

ve flexibility of the metal binding site in this enzyme. In view of the peculiar structure and chemistry of the catalytic metal binding site in HLADH, we can explore Fe-HLADH as a model system for native Fe/S proteins.

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PS3.7 — MO

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Cu^{2+} BINDING TO DOPAMINE β -MONOOXYGENASE

Dopamine β -monooxygenase contains four subunits and binds copper ions, which are necessary for catalytic activity. The questions of the stoichiometry of binding of copper ions to the enzyme, and of the number of copper ions necessary for catalytic activity have not been completely resolved [1,2]. We have now determined the stoichiometry of high affinity sites for copper in do-

pamine β -monooxygenase and the formation constants for binding to these and other sites by using a Cu^{2+} specific electrode. The electrode has been used to measure the concentration of free unbound copper during a titration of the enzyme with Cu^{2+} . The Cu^{2+} electrode shows a linear response according to the Nernst equation from 1.0 to 1×10^{-19} M of free Cu^{2+} [3]. Both native and apo(metal free) dopamine β -monooxygenase have been titrated, and the stoichiometry of high affinity binding sites for Cu^{2+} are seen in Fig. 1 to be four per enzyme tetramer. We calculated the stoichiometric formation constants using a computer program which fits the titration data by least squares regression analysis. The values are shown in Table I.

To verify the results obtained with dopamine β -monooxygenase three other metal-binding proteins have been titrated. Bovine serum albumin, apo-carbonic anhydrase and ovotransferrin bind copper with high affinity, and the binding parameters are shown in Fig. 1 and Table I. The first formation constants of the Cu^{2+} -bovine serum albumin and Cu^{2+} -(apo)carbonic anhydrase complexes agree well with published values obtained with equilibrium dialysis methods. Also, the stoichiometry of two copper sites with approximately similar affinity per ovotransferrin is in agreement with the metal binding properties of that protein.

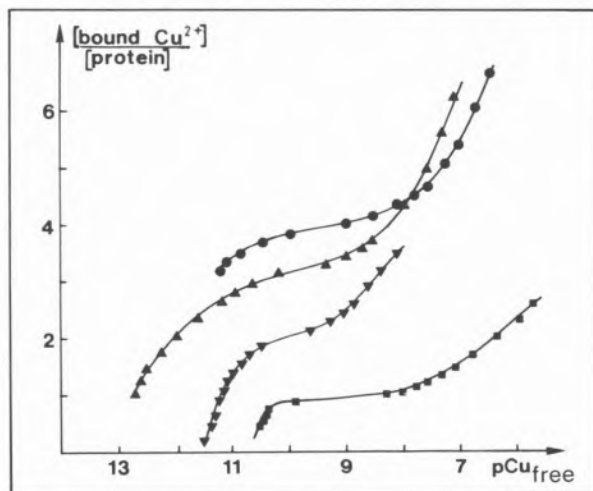


Fig. 1

Semilogarithmic plot for binding of Cu^{2+} to: (●) native dopamine β -monooxygenase, (▲) apo dopamine β -monooxygenase, (▼) ovotransferrin, (■) apo carbonic anhydrase. For dopamine β -monooxygenase the curves refer to binding to enzyme tetramer

Table I

Successive formation constants of Cu^{2+} -protein complexes. 0.1 M KNO_3 or KCl, pH 5.8-6.0 in Mes or acetate buffer. For dopamine β -monooxygenase the constants refer to binding to enzyme subunits

		CuP logK ₁	Cu ₂ P logK ₂	Cu ₃ P logK ₃	Cu ₄ P logK ₄
Dopamine β -mono- oxygenase	apo native	11.2 6.4	7.1 5.5	6.4 5.5	
Bovine serum albumin		11.2	8.7	7.0	6.0
Apo carbonic anhydrase		10.4	7.1	5.8	
Ovotransferrin*		11.2	11.4	9.1	7.8

* pH 7.9, 15 mM NaHCO_3 .

Regarding dopamine β -monooxygenase, the results establish the stoichiometry of four high affinity binding sites for Cu^{2+} ($\log K_f \sim 11$) per enzyme tetramer, and more binding sites of lower affinity ($\log K_f \sim 5-7$). While the first four Cu^{2+} represent binding to a separate class of binding sites, the next four Cu^{2+} and so forth have the same affinity as for binding of excess copper to the other three proteins analysed. Additional copper ions in excess of four per enzyme tetramer may still be necessary for maximal activity under the conditions of catalysis (presence of substrates and a reducing agent), but they should then be regarded as activating copper ions rather than being an integral part of the enzyme.

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PS3.8 — TU

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NMR STUDIES OF Ni(II)- AND Co(II)-SUBSTITUTED STELLACYANIN

Stellacyanin is a small metalloprotein which, as it occurs in nature, contains a single copper(II) ion. The metal has a distorted tetrahedral coordination that gives the protein its intense blue colour. This so-called «type 1 copper» is also found in various other small proteins and in the more complex copper oxidases; laccase, ceruloplasmin and ascorbate oxidase. It is now widely accepted that the structure of the type 1 site in all proteins is essentially the same and that the copper is coordinated to two histidine imidazole nitrogens, a cysteinyl sulfur and, at least in two small proteins, to a methionine sulfur. Stellacyanin, however, contains no methionine and the nature of the fourth ligand is still enigmatic. Since stellacyanin is known to deviate considerably in many spectroscopic and chemical properties compared to the other blue copper-containing proteins, it has attracted much interest and a great number of spectroscopic methods have been used in attempts to identify the fourth ligand and to explore the complicated structure of this copper site.

To derive more information from spectroscopic measurements, it is useful to study metal substituted proteins in which the native copper is replaced by a suitable metal ion [1]. Popular candidates for this replacement are cobalt(II) and nickel(II) and optical absorption studies of stellacyanin derivatives of these metals have been reported earlier [2]. In this work, NMR studies of Co(II)- and Ni(II)-substituted stellacyanin have been performed.