permanent interest and the object of many studies [1]. Recently, transition metal ions, including that of copper, have been reported to occur naturally in deoxyribunucleoproteins [2]. Although the interaction of Cu(II) with DNA has been thoroughly investigated [3], the specific binding ability of Cu(II) towards basic proteins at physiological pH has been overlooked. In this report we present the results of an investigation aiming to characterize complex formation between Cu(II) and one component of the protamine associated with DNA in herring sperm nuclei, namely, Clupeine Z. This protein contains 32 amino-acid residues, two-thirds of which are arginine [4].

At [Clu]/[Cu] = 1 titration curves show that at pH 6.6 two protons per Cu(II) are released. A first complex(I) is formed at this stage, the CD spectrum of which displays two negative bands at 660 and 275 nm and a positive one at 315 nm. The last is compatible with peptide involvement in coordination, while the negative peak at 275 nm is characteristic of metal binding to the terminal amino-nitrogen [5]. A second complex(II) is formed at pH 8.5 when two additional protons are removed. Its CD spectrum presents two bands: a negative one at 550 nm and a positive one at 255 nm. The first is consistent with binding to amino nitrogens of lateral chains [6]. Figure 1 illustrates the species distribution in this system obtained by treatment of titration data. The equilibrium constants defined by

$$K_I = \frac{[\text{Complex I}]\,[H^+]^2}{[Cu]\,[Clu]} \quad \text{and}$$

$$K_{II} = \frac{[\text{Complex II}]\,[H^+]^4}{[Cu]\,[Clu]}$$

where [Clu] = fully protonated [Clu] concentration, have been evaluated as: $K_I = 10^{-9}$ mol l^{-1}, and $K_{II} = 10^{-23}$ mol^3 l^{-3}.

The proposed structures of both complexes are shown in Fig. 2.

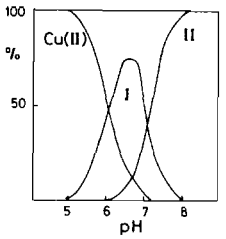

Fig. 1. Species distribution in Cu(II)–CluZ system as a function of pH.

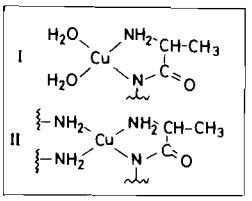

Fig. 2. Proposed structures of complexes I and II.

When [Clu]/[Cu] = 1/2, at pH 6.6 there is only one Cu(II) bound per protein molecule (site 1) and two protons are released. Spectral data are consistent with complex I formation as previously. From pH 6.6 to 8.5 the second Cu(II) bounds to the protein and four additional protons are neutralized. The spectral patterns suggest that in addition to complex II which is formed at site 1, another complex(III) is formed at site 2. In this complex two amino nitrogens of lateral chains and two oxygens of water molecules lie at the corners of the coordination square.

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Thermodynamics of Mixed Complexes of Cu(II) and Zn(II) with ATP and Some Aromatic Aminoacids

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Up to now mainly simple complexes of divalent metal ions with nucleotides have been studied in solution [1–4]; it is only for a few years that the stoichiometry, the formation constants and the structure of mixed complexes of M(II)–*NT* (*NT* = nucleotide) with biofunctional ligands have been reported [4–6]. By means of spectroscopic investigations two different types of ternary complexes have been investigated; these complexes can be represented as *L*–*NT*–M(II) and *L*–M(II)–*NT* (*L* = biogenic amines, aminoacids, *etc...*) [7]. In such mixed complexes an interesting 'secondary' bonding, due to formation of stacking adduct between the base of the nucleotide and the 'aromatic' moiety of other ligand, has been described [6]. Recently [8, 9], we have determined, by direct calorimetry, the effect of stacking interaction on the thermodynamic parameters concerning the formation of copper(II) and zinc(II) mixed com-

plexes with adenosine 5′-triphosphate (*ATP*) and some biofunctional ligands as L-tryptophan, histamine and L-histidine. We now report the thermodynamic properties of ternary complexes of Cu(II) and Zn(II) with *ATP* and aromatic aminoacids as phenylalanine and tyrosine.

The formation of stacking adducts has been checked by spectroscopic measurements. The thermodynamic data obtained by means of potentiometric and calorimetric measurements are compared with those of the corresponding parent complexes measured under the same experimental conditions (25 °C and 0.1 mol dm^{-3} in $K[NO_3]$). The $[Cu(ATP)_2]^{6-}$ and $[Zn(ATP)_2]^{6-}$ species were also found to exist; their overall stability constants were determined.

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Thermodynamics of Complex Formation: DL-Isoserine with Nickel(II) and Copper(II)

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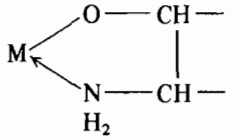
Researches in this laboratory have shown how in aqueous solution, the aminoacids, DL-4-amino-3-hydroxy-butanoic and DL-3-amino-2-hydroxypropanoic acid (isoserine) form complexes with divalent metals. The complexes contain the pentatomic chelate ring



where the alcoholic group appears to be ionized even in acidic solution [1, 2]. On the other hand the corresponding isomers, threonine and serines bind the metal *via* the α-aminoacid moiety and only in alkaline solution the hydroxyl group dissociates and is involved in chelation to the metal [3].

In order to explain the different roles of aminoethanolate and aminocarboxylate moieties in complex formation, we have now undertaken the calorimetric determination of enthalpy changes in the reactions of DL-isoserine with Ni(II) and Cu(II) in aqueous solution at 25 °C and I = 0.1 mol dm^{-3} (KCl).

The calorimetric measurements have been carried out by a modified LKB 8700-2 calorimeter. The values of the thermistor resistence in the reaction vessel were obtained by measuring the output voltage of the unbalanced Wheatstone bridge by a digital microvoltmeter HP 3455A. The e.m.f. values were printed at regular time intervals by a strip-printer HP 5150A with timer and converted to resistence values by an empirical relation.

The ΔH^0 values obtained for the protonation reaction of isoserine, $H_2NCH_2CH(OH)COOH$, H_2L, are:

HL^- ($\log K_1 = 13.2$), $\Delta H_1^0 = -53.14$ kJ mol^{-1}
H_2L ($\log K_2 = 9.139$), $\Delta H_2^0 = -43.60$ kJ mol^{-1}
H_3L^+ ($\log K_3 = 2.661$), $\Delta H_3^0 = -3.70$ kJ mol^{-1}

The enthalpy changes of Ni(II) complexes are:

NiL ($\log K_1 = 9.083$), $\Delta H_1^0 = -43.34$ kJ mol^{-1}
NiL_2^{2-} ($\log \beta_2 = 13.915$), $\Delta H_2^0 = -55.44$ kJ mol^{-1}

The data for Cu(II) complexes are:

CuH_2L^{2+} ($\log \beta_{112} = 24.120$), $\Delta H^0 = -53.43$ kJ mol^{-1}
Cu_2L_2 ($\log \beta_{220} = 34.568$), $\Delta H^\circ = -118.40$ kJ mol^{-1}

The corresponding entropy changes are rather high, particularly in the copper complexes. This stands for a remarkable entropy contribution to the stability of the complexes, coming from redistribution of water molecules involved in the process.

Some hypotheses on the structure of the complexes can be put forward. They will be tested by extending the calorimetric studies to other ligands containing the aminoethanol moiety.

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