Complex Bilding Behavior of L-Tryptophan and Related Amino Acids, a Comparative Investigation

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Abstract   The acidity constants of Tryptophan[1] were determined by potentiometric pH titration. The stability constants of the 1:1 complexes formed between M^{2+}: Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+} or Zn^{2+} and Trp^{2-}, were determined by potentiometric pH titration in aqueous solution (I = 0.1 M, NaNO3, 25°C). The order of the stability constants was reported. It is shown that the stability of the binary M(Trp) complexes is solely determined by the basicity of the carboxyl or amino group. All the stability constants reported in this work show the usual trend. The obtained order is $Ca^{2+} < Mg^{2+} < Mn^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$. The observed stability order for Tryptophan follows the Irving-Williams sequence. It is shown that regarding to M ion–binding properties vital differences on complex bilding were considered. It is demonstrated, that in M–Trp complexes, M ion is coordinated to the carboxyl group, M ion is also able to bild macrochelate over amino group. The up mentioned results demonstrate that for M–Trp complex the stability constants is also largely determined by the affinity of Cu^{2+} for amino group. It is shown that Trp can exert a direct influence on reaction mechanism through different kinds of metal ions and donor groups of Trp.

Keywords   Tryptophan, Amino Acids, Divalent Metal Ions, Potentiometric Titration, Acidity and Stability Constants

1. Introduction

Tryptophan is one of the 10 essential amino acids that the body uses to synthesize the proteins it needs (fig. 1)[1]. It's well-known for its role in the production of nervous system messengers, especially those related to relaxation, restfulness, and sleep. Tryptophan has two important functions. First, a small amount of the tryptophan we get in our diet (about 3%) is converted into niacin (vitamin B3) by the liver. This conversion can help prevent the symptoms associated with niacin deficiency when dietary intake of this vitamin is low. Second, tryptophan serves as a precursor for serotonin, a neurotransmitter that helps the body regulate appetite, sleep patterns, and mood. Because of its ability to raise serotonin levels, tryptophan has been used therapeutically in the treatment of a variety of conditions, most notably insomnia, depression, and anxiety. Vitamin B6, vitamin C, folic acid and magnesium are necessary for the metabolization of tryptophan. In addition, tyrosine and phenylalanine compete with tryptophan for absorption[2,3].

Because of the essential rols of tryptophan in biological systems is important to investigate its interactions with different metal ions and the regarding complex bilding.

It is a vital constituent of proteins and indispensable in human nutrition for establishing and maintaining a positive nitrogen balance[4]. Besides, some of its derivatives are potent drugs[5]. Trp is widely used in food industry. It is sometimes added to dietary and feed products as a food fortifier in order to maintain the amino acid balance of the food and correct possible dietary deficiencies. Trp can also be used to study structure and dynamics of the proteins because of its indole moiety[6]. In particular, Trp is the precursor of the neurotransmitter serotonin and plays an important role in brain function and related regulatory mechanisms[7]. In addition, Trp is an important and frequently used starting material in the chemical synthesis of a range of pharmaceuticals.

Among the side chains of amino acids, the indole moiety is the most potent electron donor[8]. Indeed, charge- transfer-type interactions between tryptophan or other indole derivatives and nucleosides or nucleotides occur in aqueous solution[9-15].
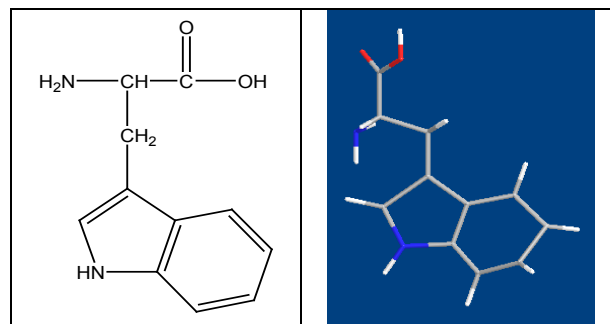
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Figure 1.   Chemical structure of and L-Tryptophan

Based on above mentioned essential role of Trp is interesting to study the interaction between other metal ions with Trp.

2. Experimental

Materials

The L- tryptophan (extra pure) was purchased from Merck, Darmstadt, Germany. The nitrate salt of Na^+, Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} (all pro analysis) were from Merck. All the starting materials were of pro analysis 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 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 Trp, an aqueous solution (0.3 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(II) : Trp, 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, $K^H_{H_2(Trp)}$ and $K^H_{H(Trp)}$ for $H_2(Trp)$ were calculated by an algebraic method. The equilibrium involved in the formation of 1:1 complex of Trp and a divalent metal ion may be expressed as equations (1) & (2).

3. 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 & 2.

Table 1. Negative logarithm of the acidity constants of $H_2(Trp)$ at 25°C, 0.1 M, $NaNO_3$, eq.(1)&(2)

No.	Species	pKa*	Site
1	$H_2(Trp)$	2.22 ± 0.04	$-CO_2H$
2	$H(Trp)$	9.14 ± 0.02	$-NH_2$

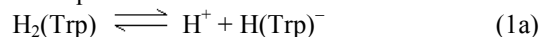*The given errors are three times the standard error of the mean value or the sum of the propabable systematic errors.

Table 2. Logarithm of the stability constants of binary complexes of M^{2+} at 25°C, 0.1 M, $NaNO_3$*, eq.(4)

No.	Species	$\log K^M_{M(Trp)}$
1	Ca^{2+}	2.55 ± 0.08
2	Mg^{2+}	2.84 ± 0.08
3	Mn^{2+}	3.34 ± 0.05
4	Co^{2+}	4.34 ± 0.07
5	Ni^{2+}	5.31 ± 0.06
6	Cu^{2+}	8.05 ± 0.05
7	Zn^{2+}	5.00 ± 0.08

*The given errors are three times the standard error of the mean value or the sum of the propabable systematic errors.

Acidity constants

Tryptophan ion (Trp^-), $RCH(NH_2)CO_2^-$, is a one-basic species, and thus it can accept one proton on the carboxyl side. On the other hand Trp^- releases at higher pH another proton from amino group, for which the following de-protonation equilibriums are hold:

$$H_2(Trp) \rightleftharpoons H^+ + H(Trp)^- \tag{1a}$$

$$K^H_{H_2(Trp)}] = H(Trp)^-[H^+]/[H_2(Trp)] \tag{1b}$$

$$H(Trp)^- \rightleftharpoons H^+ + Trp^{2-} \tag{2a}$$
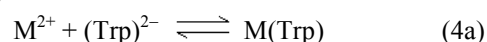
$$K^H_{H(Trp)}] = Trp^{2-}[H^+]/[H(Trp)^-] \tag{2b}$$

The two proton in $H_2(Trp)$ are certainly bound at the terminal acetate and amino groups, i.e., it is released from $RCH(NH_3^+)CO_2^-$ according to equilibrium (1) & (2). It is also closed to the de-protonation of acetate groups which occurs at the terminal acetate groups of related amino acids[16,17]. Trp can release the first proton from the terminal acetate group. Hence, here due addition to equilibrium (1) should be considered, which takes place above a pH ≈ 2.

Stability of binary and ternary complexes

If we abbreviate for simplicity associating of Ca^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+} with Trp, then one may write the following two equlibriums of (3) & (4):

$$M^{2+} + H(Trp)^- \rightleftharpoons M(H;Trp)^+ \tag{3a}$$

$$K^M_{M(H;Trp)}] = M(H;Trp)^+/[M^{2+}][H(Trp)^-] \tag{3b}$$

$$M^{2+} + (Trp)^{2-} \rightleftharpoons M(Trp) \tag{4a}$$

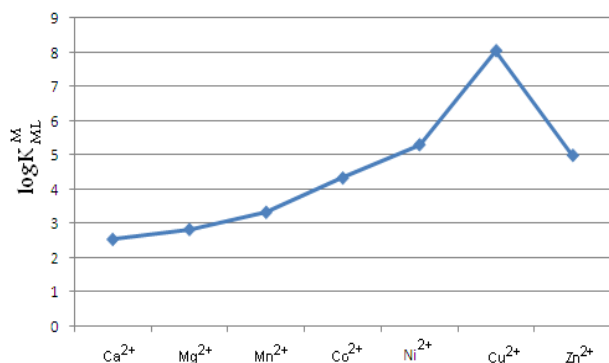$$K^M_{M(Trp)}] = M(Trp)]/[M^{2+}][Trp^{2-}] \tag{4b}$$

The experimental data of the potentiometric pH titrations may be completed by considering the above-mentioned equilibria (1) through (4), if the evaluation thereof is not carried into the pH range, where hyrdoxo complex formation occurs.

Potentiometric analyses

The results of all potentiometric pH-titration, i.e. acidity and stability constants, are summarized below in table 1 & 2. The de-protonated amino acid Trp^{2-} can accept two protons,

to give the acid $H_2(Trp)$. The first one of these two protons of carboxylate residue is released; its pKa is low (≈ 2). However, Trp^- can release one more proton from neutral $-NH_2$ site, which is pH≈ 9. The measured acidity constants in this work show good agreement with the same value received by other authors[16,18-21]. However, the carboxyl group is a far stronger acid than the amino group[22].

The stability constants of the binary complexes, such as M(Trp) 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. 3 & 4), as they were in good agreement with reported value[16,21]. We didn't receive reasonable results for $K^M_{M(H;Trp)}$. All the stability constants of table 2 show the usual trend. The obtained order is $Ca^{2+} < Mg^{2+} < Mn^{2+} < Co^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$. The observed stability order for Tryptophan follows the Irving-Williams sequence[23] (fig. 2). Based on the HSAB Principle :'Hard acids prefer to coordinate to hard bases, and soft acids prefer to coordinate soft bases.' This is the Principle of Hard and Soft Acids and Bases. This means that metal ions like Ca^{2+}, Mg^{2+}, Mn^{2+} prefer to coordinate on carboxyl site and the other above mentioned metal ions (tab.2) (as borderline metal ions) show tendency for both carboxyl group, as well as for amino group and can be coordinate bidentate.

Figure 2.   Irving-Williams sequence-type plot for the 1:1 complexes of Ca^{2+} to Zn^{2+} with Tryptophan (see table 2)

Another interesting point is the tentative and simplified structure for the macrochelated outer–sphere isomer. In the case of hard-metals such as Ca^{2+}, Mg^{2+}, and Mn^{2+} can be observed the outer-sphere complex bilding. It should be noted that the term outer-sphere is used here with regard to the $M^{2+}/^-NH-$ coordination. If an intramolecular direct $M^{2+}/^-NH-$ coordination occurs, then it is strictly more favorable to have a water molecule between M^{2+} and $^-NH-$. With an outer-sphere $^-NH-$ binding can increase the stability of binary complexes.
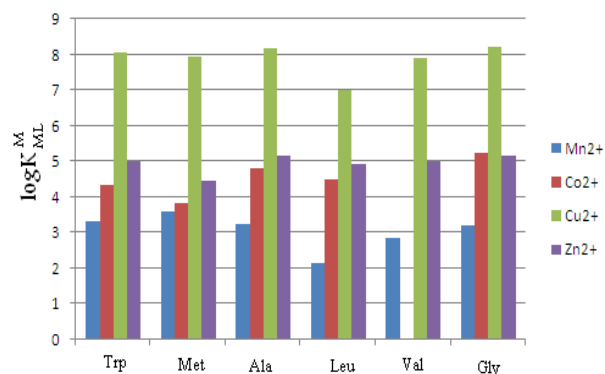
Now we are able to compare the stability constants of two species M(Am) and M(Trp) (see table 3 & fig.3). Am represent the amino acids such as Leucine, Valine, Alanine, Glycine, Methionine. It could easily distinguish that this constants of M(Trp) is generally (within the propabable systematic errors) the same of the corresponding M(Am) species. These mean that we can not distinguish a reasonable

increased stability in case of M(Trp).

Table 3.   Logarithm of the stability constants of binary complexes of M^{2+} with L at 25°C, 0.1 M, NaNO$_3^*$, eq.(4). L: Trp (tryptophan), and related compounds such as Met (methionine), Ala (Alanine), Leu (Leucine), Val (Valine), and Gly (Glycine)

No.	Ligand	log $K^M_{M(L)}$			
		Mn^{2+}	Co^{2+}	Cu^{2+}	Zn^{2+}
1	Tryptophan[a]	3.34 ± 0.05	4.34 ± 0.07	8.05 ± 0.05	5.00 ± 0.08
2	Methionine[b]	3.59 ± 0.05	3.85 ± 0.08	7.96 ± 0.02	4.46 ± 0.06
3	Alanine[c]	3.24	4.82	8.18	5.16
4	Leucine[c]	2.15	4.49	7.00	4.92
5	Valine[c]	2.84	–	7.92	5.00
6	Glycine[c]	3.20	5.23	8.22	5.16

a) from this work ; b) from[17], c) from[16]. *the given errors are three times the standard error of the mean value or the sum of the propabable systematic errors.
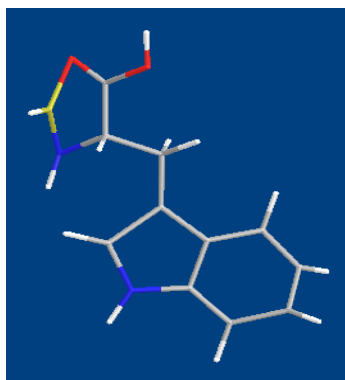

Figure 3.   stability constants of the 1:1 complexes of Mn^{2+} to Zn^{2+} with some amino acids (see table 3). Leu: Leucine, Val: Valine, Ala: Alanine, Gly: Glycine, Met: Methionine, Trp: Tryptophan

Ca^{2+} ion play numerous roles in biological systems. The intracellular level of Ca^{2+} must be kept low, as the phosphate esters are highly abundant and calcium phosphates are quite insoluble in the preceding capture. All cells have transport systems – the Ca^{2+}-ATPase and the sodium calcium exchanger – for the extrusion of Ca^{2+}.

L-Trp or D-Trp; sold for medical use as Tryptan (fig. 1)[25] is one of the 20 standard amino acids, as well as an essential amino acid in the human diet. It is encoded in the standard genetic code as the codon UGG. Tryptophan (Trp) is considered exceptional in its diversity of biological functions[26].

Research over the course of the last four decades has undeniably demonstrated, using laboratory animals, that in addition to its role as a building block of protein, glutamic acid serves as a neurotransmitter vital to the transmission of nerve impulses in many parts of the central nervous system. glutamine synthetase is an octametric enzyme, contains bound Mg^{2+} in its structure. Mg^{2+} is essential for the activity[24]. Magnesium, Vitamin B6, vitamin C, and folic acid are necessary for the metabolization of tryptophan. In addition, tyrosine and phenylalanine compete with tryptophan for absorption. This is an interesting point, because interaction of hard metals such as Mg^{2+} with amino group is considerable, which we can use from results (tab. 2).

Based on the results of this work we can draw the conclusion, that hard metal ions just with identical stability constants could have similarly interaction with Tryptophan. Even based on these results of acidity constants reported (Tab. 1) Tryptophan occurs in high organism in form of Trp^-. Earlier works have reported the structure of Tryptophan complexes with some metal ions[27] such as Co^{2+}, Ni^{2+}, Cu^{2+} and Zn^{2+}. These metal ions are able to have additional interactions with Tryptophan (Fig. 4).

Figure 4. Schematic structures of the species with interactions according to equilibrium (4) for Cu(Trp). The structure was drawn with the program CS Chem 3D, version 3.5, from Cambridge Software Corporation

Kynureninase purified from rat liver was inhibited by 3-hydroxyanthranilate, or anthranilate, and slightly by 5-hydroxyanthranilate. However, tryptophan metabolites other than anthranilate and its derivatives, and also alpha-keto acids and alanine did not affect the activity of this enzyme. Kynureninase was also inhibited and inactivated by metal ions, especially Hg^{2+} and Zn^{2+}. On the other hand monovalent cations had no effect on the activity of the enzyme[28].

The Saccharomyces cerevisiae RNA triphosphatase (Cet1) requires the presence of metal ion cofactors to catalyze its phosphohydrolase activity, the first step in the formation of the 5′-terminal cap structure of mRNAs. It has been used endogenous tryptophan fluorescence studies to elucidate both the nature and the role(s) of the metal ions in the Cet1-mediated phosphohydrolase reaction. The association of Mg^{2+}, Mn^{2+}, and Co^{2+} ions with the enzyme resulted in a decrease in the intensity of the tryptophan emission spectrum. This decrease was then used to determine the apparent dissociation constants for these ions[29].

4. Conclusions

The Tryptophan industry continues to deny that exposure to free Tryptophan found in processed food causes adverse reactions including hives, asthma etc., which is interesting to investigate. Another interesting point is the pharmacological application of new generation of Tryptophan complexes and it seems essential to understand their reaction mechanism.

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