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Il Farmaco 56 (2001) 737–740

IL FARMACO

The properties of solid Zn(II)–amino acid complexes in the form of suspensions

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Received 28 June 2000; accepted 18 April 2001

Abstract

An investigation was made into the experimental conditions for the formation of poorly soluble complexes of the divalent Zinc(II) combined with the following selected amino acids: tyrosine, tryptophan, cysteine, histidine, and alanine, in the form of suspensions for parenteral administration. The number of Zn(II)-binding sites in the amino acid (n) as well as the amino acid affinity to Zn(II) (K_a), were determined. Cysteine was found to have the highest number of Zn(II)-binding sites — 3, whereas alanine — the lowest — 1. In the conditions described herein, Zn(II)–amino acid complexes of diverse stability (durability) were obtained. The analysis of the kinetics of the binding revealed that the most stable complexes were those formed by Zn(II) in combination with tryptophan ($K_a = 405.78\ \mu M^{-1} \pm 12.17$), and with tyrosine ($K_a = 343.88\ \mu M^{-1} \pm 22.35$); whereas the least stable complexes were those formed by Zn(II) in combination with histidine ($K_a = 29.90\ \mu M^{-1} \pm 4.78$), and with alanine ($K_a = 13.0\ \mu M^{-1} \pm 1.04$). Cysteine formed complexes of intermediate stability ($K_a = 168.53\ \mu M^{-1} \pm 12.36$). The stability of the Zn(II)–amino acid complexes obtained was conditioned by both the molecular weight ($P = 0.033$) of the amino acid and its isoelectric point ($P < 0.001$).

Keywords: Amino acids; Zinc(II); Zn(II)–amino acid complex; Properties; Suspensions

1. Introduction

One of the well-known methods of sustaining the action of a parenterally administered in clinical practice is to form a poorly soluble complex of the drug substance combined with a Group 2 metal, in the form of a suspension. Examples of such a formulation of a medicine are the suspensions of insulin, corticotropin, and hirudin, in combination with Zn(II) [1–3]. These polypeptides combine with Zn(II) to form the solid Zn(II)–hormone complexes. The above method was used to develop such a formulation also with reference to other protein–peptide hormones [4–8]. The mechanism by which suspensions of hormones combined with Zn(II) in the form of poorly soluble complexes are obtained, is not, however, completely clear. For the above reason, the purpose of the present study was to determine the power to form complexes with Zn(II) for selected amino acids, and to establish the influence of the amino acid chemical structure upon the properties of the Zn(II)–amino acid complexes obtained. The most frequently encountered amino acid components of protein–peptide hormones were used as the model substances in the study suspensions. In the case of metal–amino acid complexes, the process of their formation is conditioned by the temperature applied, the concentration of the complex-forming factor, and the acidity of the solution. Various complex forms may dominate a wide range of pH [9].

2. Materials and methods

2.1. Amino acids

Cysteine (Cys) — molecular weight (m.w.) = 121.2, isoelectric point (pI) = 5.07; tyrosine (Tyr) — m.w. = 181.2, pI = 5.66; histidine (His) — m.w. = 155.2, pI = 7.59; tryptophan (Trp) — m.w. = 204.2, pI = 5.88; alanine (Ala) — m.w. = 89.09, pI = 6.02. In the experiments, the levogyrous amino acids were used (Sigma, St. Louis, USA).

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0014-827X/01/$ - see front matter
PII: S0014-827X(01)01129-6

2.2. The formation of the Zn(II)–amino acid complexes in the form of suspensions

Aliquots of the aqueous solutions containing respectively from 4 to 90 μM amino acids were added to the aqueous solution of 100 μM of zinc acetate. Then, 0.1 M sodium hydroxide was added to the solution obtained until it reached pH 7.1–7.5. The solutions were filled up to 10 cm^3 with distilled water, and stirred with a magnetic stirrer for 20 min. The suspensions remained for 1 h at room temperature, then they were centrifuged for 20 min at 5000 rpm. Next, the sediment was separated from the supernatant. The sediment was discarded, whereas the supernatant was used for further analysis.

2.3. The method of determining the amount of the bound Zn(II) with amino acid

According to the method of gel filtration on Sephadex G-25, in a 0.1 M solution of CH_3COOH, the free Zn(II) was isolated. Zinc acetate was eluted in the fraction for which R_f 0.29. After the isolation of the free Zn(II), its amount was determined by spectrophotometry. The absorbance of the supernatant from the samples prior to and following the complex formulation, was measured. The absorbance of the solution containing the free Zn(II) was measured at a wavelength of $\lambda = 538$ nm, following the formulation of the coloured complex with ditizone [10]. The absorbance values obtained ranged from 0.2–0.6. The amount of the free Zn(II) was established upon the analysis of the calibration curve. The absorbance measurements were conducted in 1-cm thick cuvettes, using the UV–Vis 'CE 3021' spectrophotometer (Cecil, UK).

2.4. The determination of the parameters describing the binding power of Zn(II) in relation to the selected amino acids

The analysis of the power of the ligand [Zn(II)] to bind with the amino acid was carried out according to Scatchard's method [11,12]. Graphs were drawn to represent the equation: $[B]/([P_c] \times [F]) = -K_a([B])/[P_c\}) + nK_a$. The x-axis represented the $[B]/[P_c]$ values, whereas the y-axis represented $[B]/([P_c] \times [F])$, where B is the number of micromoles of the bound Zn(II), F is the number of micromoles of the free Zn(II) and P_c is the total amino acid molar concentration. Upon the analysis of Scatchard's curves, the following parameters were determined: the values representing the stability (association) constants for the complexes (K_a), and the number of Zn(II)-binding sites in the amino acid (n). Ligand affinity level for amino acid was described by (K_a) — a value of the internal binding constant, of which the inverse is (K_d) — a dissociation constant. The value representing the stability constant (K_a) was found at the point of the intersection of the straight line with the y-axis, while the value representing the number of the Zn(II)-binding sites (n) was found at the intersection of the straight line with the x-axis.

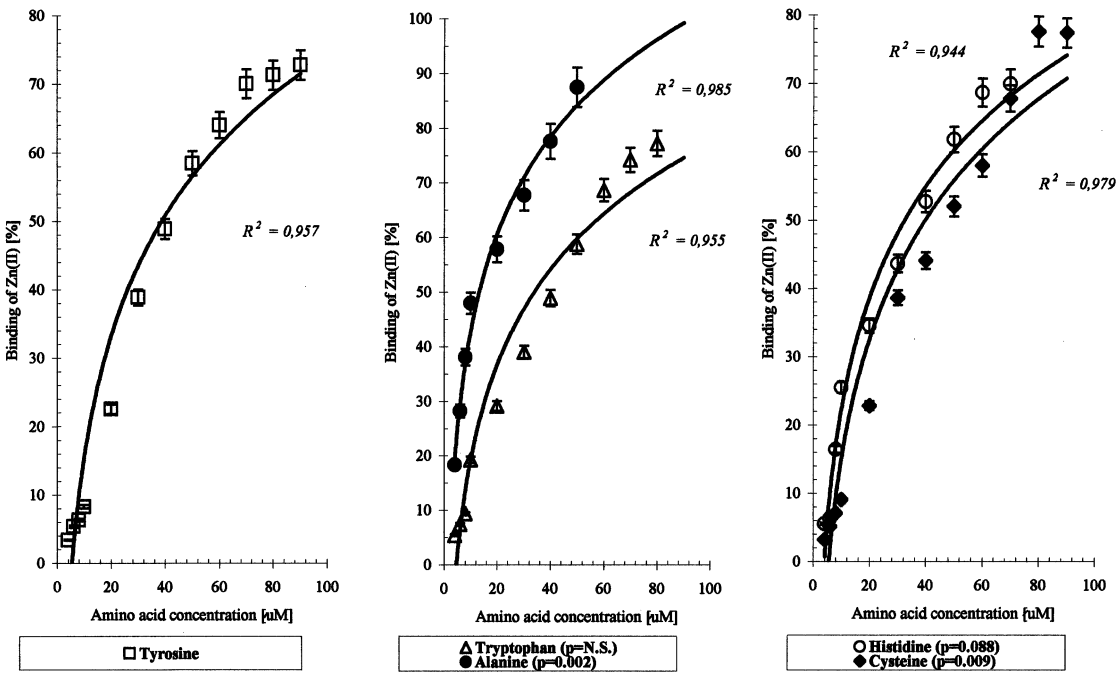

Fig. 1. The binding of Zn(II) with the amino acid as a function of its concentration.

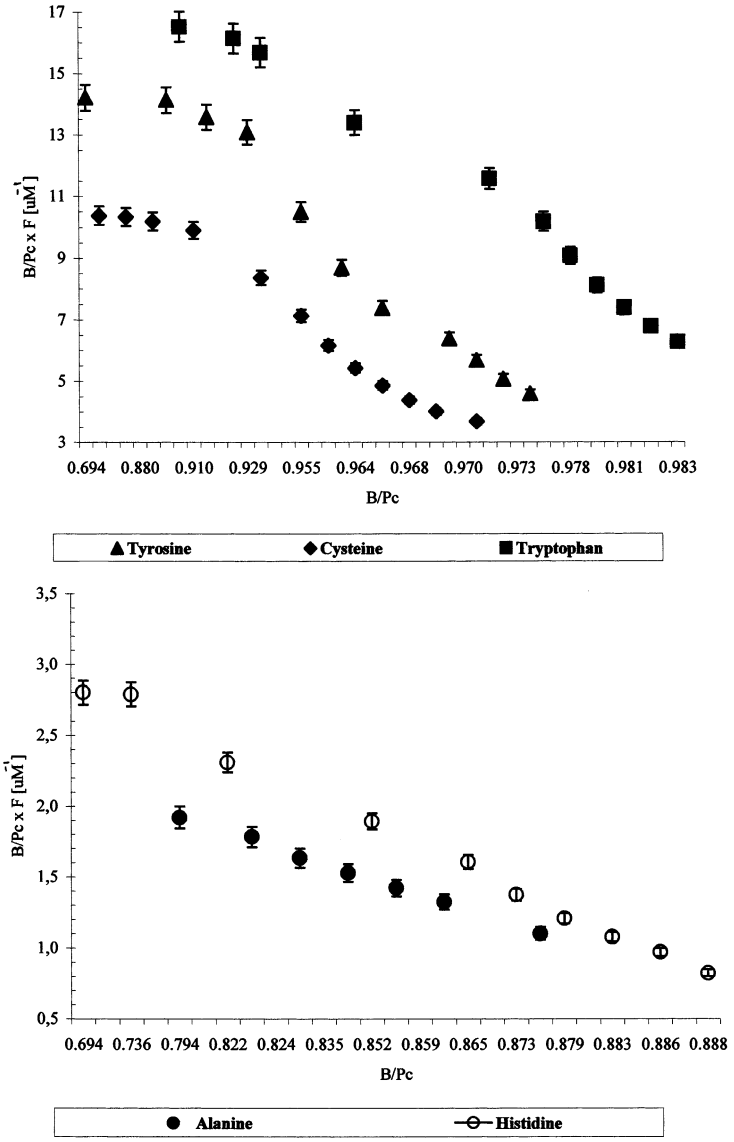

Fig. 2. Scatchard's curves representing the power of Zn(II) to bind with amino acids.

Table 1
The parameters describing the binding in Zn(II)–amino acid complexes

Amino acids	Number of binding sites (n)		Stability constant (K_a) (μM^{-1})		Dissociation constant (K_d) (μM^{-1})	
	x	SD	x	SD	$\bar{x}$	SD
Cysteine	3	±0.22	168.53	±12.36	0.0059	±0.0004
Tyrosine	2	±0.13	343.88	±22.35	0.0029	±0.0002
Tryptophan	2	±0.06	405.78	±12.17	0.0025	±0.0001
Histidine	2	±0.32	29.90	±4.78	0.0334	±0.0046
Alanine	1	±0.08	13.00	±1.04	0.0769	±0.0057

2.5. Mathematical calculations

The results were represented as the mean of three experiments. The relative standard deviation (SD) and correlation coefficient r were also calculated. The influence of the amino acid chemical structure — molecular weight (m.w.) and isoelectric point (pI) — upon the stability of the Zn(II)–amino acid complex obtained, was determined according to the method of variance analysis. The threshold of significance was $P < 0.1$.

3. Results and discussion

In neutral medium, amino acids form complexes with divalent metals. In the study described herein, the dominant complex form is the neutral chelate [9]. In the conditions described above, the selected amino acids combine with Zn(II) to form poorly soluble, colourless, solid complexes. Tyrosine and cysteine combine with Zn(II) to form solid complexes within the amino acid concentration range from 4 to 90 μM; for tryptophan the concentration range varies from 6 to 90 μM; for histidine, from 8 to 90 μM; and for alanine, from 20 to 80 μM. The percentage of the Zn(II) bound with amino acid as a function of the amino acid molar concentration, is shown in Fig. 1.

The percentage of the Zn(II) bound with amino acid increases in a direct proportion to increase in the amino acid concentration. The amounts of the Zn(II) bound with amino acid are of significance ($P < 0.1$). The significance level with regard to the power of the selected amino acids to combine with Zn(II), was determined in relation to tyrosine — the most common active centre of hormones activity. Scatchard's curves, drawn based on the above data, which determine the amino acid binding power with regard to Zn(II), are shown in Fig. 2.

The application of Scatchard's method to the analysis of the data obtained enabled us to demonstrate that alanine binds with Zn(II) by means of the class 1 binding sites. The curve obtained is a straight line. Alanine is an example of an amino acid with a non-coordinating lateral chain [9]. It is the weakest chelator among the selected amino acids. For the remaining amino acids, the shapes of the curves indicate the presence of the two classes of Zn(II)-binding sites: n_1, n_2, which considerably differ from each other in their affinity to the ligand ($K_{a1} > K_{a2}$). The number of the binding sites determined (n) is an indicator of the amino acid power to form the Zn(II)–amino acid complex in the conditions specified. The selected amino acids have two Zn(II)-binding sites, except alanine, which has one site, and cysteine, which has three sites. However, there is a greater number of Zn(II)-binding sites which participate in the formulation of the cysteine–Zn(II) complexes. In all the Zn(II)–amino acid complexes obtained, there is one site dominating, i.e. the one which forms strong links with Zn(II). This is probably connected with the presence of the coordinating group ($-NH_2$) in the amino acid. The coordinating group ($-NH_2$) has higher affinity to the metal than the acid group (COO^-) [9]. Histidine is an amino acid which has three potential coordinating sites. Its lateral chain contains a flat imidazole ring. It is not always possible, however, to take advantage of all the three binding sites [9,13]. The properties of the Zn(II)–amino acid Zn(II) complexes are compared in Table 1.

The analysis of the kinetics of the Zn(II)–amino acid binding revealed that the most stable complexes with Zn(II) are those formed by tryptophan ($K_a = 405.78\ \mu M^{-1} \pm 12.17$) and tyrosine ($K_a = 343.88\ \mu M^{-1} \pm 22.35$). The relatively least stable complexes are those formed by alanine ($K_a = 13.0\ \mu M^{-1} \pm 1.04$) and histidine ($K_a = 29.90\ \mu M^{-1} \pm 4.78$). Cysteine forms complexes of intermediate stability ($K_a = 168.53\ \mu M^{-1} \pm 12.36$). The Zn(II)–tryptophan complexes are approximately 33 times more stable than the ones formed by alanine. Variance analysis demonstrated that the amino acid molecular weight ($P = 0.033$) as well as the amino acid isoelectric point ($P < 0.001$) have considerable influence upon the stability of the complex obtained.

The higher the amino acid molecular weight ($r = 0.823$), the more stable the Zn(II)–amino acid complex provided. The results obtained indicate that the most stable complexes with Zn(II) are those formed by tryptophan. The knowledge of such factors as the number of Zn(II)-binding sites in the amino acid, the amino acid affinity to Zn(II), and the stability of the Zn(II)–amino acid complex obtained, may be of significance for the development of a formulation for sustained-action suspensions which would have specified, programmed, determined in advance, parameters, such as: maintenance dose, half-time, and the dynamics of the release. In the present study, both the amino acid power to combine with Zn(II) and the influence of the amino acid chemical structure upon the properties of the Zn(II)–amino acid complexes obtained in the solid form, were determined.

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