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Zinc Ion Coordination Equilibria of Thymopoietin Fragments and of an Angiotensin Antagonist Octapeptide

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The pentapeptide Arg–Lys–Asp–Val–Tyr (TP5) is a biologically active fragment of thymopoietin [1]. Only the formation of its lanthanide [2] and copper(II) complexes [3] have been studied so far. The arginine guanidino and aspartate β-carboxylate groups were shown to take part in the coordination of lanthanide ions, while the coordination of copper(II) occurs through the terminal amino and two-backbone N-donor atoms. The tetrapeptide (TP4), Ary–Lys–Asp–Val exerts similar biological properties as TP5 [4]. The comparison of the complex formation properties of the two peptides may give further information on the binding sites of zinc.

The octapeptide Sar–Arg–Val–Tyr–Ile–Pro–Lac (A8) is an angiotensin antagonist [5] which differs from angiotensin only in the two terminal moieties. Its metal ion coordination equilibria have not been investigated so far.

All the three peptides were synthetic products of high purity containing no free amino acids, smaller peptides or other complexing contaminations. Therefore, they proved to be suitable for exact coordination chemical studies.

Zinc ion coordination may hinder the enzymatic degradation of peptides, leading to the preparation of drugs with retarded pharmaceutical effect (e.g. corticotropin, insulin, etc.). The study of the zinc ion coordination of peptides is therefore important in pharmaceutical research as well as in coordination chemistry [6].

Because of the small amount of peptides available in such high purity, the studies have to be performed on micro scale. Therefore, polarography was chosen instead of classical potentiometric methods for these investigations. The polarographic evaluation method used in the study of mononuclear systems [7] had to be modified, however, to make it suitable for the investigation of polynuclear complexes. This letter presents the results of our zinc ion coordination studies performed with the three peptides.

Experimental

The peptides were provided by Gedeon Richter Chemical Works, Budapest. All chemicals used were of analytical purity. The solutions were prepared using twice-distilled water. During measurement, the pH of the solutions was kept constant at pH = 5.5 for TP4 and TP5 and pH = 6.50 for A8. All the measurements were performed at 298 K in aqueous solutions of potassium nitrate at a constant ionic strength of 0.2. Purified nitrogen was bubbled through the solutions before the measurements to remove oxygen.

The polarograms were recorded with a Radiometer PO4 Polarograph. Characteristic data of the capillary ($m = 2.22$ mg s^{-1}, $t = 3.06$ s; $m^{2/3}t^{1/6} = 2.05$ mg$^{2/3}$ s$^{1/2}$) were determined in 0.2 mol dm^{-3} potassium nitrate solution with an open circuit. The half-wave potentials ($E_{1/2}$) were determined by the logarithmic analysis of the polarographic waves and were reproducible for ±1.5 mV.

For the determination of the number of zinc ions bound by one ligand and of the corresponding stability constants from the ligand concentration dependence of the $E_{1/2}$ values, the DeFord–Hume equation [7] was modified in the following way.

The basic equation for the potential of a dropping mercury electrode is

$$E_{de} = E_a{}^0 - \frac{RT}{nF} \ln C_a{}^0/[\mathrm{M}]_0 \tag{1}$$

where $E_a{}^0$ is the standard potential of the amalgam, $C_a{}^0$ the concentration of the amalgam formed at the surface of the mercury drop and $[\mathrm{M}]_0$ the concentration of the reducible metal ion in the solution layer at the surface of the mercury drop.

The total metal ion concentration (C_M) in the presence of a ligand when both mono-and polynuclear complexes are formed is

$$C_\mathrm{M} = \sum_{j=0} \beta_{1j}[\mathrm{M}][\mathrm{X}]^j + \sum_{i=2} i\beta_{i1}[\mathrm{M}]^i[\mathrm{X}] \tag{2}$$

where β-s denote the corresponding complex products and $\beta_{10} = 1$ by definition, [M] and [X] are the free metal ion and ligand concentrations, respectively, and i is the number of metal ions bound by one ligand.

The total current at any part of the polarographic wave

$$\bar{i} = kI_\mathrm{compl}\left\{\sum_{j=0}\beta_{1j}([\mathrm{M}][\mathrm{X}]^j - [\mathrm{M}]_0[\mathrm{X}]_0{}^j) + \sum_{i=2} i\beta_{i1}([\mathrm{M}]^i[\mathrm{X}] - [\mathrm{M}]_0{}^i[\mathrm{X}]_0)\right\} \tag{3}$$

where $k = m^{2/3}t^{1/6}$ (capillary constant), $I_\mathrm{compl} = 607$ n $D^{-1/2}$ (D being the measurable average diffusion constant), and $[\mathrm{M}]_0$ and $[\mathrm{X}]_0$ denote the concentrations of the metal ion and ligand at the surface of the mercury drop, respectively. When the current reaches its limiting value (i_d), the concentrations at the electrode surface become zero, thus

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$$i_d - \bar{i} = kI_{\mathrm{compl}}\left\{\sum_{j=0} \beta_{1j}[X]_0^{j}[M]_0 + \sum_{i=2} i\beta_{i1}[X]_0[M]_0^{i}\right\} \qquad (4)$$

By expressing the metal ion concentration $[M]_0$ we obtain

$$[M]_0 = \frac{i_d - \bar{i}}{kI_{\mathrm{compl}}\left\{\sum_{j=0} \beta_{1j}[X]_0^{j} + \sum_{i=2} i\beta_{i1}[M]_0^{i-1}[X]_0\right\}} \qquad (5)$$

On the other hand

$$\bar{i} = kI_a C_a^0 \qquad (6)$$

where I_a is proportional to the diffusion constant of the metal ions in the amalgam, ($I_a = 607\ n\ D_a^{1/2}$). From eqn. (6) C_a^0 can be expressed, and substituting C_a^0 and $[M]_0$ into eqn. (1) we obtain

$$E_{de} = E_a^0 - \frac{RT}{nF}\ln\frac{I_{\mathrm{compl}}}{I_a}\frac{\bar{i}}{i_d - \bar{i}}\left\{\sum_{j=0}\beta_{1j}[X]_0^{j} + \sum_{i=2} i\beta_{i1}[M]_0^{i-1}[X]_0\right\} \qquad (7)$$

The half-wave potential at $i = i_d/2$

$$(E_{1/2})_{\mathrm{compl}} = E_a^0 - \frac{RT}{nF}\ln\frac{I_{\mathrm{compl}}}{I_a}\left\{\sum_{j=0}\beta_{1j}[X]_0^{j} + \sum_{i=2} i\beta_{i1}[M]_0^{i-1}[X]_0\right\} \qquad (8)$$

and the shift in the half-wave potential due to complex formation

$$\Delta E_{1/2} = (E_{1/2})_{\mathrm{simple}} - (E_{1/2})_{\mathrm{compl}} = \frac{2303RT}{nF}\log\frac{I_{\mathrm{compl}}}{I_{\mathrm{simple}}}\left\{\sum_{j=0}\beta_{1j}[X]_0^{j} + \sum_{i=2} i\beta_{i1}[M]_0^{i-1}[X]_0\right\} \qquad (9)$$

where $I_{\mathrm{simple}} = 607\ n\ D^{-1/2}$ for the simple ion and

$$\frac{nF}{2303RT}\Delta E_{1/2} + \log\frac{I_{\mathrm{simple}}}{I_{\mathrm{compl}}} = \log\left\{\sum_{j=0}\beta_{1j}[X]_0^{j} + \sum_{i=2} i\beta_{i1}[M]_0^{i-1}[X]_0\right\} = \log\frac{(C_M)_0}{[M]_0} \qquad (10)$$

In the calculations both ligand and metal ion concentrations on the electrode surface were taken into consideration according to Ringbom and Eriksson [8]. The free ligand concentrations were computed by iteration.

The computer program used to evaluate the experimental data based on the above equations simulated the experimental $\Delta E_{1/2}$-ligand concentration curves, with the help of the calculated equilibrium constants to control their reliability.

Results

1. Zinc Coordination of TP5 and TP4

The reduction waves of zinc ions in the presence of either ligand TP5 or TP4 showed reversible character, and their limiting currents were diffusion-controlled in the ligand concentration range 10^{-4}–2×10^{-2} mol dm^{-3}. With increasing ligand concentration the half-wave potentials ($E_{1/2}$) shifted in negative direction indicating the coordination of zinc ions (Fig. 1).

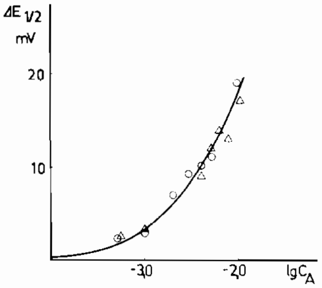

Fig. 1. Measured and calculated $\Delta E_{1/2}$ values plotted as a function of lg[C_a] for the zinc complexes of TP5 and TP4. Points are experimental data, full line represents the calculated values.

The computer evaluation of the data for both systems has shown the formation of only one complex with 1:1 zinc:ligand composition in the studied concentration range. The stability constants for complexes TP5 and TP4 were found to be equal, within experimental error, $\log K_1 = 2.46 \pm 0.05$, indicating that the part of the peptide containing the N-terminal four amino acids is responsible for the complex formation in TP5, and that the structures of the zinc complexes of TP5 and TP4 are analogous. Figure 1 shows the good agreement of experimental and computer-simulated $\Delta E_{1/2}$ values plotted as a function of the ligand concentrations.

2. Zinc Coordination of A8 Octapeptide

Polarographic zinc waves, recorded in solutions of constant 10^{-4} mol dm^{-3} zinc ion concentration and of ligand concentrations varying from 10^{-5} to 10^{-3} mol dm^{-3}, were found to be reversible and diffusion-controlled. The half-wave potentials showed significant shifts at relatively low ligand concentrations, as seen in Fig. 2. Surprisingly, in a solution with a total metal:ligand concentration ratio of 1:1, the $E_{1/2}$ shift reached 30 mV.

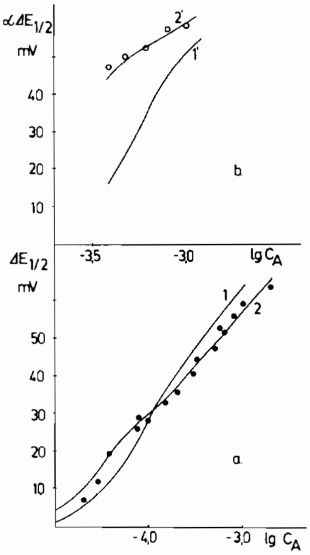

Fig. 2. Measured and calculated $\Delta E_{1/2}$ values plotted as a function of $\log[C_a]$ for the zinc ion coordination of A8. Points are the experimental values. Curve 1 and 1': calculated values assuming the formation of a single 1:1 complex. Curve 2 and 2': calculated values by assuming the presence of two complexes with metal–ligand ratios of 1:1 and 2:1. Zinc concentrations in system: (a) 10^{-4} mol dm^{-3}, (b) 10^{-3} mol dm^{-3}.

The latter system could not be characterized using a model base on the formation of only mononuclear species (see curve 1 in Fig. 2). It was necessary to use the modification of the DeFord–Hume equation discussed in the experimental part. Curve 2 in Fig. 2 was calculated using this modified equation, assuming the presence of complexes of 1:1 and 2:1 metal: ligand ratio. It can be seen that the latter model reflects well the behaviour of the system.

In contrast to the original DeFord–Hume equation, our equation contains the concentration of the free ligands as well as that of the free metal ions; this reflects the experimental fact that in polynuclear systems the complex formation function is metal-ion dependent. Figure 2 shows the metal concentration dependence of the $\Delta E_{1/2}$-ligand concentration curves in our system. The zinc waves recorded for solutions of higher zinc concentrations (e.g. in Fig. 2 at 10^{-3} mol dm^{-3}) proved to have an irreversible character with a transfer coefficient (α) of 0.47. For such a system, $\alpha\Delta E_{1/2}$ values are used in the calculations instead of the $\Delta E_{1/2}$ values, according to eqn. (10). The good agreement between the measured and calculated values for data measured in solutions of different metal concentrations indicates the validity of the model which led to the following stability constants:

$\log \beta_1 = 4.9 \pm 0.1$

$\log \beta_2 = 9.9 \pm 0.15$

The unusual high $\beta_2:\beta_1$ ratio shows a stabilization effect caused by the coordination of the second zinc ion.

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