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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U20

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_{2el} 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:

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

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-

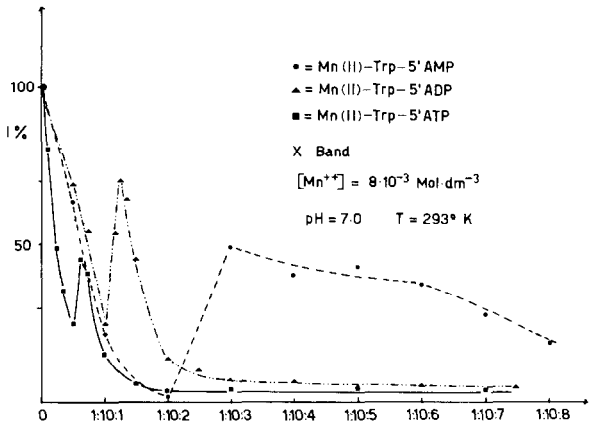

Fig. 1.

centration, the predominant species becomes Mn(II)–$L_2)_2$, E.S.R. undetectable [7].

The intensity minima of ternary systems occurring at different molar ratios point to the fundamental role of the phosphate groups in the stabilization of mixed coordination.

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U21

About the Origin of a Novel Fluroescence Observed in Metallo-proteins

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Very recently [1] new fluorescence bands at 415–445 nm have been described for ceruloplasmin, tyrosinase and haemocyanin, when these copper-proteins are excited within their absorption band at 325–345 nm. The excitation spectra show the occurrence of a chromophore absorbing at *ca.* 330 nm and the fast decay time (≤10 ns) should exclude any phosphorescence.

Although in the apo-proteins the absorption band at 325–345 nm, typical of Type 3 sites, has nearly completely disappeared, the excitation peak at 330 nm is still present and moreover the quantum yield of the fluorescence strongly increases. Therefore the absorption due to the copper(II) pair should hide the less intense residual absorption produced by the true fluorophore, while the only role of the copper is the quenching of the fluorescence.

Furthermore a quite analogous fluorescence has been found in several proteins obtained from different sources and having different catalytic functions (Table I), and in carboxylic and amino acids as well.

TABLE I. Behaviour of Proteins and Peptides under Excitation at 330 nm.

Compound	Fluorescence at 400–450 nm
Ceruloplasmin	yes
Haemocyanin	yes
Apo-haemocyanin	yes
Tyrosinase	yes
Catalase	yes
Peroxidase	yes
Cytochrome *c*	yes
Dopamine-β-hydroxylase	yes
Subtilisin	yes
Valinomycin	no
Gramicidin D	no

Measurements of the intensity of the fluorescence as a function of pH, concentration and different added metal ions, performed on solutions of pure carboxylic and amino acids, seem to indicate free carboxylate groups as mainly responsible for such a fluorescence, while an inter- or intra-molecular hydrogen bond probably also plays an important role.

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