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Metal-Ion-Coordinating Properties of Various Amino Acids, Investigation of the Essential Function in Biological Systems regarding to their Nano-Structure

Seyed Ali Akbar Sajadi

Sharif University of Technology, Institute of Water & Energy, Tehran P.O.Box 11155-8639, I.R. Iran

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ABSTRACT

The acidity constants of some amino acids (Am) 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 Am^{2-}, were determined by potentiometric pH titration in aqueous solution (I = 0.1 M, $NaNO_3$, 25°C). The order of the stability constants was reported. It is shown that the stability of the binary M(Am) 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 Am 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 MAm 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 MAm complex the stability constants is also largely determined by the affinity of Cu^{2+} for amino group. It is shown that Am can exert a direct influence on reaction mechanism through different kinds of metal ions and donor groups of Am.

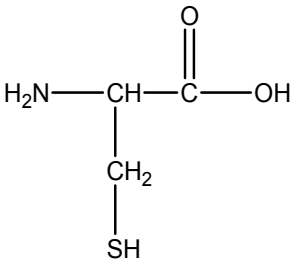
Keyword: Tryptophan; methionine; cysteine; glutamine; aspartic acid; glycine; amino acids; divalent metal ions; potentiometric titration; acidity and stability constants.

1. INTRODUCTION

The a-amino and α-carboxyl groups of amino acids play a prominent role in metal ion binding. There are many examples of the side Chain functional groups that also interact with metal ions. Peptides interact with metal ions primarily through side chain functional groups, although there are many examples of peptide amide nitrogens functioning as donor atoms with certain metal ions. Many physiologically important peptides function as metal complexes. In Figure 1 is shown the chemical structure of some amino acids [1].

Methionine is an essential amino acid. It is one

(*) Corresponding Author - e-mail: sajadi@sharif.ac.ir



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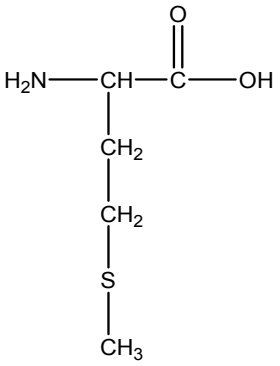

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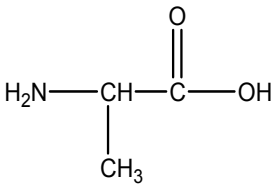

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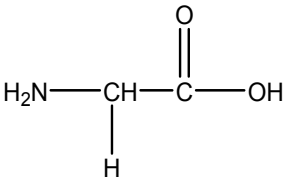

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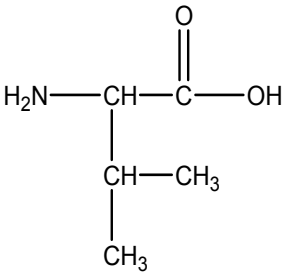
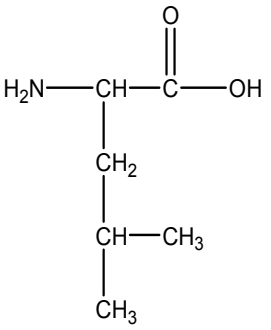
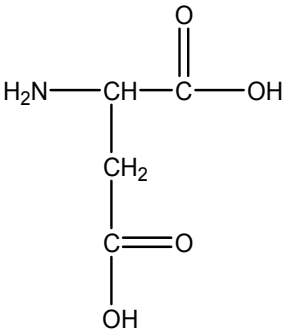


Val



Leu



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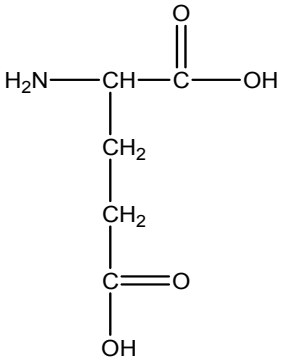

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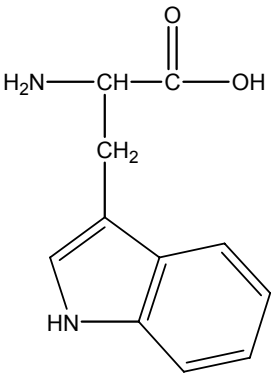
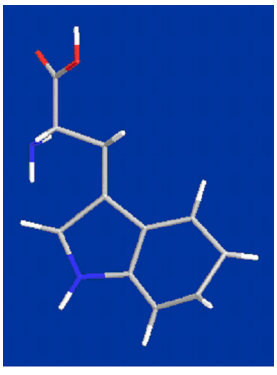


Trp



Trp

Figure 1: *Chemical structure of L-cysteine, L-methionine, alanine (Ala), glycine (Gly), valine (Val), leucine (Leu), aspartic acid (Asp), glutamic acid (Glu), and tryptophan (Trp).*

of the two sulfur-containing amino acids. The side chain is quite hydrophobic and methionine is usually found buried within proteins [1-2]. In contrast to cysteine, the sulfur of methionine is not highly nucleophilic, although it will react with some electrophilic centers. It is generally not a participant in the covalent chemistry that occurs in the active centers of enzymes. The thiol ether can be chemical linkage of the sulfur in methionine. We can compare this terminology with that of the oxygen containing ethers. The sulfur of methionine, as with that of cysteine, is prone to oxidation. Data on the complexation of essential metal ions and the bioactive ligands methionine and cysteine give insight into many physicochemical processes. The significance of these amino acids is enhanced by the fact that they display independent therapeutic activity [1-3]. Results of previous study revealed that both vanadyl sulfate and vanadium (III)-cysteine complex possess significant antitumor properties as it is evident by their effects on malignant cell lines (HeLa) and rats bearing and soft tissue malignant tumors which are considered resistance to chemotherapy and radiation [4]. The electronebulization of a Co^{2+}/Cys mixture in water/methanol(50/50) produced mainly Co-cationized species. Three main groups of this can be distinguished in the ESI-MS spectrum. The resulting product ion structures the high affinity of Co^{2+} for the sulfur atoms of Cys [5]. The amino acid Cys appears to be of particular interest because the sulfur atom is believed to be of major importance in the complexation of toxic metals either by Cys alone or by the phytochelatins (PCs) [6,7]. Based on above mentioned essential role of Cys and Met is interesting to study the interaction between other metal ions with Met and Cys. The interesting question is also, is there any interaction between sulfur group and metal ions. In other words, is it possible to detect the last mentioned interaction in aqueous solution?

Tryptophan is one of the 10 essential amino acids that the body uses to synthesize the proteins it needs (Figure 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 role of amino acids in biological systems it is important to investigate their 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].

Based on above mentioned essential role of amino acids it is interesting to study the interaction between other metal ions with Am.

2. EXPERIMENTAL

2.1. Materials

The amino acids (extra pure) were 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.

2.2. pH titrations and reagents

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).

2.3. 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).

2.4. Procedure

For the determination of acid dissociation constants of the ligand Am, 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): Am, 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.

2.5. Calculation

The acid dissociation constants, $K_{H2(Am)}^{H}$ and $K_{H(Am)}^{H}$ for $H_2(Am)$ were calculated by an algebraic method. The equilibrium involved in the formation of 1:1 complex of Am 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.

3.1. Acidity constants

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

Table 1: *Negative logarithm of the acidity constants of some amino acids at 25 C, 0.1 M, NaNO3*, eq.(1)&(2) .*

No.	Species	pKa	Site
1	H_2(Met)	2.80±0.02	$-CO_2H$
2	H(Met)	9.85±0.01	$-NH_3$
3	$H_3(Cys)^+$	2.0 [a]	$-CO_2H$
4	$H_2(Cys)^±$	8.2 [a]	-SH
5	$H(Cys)^-$	10.3 [a]	$-NH_3$
6	H_2(Trp)	2.22 ± 0.04	$-CO_2H$
7	H(Trp)	9.14 ± 0.02	$-NH_2$
8	H_2(Asp)	3.72±0.03	$-CO_2H$
9	H(Asp)	9.90±0.03	$-NH_3$
10	$H_3(Glu)^+$	2.05 ± 0.13	$-CO_2H$
11	H_2(Glu)	4.37 ± 0.03	$-CO_2H$
12	H(Glu)	9.98 ± 0.02	$-NH_3$
13	H_2(Gly)	2.49±0.08	$-CO_2H$
14	H(Gly)	9.36±0.03	$-NH_2$

(*) The given errors are three times the standard error of the mean value or the sum of the propabable systematic errors. a from [8b].

Table 2: *Logarithm of the stability constants of binary complexes of M^{2+} at 25°C, 0.1 M, $NaNO_3^*$, eq.(5). Cys: cysteine, Met: methionine, Trp: tryptophan, Asp: aspartic acid, Glu: glutamic acid, and related compounds such as Alanine, Leucine, Valine, and Glycine. L : Am.*

No.	Ligand	log $K_{M(L)}^{M}$					
		Ca^{2+}	Mg^{2+}	Mn^{2+}	Co^{2+}	Cu^{2+}	Zn^{2+}
1	Cysteine [a]	-	-	4.1	9.3	19.2	9.8
2	Methionine [b]	-	-	3.59±0.05	3.85±0.08	7.96±0.02	4.46±0.06
3	Alanine [a]	-	-	3.24	4.82	8.18	5.16
4	Leucine [a]	-	-	2.15	4.49	7.00	4.92
5	Valine [a]	-	-	2.84		7.92	5.00
6	Glycine [a]	-	-	3.20	5.23	7.06±0.08	5.16
7	Tryptophan	2.55 ± 0.08	2.84 ± 0.08	3.34 ± 0.05	4.34 ± 0.07	8.05 ± 0.05	5.00 ± 0.08
8	Glutamic acid	1.41 ± 0.02	1.82 ± 0.06	3.19 ± 0.08	4.15 ± 0.09	7.70 ± 0.09	5.84 ± 0.03
9	Aspartic acid	1.26±0.06	2.50±0.06	3.91±0.03	6.69±0.06	8.78±0.02	5.35±0.06

[a] from [8]; [b] from this work, *the given errors are three times the standard error of the mean value or the sum of the probable systematic errors.

$$H_2(Am) \Leftrightarrow H^+ + H(Am)^- \quad (1a)$$

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

$$H(Am)^- \Leftrightarrow H^+ + Am^{2-} \quad (2a)$$

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

$$M^{2+} + H(Am)^- \Leftrightarrow M(H;Am)^+ \quad (3a)$$

$$K^{M}_{M(H;Am)} = [M(H;Am)^+]/[M^{2+}][H(Am)^-] \quad (3b)$$

$$M^{2+} + (Am)^{2-} \Leftrightarrow M(Am) \quad (4a)$$

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

The two proton in H_2(Am) 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]. Am can release the first proton from the terminal acetate group. Hence, here due to addition of equilibrium (1) should be considered, which takes place above a pH≈2.

3.2. 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 Am, then one may write the following two equilibriums of (3) & (4):

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.

3.3. Potentiometric analysis

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 Am^{2-} can accept two protons, to give the acid H_2(Am). The first one of these two protons of carboxylate residue is released; its pKa is low (≈2). However, Am^- 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 the 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(Am) 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 a good agreement with reported value [16,21]. We didn't receive reasonable results for $K_{M(H;Trp)}^{M}$. 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 Am follows the Irving-Williams sequence [23] (Figure 2). Based on the HSAB Principle: Hard acids prefer to coordinate to hard bases, and soft acids prefer to coordinate to 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 amino group and can be cordinated bidentate.

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

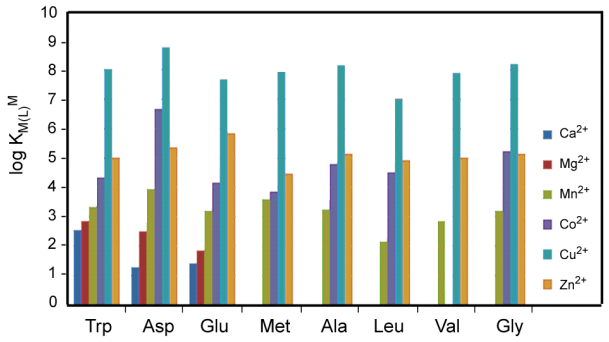


Figure 2: *Irving-Williams sequence-type plot for the 1:1 complexes of Ca^{2+} to Zn^{2+} with Am (see table 2).*

between M^{2+} and $^{-}$NH. With an outer-sphere $^{-}$NH binding we can increase the stability of binary complexes.

As we can use from table 1, $H_3(Cys)^{-}$ is a three protonic species, which can release the first proton from carboxyl group, the second one from -SH group and the third proton from amin group $-NH_3^{+}$. The related acidity constants are listed.

The stability constants of the binary complexes, such as M-Met 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 a good agreement with reported value [8,13]. We didn't receive reasonable results for $K_{M(H;Trp)}^{M}$. All the stability constants of table 2 show the usual trend [15-18].

Now we are able to compare the stability constants of two species M(Am) and M(Cys). Am represents the amino acids such as Leucine, Valine, Alanine, Glycine, Methionine, all are without SH group. It could be easily distinguished that this constants of M(Cys) are generally much larger than those of the corresponding M(Am) species. This increased stability from the difference between the stability constants as defined in eq.(5) [19]:

Ca^{2+} ion plays numerous roles in the 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; which is sold for medical use as Tryptan (Figure 1)[24,25] 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, that using laboratory animals, which 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, that 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, that we can use we can use from its results (Table 2).

Based on the results of this work we can come to this 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 (Table 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 (Figure 3).

Kynureninase which 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].

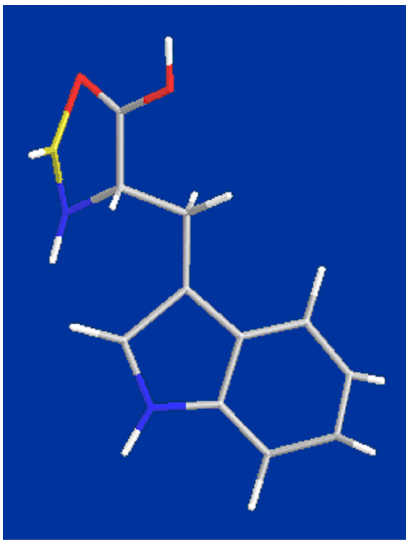


Figure 3: *Schematic structures of the species with interactions according to equilibrium (4) for Cu(Trp). The structure was drawn with the program CS Chem 3D, version 3.5, from Cambridge Software Corporation.*

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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