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Research paper

The effect of carboxylate groups on the complexation of metal ion with oligopeptides – Potentiometric investigation

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This paper was dedicated to Prof. Imre Sóvágó on the occasion of his 70th birthday.

ABSTRACT

Iron(II), iron(III), cobalt(II), zinc(II) cadmium(II) and lead(II) complexes of Asp_2 and Asp_3 and other Asp/Glu containing ligands were studied by potentiometry. Our goal was to study the influence of β- and γ-carboxylate groups as well as the increased negative charge of the ligands on the complex formation processes. Mainly 1:1 species are present, the formation of bis(ligand) complexes is not typical for these systems. It can be concluded that the effect of side chain carboxylate groups on the stability of complexes is especially significant in the case of lead(II) and cadmium(II). The greater the number of carboxylic groups there are in the system, the more stable complexes are formed with the studied metal ions. The influence of β-carboxylate groups of aspartic acid is unambiguous, they are directly bounded to the metal ion, which results in increased stability of the complexes. On the other hand, the repulsion between the negative charged residues becomes conspicuous resulting less stable species in the case of peptides containing glutamic acid.

1. Introduction

Imidazole nitrogen of histidyl residues and thiol sulfur atoms of cysteinyl residues are quite generally known metal-binding sites in proteins. On the other hand, the carboxylate group of aspartic and glutamic acid attracted less attention on this field. However, several studies proved that these groups also play important role in metal binding; are especially preferred binding sites of metalloenzymes for several types of metal ions. For example Human Serum Albumin binds Cu(II) as well as Ni(II) ions in a square–planar geometry with *N*-terminal amino nitrogen, two deprotonated amide nitrogens and the His imidazole donor in addition to the weak axial coordination of the carboxylate group of aspartic acid [1–3]. Co(II) ions have similar binding mode and the same role of the carboxylate group is supported [4].

In Carboxypeptidase (A and B) and Thermolysin Zn(II) bounds to the proteins via two histidine imidazoles and the carboxylate group of one glutamyl residue [5], while in Alkaline Phosphatase the aspartate carboxylate occupies the third site.

A peptide motif Glu-Xaa-Xaa-Glu has been proposed as a ferric iron-binding site in several proteins involved in different aspects of iron metabolism [6–8]. The iron-binding site of transferrin includes one aspartic acid (Asp61) besides one histidine (His254) and two tyrosine (Tyr93 and Tyr193) side chains [9].

The polymorphic states of Cu(II)-bonded Aβ(1−16) peptides were characterized by computer simulation, too. Glu3, His6, His13 and His14 residues were suggested as the major copper binding sides [10]. On the other hand, the *N*-terminal group of Asp1 has been proposed as one possible coordination site for Zn^{2+} in native Aβ [11–13]. Continuous-wave electron paramagnetic resonance (CW-EPR) spectra and hyperfine sublevel correlation (HYSCORE) have identified that at low pH (pH 6.3–6.9) the Cu^{2+} binding sites include two His (His6 and His13/His14) and Asp1 [14].

In binary complexes with Pb(II), in addition to the (NH_2, CO) chelate, only the weak coordination of the imidazole moiety of His and the carboxylate moiety of Asp were suggested in a solution equilibrium work. Moreover, these ligands, did not show good selectivity for binding Pb(II) ion against Zn(II) [15].

The effect of the γ-carboxylate of glutamyl residue was investigated via the Cu(II) and Zn(II) complexes of peptides containing the HEXXH and HXXEH motifs. The direct role of the glutamate carboxylate group was found. Interestingly it was shown that the non-covalent interactions also have a significant contribution to the overall stability of the Cu(II) complexes, while an unusual Zn(II) promoted amide deprotonation and coordination was observed in the corresponding Zn(II) containing systems [16].

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Copper(II), nickel(II) and palladium(II) complexes of di- tri- and tetrapeptides containing Asp and/or Glu residues were studied earlier in our research group. These results proved that there is significant interaction with these donor functions; they have effect on the stability constant of the complexes, occasionally modifying even the stoichiometry of the formed species compared to the common peptides [17–19].

These measurements are now completed by the studies of complexation of various oligopeptides containing this moiety with additional metal ions, namely the biogenic iron(II), iron(III), cobalt(II), zinc(II) and the toxic cadmium(II) and lead(II) ions. Normally cadmium(II) and lead(II) ions are not present in the biological systems, have no biological functions in humans, but theirs toxicity is highly based on the fact that these ions are able to replace the essential metal ions.

The complexation of all the above mentioned metal ions were studied with the peptides Asp_2, Asp_3 and with other Asp/Glu containing selected ligands. The structure of the studied peptides are presented on Scheme 1.

For comparison we also summarize the previous data reported for the copper(II) and nickel(II) complexes. Our goal was to study the influence of β- and γ-carboxylate groups as well as the increased negative charge of the ligands on the complex formation processes. These results may provide additional information about the interaction of metal ions with proteins.

2. Experimental

2.1. Materials

The peptides AspAsp (abbreviated as Asp_2), AspAspAsp (abbreviated as Asp_3), AspAspAspAsp (abbreviated as Asp_4), AspAla, AspGlu, GluGlu (abbreviated as Glu_2), GlyAsp, and GluGluGlu (abbreviated as Glu_3) were purchased from Bachem AG (Switzerland), while GlyAspGly was from GenScript Co. (USA). Concentration of the peptide stock solutions was determined by pH-potentiometric titrations.

Stock solutions of metal ions were prepared from analytical grade reagents ($ZnCl_2$, $CoCl_2$, $Fe(NO_3)_3$, $Cd(NO_3)_2$ and $Pb(NO_3)_2$ (Reanal)) and their concentrations were checked gravimetrically via the precipitation of oxinates or using pH-potentiometric titrations with EDTA. In the case of iron(III) stock solution the concentration was checked permanganometrically. Stock solution of $FeCl_2$ was prepared by dissolving solid iron (Reanal) in hydrochloric acid. During the dissolution, argon was bubbled through the solution and it was stored under argon. Concentration of iron(II) has also been checked permanganometrically, moreover, the contamination of iron(III) was checked via the complex formation between iron(III) and thiocyanate monitored at absorbance of 480 nm. The contamination was less than 0.6%.

2.2. pH-potentiometric measurements

pH-potentiometric titrations were performed in different volume of samples depending on the metal ions. 3 and 4 cm^3 of samples were used in the case of zinc(II), cobalt(II) and cadmium(II) while 10 and 15 cm^3 of samples were used in the presence of iron(II/III) and lead(II) at 2–5 mM ligand concentration. The metal to ligand ratio was selected between 1:1 and 1:5. In the case of zinc (II), cobalt(II) and cadmium(II), the titrations were performed with a MOLSPIN pH meter equipped with 6.0234.110 combined glass electrode (Metrohm) and a titrant was dosed by means of MOLACS microburette controlled by computer. In the case of iron(II/III) and lead(II) the titrations were carried out with Mettler Toledo T50 automatic titrator equipped with a Mettler Toledo DG 101-SC combined electrode for iron(II/III) and Metrohm 6.0255.100 for lead(II). The latter electrode is a so-called double-junction electrode that contains replaceable bridge electrolyte (in our case KNO_3) for protecting the lead(II) from the interference of chloride ions. The titration was controlled by computer with LabX titration software (V. 3.1.1.0).

The titrations were performed with carbonate free stock solution of potassium hydroxide of known concentration. During the titration, argon was bubbled through the samples to ensure the absence of oxygen and carbon dioxide and for the stirring of the solutions. In the case of iron(II) samples, the dosage of iron(II) solution and the titrations were performed under argon atmosphere with argon being purified by chromium(II) chloride in zinc/hydrochloric acid system. All pH-potentiometric titrations were carried out at a constant ionic strength of 0.2 M KNO_3 (in the case of iron(III), cadmium(II) and lead(II)) and 0.2 M KCl (in the case of other metal ions) and at constant temperature 25.0 °C ± 0.1 °C.

The recorded pH values were converted into hydrogen ion concentration as described earlier [20].

Protonation constants of the ligands and the overall stability constants ($\log\beta_{pqr}$) of the complexes formed in the investigated systems were calculated by means of general computational programs, SUPERQUAD [21] and PSEQUAD [22] using Eqs. (1) and (2). The standard deviations of the protonation and stability constants are in parenthesis.

$$pM + qH + rL = M_pH_qL_r1q \quad (1)$$

$$\beta_{pqr} = \frac{[M_pH_qL_r]}{[M]^p \cdot [H]^q \cdot [L]^r} \quad (2)$$

It is important to note that if the hydrolytic processes compete with the complex formation, the hydrolysis of the metal ions has to be taken into account. This is the case for iron(III) and lead(II) ions. The hydrolysis models of these metal ions were determined in previous works. The following stability constants ($\log\beta$) were taken into account; $[Fe(OH)]^{2+}$ ($\log\beta = -2.77$), $[Fe(OH)_2]^{+}$ ($\log\beta = -6.61$), $[Fe_2(OH)_4]^{2+}$ ($\log\beta = -3.22$), $[Fe_3(OH)_4]^{5+}$ ($\log\beta = -6.98$) [23] and $[Pb(OH)]^{+}$ ($\log\beta = -7.32$), $[Pb_4(OH)_4]^{4+}$ ($\log\beta = -19.98$) and $[Pb_6(OH)_8]^{4+}$ ($\log\beta = -42.62$) [24].

3. Results and discussion

3.1. Protonation studies of the ligands

All the studied peptides contain one amino and two to five carboxylate donor functions. The structural formulae of the studied ligands are depicted on Scheme 1. The charge of the fully deprotonated form of the ligands depends on the number of Asp/Glu residues; 2– for AspAla, GlyAsp and GlyAspGly, 3– for Asp_2, AspGlu and Glu_2, 4– for Asp_3 and 5– for Asp_4. Due to this fact the complexes with the same stoichiometry also have different charges; therefore the charges of the formed species are not shown.

The pK values of the ligands were determined and published earlier at a constant ionic strength of 0.2 M KCl [17], except GlyAspGly, and all the data are collected in Table 1. Most of the pK values are recently determined at a constant ionic strength of 0.2 M KNO_3, and the values are included in Table 2.

Although, these pK values are macroscopic dissociation constants, but based on chemical evidences, the highest pK values belong to the protonation equilibria of the terminal amino groups, while the lowest and intermediate values correspond to the deprotonation of the terminal and side chain carboxylate donor functions, respectively.

It can be seen by the comparison of the data of the two Tables, that there is no significant difference in the pK values depending