Determination of the Cd:S cluster stoichiometry in *Fucus vesiculosus* metallothionein

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The seaweed *Fucus vesiculosus* is unusual when compared with other algal species in that it can survive in heavy-metal-contaminated aquatic environments. Metallothioneins are known to provide protection against a number of metals in many species through chelation. The metallothionein gene was identified in *Fucus vesiculosus* by Kille and co-workers (Morris, C.A., Nicolaus, B., Sampson, V., Harwood, J.L. and Kille, P. (1999) Biochem. J. 338, 553). We report the first detailed study of the metal binding properties of *Fucus vesiculosus* metallothionein using electrospray mass spectral and UV absorption data. The overall metal-to-sulfur ratios of this novel algal protein when bound to divalent cadmium and zinc are Cd_6S_{16} and Zn_6S_{16}, respectively. Mixed Cd/Zn species were also formed when Cd(II) was added to the Zn-containing *Fucus* metallothionein. Analysis of the charge states observed in the mass spectral data at both acidic and neutral pH showed that *Fucus* metallothionein is monomeric in solution. Only one conformation was identified at low pH for the native protein, however, the mass spectral data show the presence of two distinct conformations for *Fucus* MT that was studied with the S-tag peptide tail (fMT(s)). Analysis of the UV-absorption and ESI-MS data recorded during step-wise, acid-induced demetallation supports a two-domain structure for the protein, with 3- and 4- metal binding sites. The data suggest that one of the domains is significantly less stable than the other and we propose from the arrangement of cysteines in the sequence that the two domains are M_3S_7 and M_3S_9 where M = Cd(II) or Zn(II)). While the M_3S_9 cluster is known in the β clusters of mammalian metallothioneins, the M_3S_7 is an hitherto unknown cluster structure.