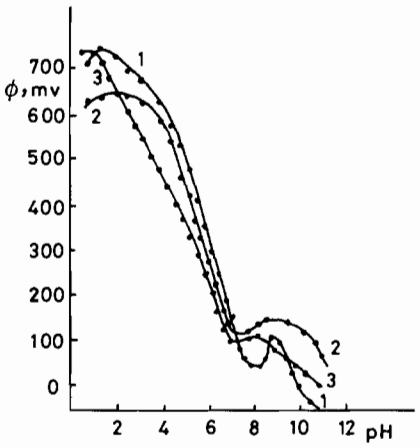

Fig. 1. Oxidizing potential dependence on pH. $T = 308$ K; $C_L = 0.1$ *M*; $C_{Fe^{2+}} = C_{Fe^{3+}} = 1 \times 10^{-5}$.

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.

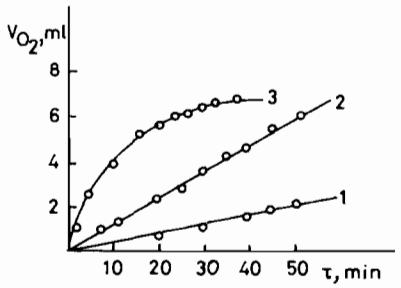

Fig. 2. Oxygen absorbance by L-cysteine aqueous solution (10 ml) in the presence of iron complex compounds. $T = 308$ K; pH = 7.0; $C_{Cys} = 0.2$, $C_{Fe^{2+}} = 1 \cdot 10^{-5}$, $C_{Cys} = 0.1$ *M*; curve 1, L = Gly; curve 2, L = $(Gly)_2$; curve 3, L = $(Gly)_3$.

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],

none of the enzyme stuck to the column. Addition of increasing amounts of Sm^{3+} progressively enhanced binding, with complete sequestration of clostridiopeptidase A occurring at a Sm^{3+} concentration of 75 μM. Comparison of the efficiencies of Lu^{3+}, Er^{3+}, Sm^{3+} and La^{3+} at a concentration of 75 μM (Table I), gave results in complete accord with those of the kinetic studies.

TABLE I. Clostridiopeptidase A which Bound to the Column in the Presence of Ln^{3+} Eluted with a Solution of EDTA or EGTA.

Lanthanide	Conc. (*M*)	% of enzyme bound to column
		0
Sm^{3+}	10	0
	20	10
	50	70
	75	100
	100	100
Lu^{3+}	75	70
Er^{3+}	75	70
La^{3+}	75	0

Co^{2+} enhanced the rate of hydrolysis of peptide by clostridiopeptidase A, a maximum stimulation of 2.5–3 fold being obtained with 2.5 mM Co^{2+}. Lineweaver-Burk analysis of this stimulation revealed an elevation of both the V_{max} and K_m by a factor of about 2.5 in the presence of 2.5 mM Co^{2+}. Taken at face value, these findings indicate that Co^{2+} increased the catalytic efficiency of the enzyme, despite reducing its affinity for the substrate. If Co^{2+} specifically replaced Zn, as happens with many other enzymes, the data suggest a previously unrecognized role for Zn in substrate-binding. However, we are also investigating possible alternative explanations, such as an action of Co^{2+} at the Ca^{2+}-binding site of the enzyme, or its interaction with the substrate.

Both Ln^{3+} and Co^{2+} provide useful spectroscopic properties which should greatly aid further investigation of these matters.

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A Magnetic Resonance Study of Metal Ion Coordination in Ternary Systems

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The aromatic moieties of biomacromolecules as proteins and nucleic acids specifically interact by means of 'stacking' interactions [1]. In many cases, the stacking adducts are stabilized by ionic bridges and divalent metal ions play a prominent role in $M-L_1-L_2$ mixed coordination [2].

Magnetic resonance techniques yield important information on the structure and the stability of the ternary systems [3]. When a paramagnetic metal ion such as Mn(II) is involved in mixed coordination, the E.S.R. analysis is useful to explain the features of the ternary interaction. The E.S.R. parameters of the manganous ion paramagnetic probe are strictly dependent on the Zero Field Splitting modulation [4, 5]. In particular, the mixed coordination $Mn(II)-L_1-L_2$ determines large Z.F.S. terms leading to undetectable E.S.R. spectra, while $T_{2_{el}}$ of Mn(II) senses the molecular dynamics around the metal ion [6]. In the slow motion limit ($\omega_o^2\tau_c^2 \gg 1$), a second order correction of the linewidth is necessary:

$$T_{2_{el}}^{-1} = -\frac{D:D}{5}\left\{16J_1 + 56J_2 + 64\left(\frac{a}{\omega_o}\right)^2 \times \left[I(I+1) - m_I^2\right] J_o\right\}$$

The Mn(II)–tryptophane-5′AMP/5′ADP/5′ATP complexes are suitable model systems to explain protein–nucleic acid specific interactions.

Figure 1 shows the E.S.R. intensity of the X-Band spectra of the ternary systems Mn(II)–Trp-5′AMP, Mn(II)–Trp–5′ADP and Mn(II)–Trp–5′ATP under the same experimental conditions.

The analysis of E.S.R. intensity changes of the binary mixtures upon addition of the second ligand allows the characterization of the ternary species existence. The minimum intensity is observed for molar ratios $Mn(II)-Trp-L_2 = 1:10:2$ (L_2 = 5′AMP); $Mn(II)-Trp-L_2 = 1:10:1$ (L_2 = 5′ADP); $Mn(II)-Trp-L_2 = 1:10:0.5$ (L_2 = 5′ATP).

The intensity loss points to the quantitative formation of ternary complexes: the T_2 values are much shorter than those measured for the free manganous ion [6]. Further nucleotide additions cause a recovery of E.S.R. intensities due to the shift of the ternary equilibrium toward the binary $Mn(II)-L_2$ species formation. At higher nucleotide con-