been studying recently metal ion interaction with two hypothalamic hormones: thyroliberin (TRF) and melanostatin (MIF). These two hormones are the only tripeptides among all the pituitary hormones described until now [1–4]. TRF (Pyr–His–Pro–NH_2) and MIF (Pro–Leu–Gly–NH_2) have been extensively studied in various biological aspects [5], among others their possible use in the clinical treatment of mental diseases [6]. Many hormone analogues have been synthesized in order to solve the problem of the relation between biological activity and chemical structure [7]. In most papers the critical role of the histidine residue in TRF biological activity is suggested.

Our main interest concerns the coordination abilities of both hormones and some of their analogues and the relation between the binding modes and the ligand conformation (structure). Spectroscopic (NMR, EPR, absorption and CD spectra) and potentiometric techniques were used to solve these problems.

Though the MIF binding mode to Cu(II) and Ni(II) ions looks classical, the strongly basic Pro nitrogen donor on the N-terminal leads to the formation of very low concentration of 110 (1N) complex species [8].

The histidine residue of TRF ligand is a major binding site of metal ions. The other two residues however, have a critical importance for the complex equilibria and the structure of the formed species [9, 10].

The replacement of pyroglutamic acid with picolinic acid in the TRF molecule causes a major change in the structure of its Cu(II) and Ni(II) complexes. *E.g.*, in the Cu(II)Pic–His system a very specific equilibrium between dimeric and monomeric species was observed [11].

The introduction of tyrosine residue in the TRF sequence in place of histidine also changes the peptide coordination ability. In the Ni(II)Pyr–Tyr–Pro–NH_2 system a direct involvement of Pro–NH_2 residue in the metal binding was proposed [11].

TRF analogue with nicotinic acid in place of pyroglutamic acid forms very unstable Cu(II) complexes due to the unfavourable meta-position of the nitrogen donor with respect to carbonyl in the pyridine ring. Also the TRF analogue with Pro–NH–NH_2 residue in the C-terminal position forms much weaker Cu(II) and Ni(II) complexes than the TRF peptide.

The studies which were done for both hormones show that naturally occurring peptides may form relatively strong complexes with some metal ions and their chemical and physical properties may be very specific for each ligand. The latter conclusion becomes evident when the coordination abilities of the different chemical analogues (*e.g.* of TRF) are compared.

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U18

Iron Coodination Compounds with Glycine, Glycylglycine and Diglycylglycine

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The investigation of biometals complex-forming processes with aminoacids and peptides is of interest as the model of metal–protein interactions.

In this work, the composition of coordination compounds at T = 308 K and pH interval from 0 to 11 units was established on the basis of the obtained partial dependences of oxidizing potential value (ϕ) on iron(III), iron(II), glycine (Gly), glycylglycine $(Gly)_2$, and diglycylglycine $(Gly)_3$ concentrations.

The fall of the oxidizing potential value with pH of the solution (Fig. 1) in pH sphere $< pK_1$ is accounted for by the stepped iron complex-forming processes in aminoacid and peptide aqueous solutions.

From the oxidizing potential dependence on (C_L)-glycine and glycylglycine and diglycylglycine ligand concentrations obtained at different pH values, it has been found that in pH sphere = 2.0 + 5.0 iron(III) complex compounds are formed where only one acidoligand is formed for the complex-forming ion and two acidoligands are formed as the aminoacid and peptide concentrations increase. These results confirm the competition for complex-formation between Fe(III) and Fe(II) in this pH sphere. The idea that protolytic processes proceed in aminoacid

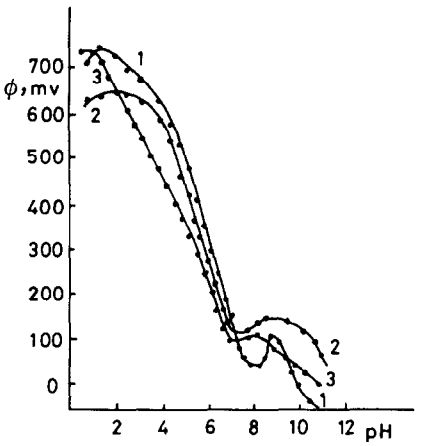

Fig. 1. Oxidizing potential dependence on pH. $T = 308$ K; $C_L = 0.1$ *M*; $C_{Fe^{2+}} = C_{Fe^{3+}} = 1 \times 10^{-5}$.

Curve	L	pK_1	pK_2
1	Gly	2.35	9.7
2	$(Gly)_2$	3.06	8.13
3	$(Gly)_3$	3.26	7.91

and peptide aqueous solutions made it possible to establish the coordination form of the ligands in the complexes.

Graphical analysis of the experimental oxidizing potential dependences on the concentration of redox iron forms obtained at different fixed pH values made it possible to establish the formation of polynuclear acidohydroxyl iron(III) complex compounds and iron mononuclear complexes in neutral and alkaline solutions. The formation of heterovalent compounds may be suggested as well.

The catalytic activity of iron coordination compounds with glycine, glycylglycine and diglycylglycine in the process of L-cysteine (Cys) liquid-phase oxidation by molecular oxygen in aqueous solutions at pH = 7.0 (Fig. 2) has been determined.

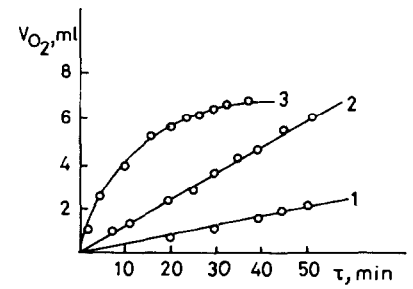

Fig. 2. Oxygen absorbance by L-cysteine aqueous solution (10 ml) in the presence of iron complex compounds. $T = 308$ K; pH = 7.0; $C_{Cys} = 0.2$, $C_{Fe^{2+}} = 1 \cdot 10^{-5}$, $C_{Cys} = 0.1$ *M*; curve 1, L = Gly; curve 2, L = $(Gly)_2$; curve 3, L = $(Gly)_3$.

The increase in the iron coordination compounds' catalytic activity by the ligand's nature change in the series: aminoacid < dipeptide < tripeptide, may be accounted for by the increase in the strength of mixed iron(III) polynuclear complexes and mononuclear iron(II) complexes in the above-cited sequence.

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Metal Ions and Clostridiopeptidase A

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Clostridiopeptidase A is a collagenolytic enzyme produced by the bacterium *Clostridium histolyticum*. It contains four identical sub-units, each with a molecular weight of about 25,000. The enzyme contains Zn and requires Ca^{2+} for activity [1]. We are using lanthanide ions (Ln^{3+}) and Co^{2+} to investigate the role of Ca^{2+} and Zn, respectively, in the catalytic activity of clostridiopeptidase A. For this purpose, it is convenient to use an artificial pentapeptide ('Pz-peptide') as the substrate in place of collagen whose properties are altered in the presence of Ln^{3+} [2].

Previous kinetic analyses, conducted in this laboratory, of the effect of Ln^{3+} on the hydrolysis of Pz-peptide by clostridiopeptidase A have suggested that Ca^{2+} is required for the binding of enzyme and substrate [3]. Sm^{3+} lowers the K_m nearly 15 fold, but it also lowers the V_{max} by a similar amount, suggesting that the enzyme-substrate complex is an abortive one. The relative ability of different Ln^{3+} to inhibit clostridiopeptidase A is Lu < Er < Sm ≫ La. As the radius of La^{3+} (1.016 Å) exceeds that of Ca^{2+} (0.990 Å), while Sm^{3+} (0.964 Å), Er^{3+} (0.881 Å) and Lu^{3+} (0.850 Å) are smaller, the Ca^{2+}-binding site on the enzyme appears to be sterically constrained, such that ions of greater radius than that of Ca^{2+} have restricted access. Heat inactivation studies also revealed a thermostability role for Ca^{2+} [3].

The ability of Ln^{3+} to inhibit clostridiopeptidase A while enhancing its substrate-binding, suggests a role for these cations in the purification of this enzyme by affinity chromatography; previous attempts to do this have been foiled by collagenolysis, even at 4 °C [4]. Experiments to investigate this possibility have qualitatively confirmed the conclusions from the kinetic analyses mentioned in the preceding paragraph. When a Ca^{2+}-free solution of clostridiopeptidase A was passed through an affinity column packed with calf skin collagen trapped within particles of polyacrylamide [4],