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Lanthanide Spectral Properties as a Probe of Calcium-Binding Sites in Mesentericopeptidase

FERNANDA RICCHELLI, GIULIO JORI

C.N.R. Centro Emocianine e altre Metallo-Proteine, Istituto di Biologia Animale, Università di Padova, Padua, Italy

MARIA SHOPOVA, RAINA BOTEVA and NICOLAY GENOV

Bulgarian Academy of Sciences, Institute of Organic Chemistry, 1113 Sofia, Bulgaria

Mesentericopeptidase is a subtilisin-like bacterial protease, isolated from strains of *Bacillus mesentericus* [1]. Its physico-chemical and conformational properties are closely similar to those of subtilisin Novo (or BPN′) [1]. Like other subtilisins, mesentericopeptidase binds Ca^{2+} ions which play a role in the maintainance of the conformational stability and in the resistance to autolysis. Atomic absorption spectroscopy indicates that two Ca^{2+}-binding sites are present in mesentericopeptidase. The widely studied subtilisins Carlsberg and Novo were shown to bind at least one calcium ion with high affinity [2].

To probe the nature of the metal-binding sites in mesentericopeptidase, we replaced Ca^{2+} by the lanthanide ion Tb^{3+}. Such a replacement is often successful in biological systems [3]. Owing to their particular electronic configuration, lanthanide ions exhibit many spectral and magnetic properties. On the contrary, Ca^{2+} is silent towards most spectroscopic techniques [3].

The interaction of Tb^{3+} with mesentericopeptidase induces changes in the absorption spectrum of the protein with maxima ΔA at 245 nm and 300 nm. The shape of the titration plot for Tb^{3+} binding (Fig. 1), obtained by following the absorption changes at 245 nm, indicates that this protein possesses two non-equivalent Tb^{3+}-binding sites. Subtilisin Novo too was shown to contain at most two Tb^{3+}-binding sites [3].

Upon excitation in the aromatic region (λ_{exc} = 280 nm), Tb^{3+}-substituted mesentericopeptidase shows a green fluorescence emission (λ_{max} = 545 nm) characteristic of many proteins after addition of lanthanide ions [3]. The shape of the fluorescence excitation spectrum indicates the occurrence of resonance energy transfer to Tb^{3+} from tryptophan (Trp) residues. Mesentericopeptidase contains three Trp residues: one of them is virtually non-fluorescent, another one accounts for about 15–18% of the total fluorescence while the third residue accounts for about 60–80% [1]. Only 10–15% of the overall emission of the protein Trp residues is quenched upon Tb^{3+} binding. Therefore, it is likely that the Tb^{3+}-binding sites are close to a particular Trp residue.

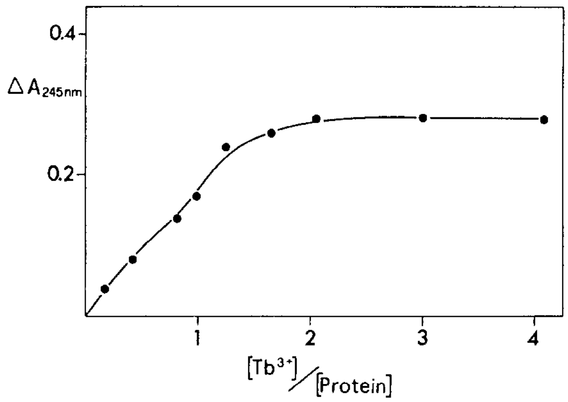

Fig. 1. Absorbance change at 245 nm of mesentericopeptidase during Tb^{3+} binding. Protein concentration = 1.2×10^{-4} *M*. 0.1 *M* Tris/HCl buffer. pH = 6.3 plus 0.5 *M* KCl.

The spectral changes associated with the lanthanide binding can be observed only after removal of Ca^{2+} by dialysis of mesentericopeptidase against EDTA. This is indicative of the replacement of Ca^{2+} by Tb^{3+} and also suggests that the binding constant of mesentericopeptidase with Ca^{2+} is higher than that with Tb^{3+}.

This preliminary report is part of a larger programme which aims to establish some intramolecular distances in mesentericopeptidase by using both lanthanide-derivatives of this protein and the dansyl-derivative in which the active site serine residue has been inactivated by dansyl binding. On the basis of the spectral properties of lanthanide ions and the dansyl group it would be possible, in particular, to calculate the distance between the Ca^{2+}-binding sites and the active site in mesentericopeptidase.

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Spectroscopic Studies and Characterization of Metallothioneins containing Mercury, Lead and Bismuth

WERNER BERNHARD, MEINRAD GOOD, MILAN VAŠÁK and JEREMIAS H. R. KÄGI

Biochemisches Institut der Universität Zürich, Zürichbergstrasse 4, CH-8028 Zürich, Switzerland

Metallothioneins are widely distributed metal-binding proteins which are involved in the meta-

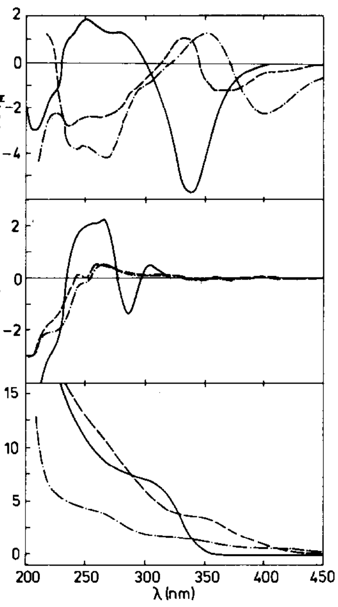

Fig. 1. MCD (top), CD (middle) and absorption (bottom) spectra of Hg(II)$_7$-metallothionein (———), Pb(II)$_7$-metallothionein (-----) and Bi(III)$_7$-metallothionein (—·—). Units employed: ϵ (M^{-1} cm), θ (deg cm^2 dmol^{-1}), θ_M (deg cm^2 dmol^{-1} gauss^{-1}).

bolism, storage and detoxification of essential and nonessential trace metals. The vertebrate forms characterized to date contain a single polypeptide chain of 61 amino acid residues, among them 20 cysteines providing the ligands for 7 metal-binding sites. Native metallothioneins are usually heterogeneous in metal composition with Zn, Cd, Cu and occasionally other metals occurring in varying proportions [1]. However, forms containing only a single metal species, *i.e.*, Zn, Cd, Ni, and Co, have been prepared by reconstitution from the metal-free apoprotein [2, 3]. By spectroscopic analysis of such derivatives it was established unambiguously that all cysteine residues provide their thiolate ligands for metal binding, that each metal ion is bound to 4 thiolate ligands, that the symmetry of each complex is tetrahedral, and that in order to satisfy the requirements of the overall Me$_7$-(Cys$^-$)$_{20}$ stoichiometry, these ceomplexes are combined to form metal–thiolate clusters [4].

Mercury and bismuth are also occasional constituents of native metallothionein [5]. To explore the mode of binding of these and related ions, we have now prepared derivatives containing Hg(II), Pb(II) or Bi(III). With Hg(II) and Pb(II) different types of complexes are formed depending on pH and metal-to-protein ratio. At a stoichiometry of 7 metal ions per molecule with each of the three metals unique complexes are obtained in which all cysteine residues are likely to participate in metal binding. The absorption, circular dichroism (CD) and magnetic circular dichroism (MCD) spectra of these forms are highly characteristic (Fig. 1) and can be interpreted in terms of Jørgensen's optical electronegativity theory of electron transfer (CT) transitions of metal ligand complexes [6]. From the coincidence of the location of the first CT-transition of Hg$_7$- and Pb$_7$-metallothionein with those predicted theoretically for T$_d$-thiolate coordination of these metals, it appears very likely that in these derivatives of metallothionein, too, these metals are bound in T$_d$ microsymmetry. The close similarity of the spectra of Bi$_7$- and Pb$_7$-metallothionein allows, furthermore, the inference that Bi(III) which is isoelectronic with Pb(II) is also bound in this fashion. Since T$_d$ coordination is highly unusual for these metals, it appears that as in the earlier case of Ni$_7$-metallothionein [3], this geometry is imposed by the rigidly maintained thiolate-binding sites of the protein. That all three derivatives have the same overall structure is suggested independently by the observation that their Stokes' radii are identical.

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Metal–Protein Interaction in *Carcinus Maenas* Hemocyanin

PAOLO ZATTA and FERNANDA RICCHELLI

Centro di Studio C.N.R. per la Biochimica e la Fisiologia delle Emocianine ed altre Metallo-Proteine, V. Loredan 10, 35100 Padua, Italy

Hemocyanin (Hc) is the respiratory pigment next in importance to hemoglobin for its distribution in two large phyla of Invertebrates: Arthropoda and Mollusca. The characteristic of Hc as oxygen carrier has overshadowed interest in other properties of possible physiological meaning such as the pseudo-