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

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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

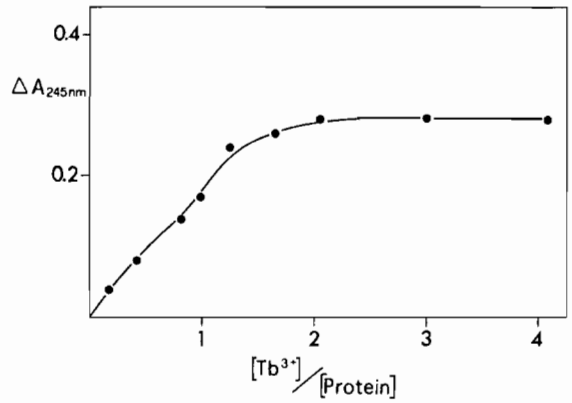

Fig. 1. Absorbance change at 245 nm of mesentericopeptidase during Tb^{3+} binding. Protein concentration = 1.2×10^{-4} *M*. 0.1 *M* Tris/HCl buffer. pH = 6.3 plus 0.5 *M* KCl.

Tb^{3+}-binding sites are close to a particular Trp residue.

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

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