asp)_{cl}$ eq. (11) comprise the sum of percentages for both isomers.

However, as we have seen above, for the present cases Δlog K holds and hence values for the chelate building, which then also allow calculation of percentage of the closed isomer $M(Asp)_{cl}$ with eq. (11). The chelate – building can occur in inner-sphere or outer-sphere form *via* amid group. The results show that the total percentage of chelate – building according eq. (11) for Mg^{2+} is about 75% and for Zn^{2+} is ≈100%. Because of high increased stability we can draw conclusion, that the releasing of Zn^{2+} in biological systems is relatively strong. But also in comparison with "hard" metal ions like Mg^{2+} and Ca^{2+} is the releasing much easier. These properties can play a significant role for catalytic activity of such metal ions in biological systems.

Biological systems have the ability to selectively bind to metals taking advantage of the array of protein binding functionalities [25]. Short chain synthetic biopolymers also have unique, strong and selective binding properties offered by their constituent amino acids.

Interactions between aspartic acid (Asp) and cytidine-5-monophosphate (CMP) in metal-free systems as well as the coordination of Cu(II) ions with the above ligands were studied. The composition and overall stability constants of the species formed in those systems were determined [26]. Amino acid chelated minerals, also referred to as chelated minerals or mineral chelates, are minerals that have been chemically engineered to become more bio available to our body. Amino acids act as carriers to ship the much-needed minerals to the destination (the small intestine) where consumption takes place.

Elixir Industry has tested many self-claimed "mineral chelates" available on the market and found most of them are merely mixtures of amino acids and inorganic minerals [27]. Why are amino acid chelated minerals superior to common inorganic minerals?

New products are anhydrous chelates of two L-Aspartic Acid molecules and a single metal ion. The technology of achieving this structure is patented and was jointly developed by Elixir Industry and the Chinese National Institute of Pharmaceutical Industry [27]. Mineral chelates to be completely soluble over a wide range of pH values. The chelated minerals are soluble in the small intestine for absorption and subsequent bio-utilization. These products are compatible with most nutritional additives commonly used in formulations and tableting applications. There are different types of amino acids. Nutrition scientists selected L-Aspartic acid based on many additional benefits that come with it.

Table 1. Comparison of the Stability Constants of Binary Complexes of Asp and TTA with M^{2+} at 25°C, *I* = 0.1 M, $NaNO_3$*

No.	Species	Log $K_{(Asp)}$	Log $K_{(TTA)}$	Δlog K
1	$H_2(L)$	3.72±0.03	3.09±0.07	
2	H(L)	9.90±0.03	4.19±0.05	
3	Mg^{2+}	2.50±0.06	1.90±0.05	0.60±0.05
4	Ca^{2+}	1.26±0.06	1.80±0.05[1]	–0.54±0.08
5	Zn^{2+}	5.35±0.06	2.69±0.07	2.66±0.09

*The given errors are three times the standard error of the mean value or the sum of the probable systematic errors. [1][6, 23].

CONCLUSION

The obtained results demonstrate that for Zn(Asp) complex the stability constant is largely affected by the affinity of metal ion for amine group. This shows a kind of selectivity of metal ions and transfer them *via* complex – building with the aspartate. All three metal ions complexes could be considered as mineral carriers . These complexes can release the minerals in human body at certain conditions.

Chelated minerals are well shielded by bonded organic ligands. They will not come in contact with vitamin molecules; thus, the vitamins will be protected from oxidation and degradation.

Inorganic metal ions may serve as a catalyst to further the oxidation and degradation of vitamins. Chelated minerals will not absorb vitamins and cause them to become non absorbable - problems that common inorganic mineral are known to cause. TTA is not able to build three dentate chelate like Asp, so that metal ions are not shielded enough. As a consequence, metal ions can take part in substitution reactions. In contrast to the high-tech nature of chelated minerals, common inorganic minerals that are used in majority of vitamin and mineral supplements today are minerals that are easily found in nature or in the earth, in the forms of rock or limestone. First of all, chelated minerals are substantially more bio available than common inorganic minerals. For mineral to be absorbed by our body, it has to be soluble in the luminal fluid of the small intestine. The pH of the small intestinal fluid below the duodenum is 7.0-7.2. Most inorganic minerals will form insoluble hydroxides and become non absorbable at this pH. Chelated minerals, on the other hand, are well shielded by amino acids, and will not precipitate to cause absorption problems.

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