comparing with that of the Zn aminoacylase. For these reasons, we tentatively assign the peak to the vibrational absorptions of the metal ions and the coordinated atoms in the active sites of aminoacylase. One prerequisite for this assignment is that the conformation of the aminoacylase remains the same when Zn is removed or when Co is put in. And an experimental evidence which supports this hypothesis is that the fluorescence emission spectra of aminoacylase with metal ions are fundamentally the same as that of the apoaminoacylase, while it has been known that aminoacylase has twelve tryptophan residues, and one of them is necessary for the activity.

The specific activity of the Co-substituted aminoacylase is higher in the presence of free Co(II) ions than in their absence. For Zn aminoacylase, the existance of some free Co(II) ions can also increase its specific activity [1]. These facts indicate that there are some metal ions, other than those firmly situated in the active sites, which can activate aminoacylases somehow. It can be found from Fig. 2 that the Co-substituted aminoacylase has very similar absorbance spectra under the two different conditions mentioned above. The positions of the peak which is believed to reflect the vibration between metal ions and the coordinated atoms are the same in the two cases. This indicates that free Co(II) ions do not influence the environment of the Co(II) ions in the active sites very much. More experiments will be needed to explain how free Co(II) ions influence the activity of aminoacylase.

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BROMOPEROXIDASES FROM SEAWEED: A NOVEL CLASS OF ENZYMES CONTAINING VANADIUM?

Marine organisms, and in particular algae, accumulate a wide variety of halogenated compounds which show antimicrobial activity and which may have a function in systems involved in chemical defence [1,2]. In the biosynthesis of the brominated compounds bromoperoxidases participate. Bromoperoxidases from red [3] or green algae [4] have been studied in some detail, but little is known about the enzyme found in brown algae [5,6]. In this contribution data are presented on the properties of bromoperoxidase from the brown algae *Ascophyllum nodosum* which was recently purified by us [7].

It is known [8] that bromoperoxidases from marine origin show a pH optimum in the bromination reaction and this was also observed in the bromoperoxidase from *Ascophyllum nodosum*. The enzyme shows distinct pH optima, the position of which was determined by the concentration of H_2O_2 and Br^-.

At low pH values, the enzyme is inhibited by an excess of Br^-. However, high concentrations of H_2O_2 (1-5 mM) do not inhibit the enzyme. The complex steady-state kinetics correspond to a ping-pong mechanism in which H_2O_2 first has to oxidise the enzyme before Br^- or H^+ are bound. When Br^- or H^+ are bound to the enzyme before H_2O_2 has reacted, these substrates act as inhibitors.

Surprisingly, although the brominating activity (90 μmoles monochlorodimedone brominated per min

per mg of protein) is comparable to that of other haloperoxidases that contain haem, the bromoperoxidase even at high protein concentration (10 mg/ml) does not exhibit a Soret peak typical of a haemoprotein. Recently, it was reported [6] that the bromoperoxidase activity, after inactivation of the enzyme at low pH, could be restored at high pH by addition of vanadium. This observation [6], which we could confirm, prompted us to determine the vanadium content of our preparation. Indeed, analysis demonstrated that a considerable amount of vanadium was present in our purified bromoperoxidase preparation. The amount of vanadium per mg of protein was high enough to be studied by EPR at low temperature.

In the oxidised state, the enzyme as isolated does not show signals that can be attributed to vanadium. Also, addition of Br^- or H_2O_2 was without effect. However, upon reduction with dithionite, an EPR spectrum was observed which showed the typical hyperfine splitting of vanadium ($I = 7/2$). EPR experiments are in progress to determine whether the valence of this metal ion changes during catalysis. If this should be the case, a novel class of enzymes that contain vanadium as a prosthetic group has been discovered.

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ISOLATION OF THE DNA SEQUENCE CODING FOR *PSEUDOMONAS AERUGINOSA* AZURIN

The mechanism of electron transfer by metalloenzymes has been the basis of extensive research. One of the unanswered questions in the field is the importance of relative metal ion and protein orientations to the electron transfer rate. Important data have been accumulated for protein-protein interactions [1,2] and for protein--small molecule electron transfer reactions [3-5] with the "blue" copper proteins. Azurin, a "blue" copper protein isolated from *P. aeruginosa*, is an ideal donor/acceptor protein to study because: a) the protein sequence is known [6]; b) the X-ray structure is reported [7]; and c) electron transfer rates between azurin and other proteins and small molecules are tabulated [1-5].

Our entry into this field hopes to exploit the new biotechnology known as site specific mutagenesis. The single "blue" copper atom in azurin is coordinated in a distorted tetrahedral geometry by nitrogen atoms from His-46 and His-117 and sulfur atoms of Cys-112 and Met-121 [7]. We propose to develop a retroazurin in which residues 112 and 121 are changed to Met and Cys, respectively. This alteration of the protein sequence will result in a change in the long axis (Cu-Met) in the distorted tetrahedral environment of the copper. Besides generating a unique modification of the