We have now studied the binding of ^{64}Cu to this enzyme in reconstitution and exchange experiments. High performance size-exclusion gel chromatography with the protein analysis column I-125 from Waters was used to separate the enzyme-bound and free ^{64}Cu, and the amount of ^{64}Cu bound to the protein was determined from the radioactivity eluting together with the protein. Experiments with binding of $^{64}Cu(II)$ to the apoenzyme give further evidence for a specific binding of 4 copper atoms per tetramer, but some weaker copper-binding sites were observed in the presence of an excess of copper. When the apoenzyme was incubated with 4 atoms of $^{64}Cu(II)$ per tetramer, about 3.5 copper atoms were eluted with the protein indicating that the binding of Cu(II) is not extremely tight. Similar amounts of ^{64}Cu were bound to the apoenzyme in the presence of ascorbate indicating the binding of Cu(I) is similar to that of Cu(II).

The exchanges of both Cu(I) and Cu(II) in the holoenzyme are rapid and a half-life of about 1 min was estimated for the exchange of the enzyme-bound Cu(II) in the presence of a two-fold excess of $^{64}Cu(II)$ at pH 6.1. Experiments in the presence of ascorbate revealed that the exchange of Cu(I) was complete in 1 min at similar conditions. The exchange of the copper atoms in dopamine β-monooxygenase are thus much more rapid than reported for other copper proteins, and the present results point to a unique copper-binding site in this protein.

1 T. Skotland and T. Ljones, *Inorg. Perspect. Biol. Med., 2,* 151 (1979).
2 T. Skotland and T. Ljones, *Eur. J. Biochem., 94,* 145 (1979).
3 T. Skotland, L. Petersson, D. Bäckström, T. Ljones, T. Flatmark and A. Ehrenberg, *Eur. J. Biochem., 103,* 5 (1980).

O6

Zinc in Aminolevulinic Acid Dehydratase

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5-Aminolevulinic acid dehydratase (EC 4.2.1.24) catalyzes the formation of the tetrapyrrole precursor porphobilinogen from 5-aminolevulinic acid. The isolated enzyme contains variable amounts of zinc but binds a maximum of 8 Zn(II) per octameric protein. Removal of zinc by chelators increases K_M 60-fold and decreases V_{max} to about 10 to 20% of its original value. After removal of zinc the enzyme activity may be restored to its original value by Zn(II) or Cd(II). The ^{113}Cd-NMR spectrum of the enzyme reconstituted with $^{113}Cd(II)$ exhibits a single sharp resonance signal at 79 ppm which is not changed when substrate is added. Obviously there is no immediate interaction between the metal and the substrate. On the other hand zinc is located in the substrate binding domain since the enzyme is specifically inhibited by the affinity reagent 2-bromo-3-(5-imidazolyl) propionic acid.

It is concluded that zinc in 5-aminolevulinic acid dehydratase is a constituent of the active centre but does not have an immediate catalytic function.

O7

Preliminary Crystallographic Data for the Copper Enzyme Ascorbate Oxidase

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Ascorbate oxidase (EC 1.10.3.3) is a copper enzyme belonging to the group of so-called 'blue oxidases' together with laccases and ceruloplasmin. The enzyme, widely distributed in several plant species, catalyzes the oxidation of L-ascorbate, transferring the reducing equivalents to molecular dioxygen. The biological function of the enzyme is still in question. Ascorbate oxidase activity is highest in those parts of plants which grow faster; on the other hand some authors suggested a possible role of the enzyme in plant respiration [1].

The native enzyme is a non-covalent dimer, whose subunits (respectively 75,000 and 72,000 Mr) contain 8 Cu^{2+} ions; these can be classified, according to their coordination environments, as of type-1, type-2 and type-3 [2, 3]. Ascorbate oxidase is known to undergo fully reversible association-dissociation phenomena. Its ultracentrifuge pattern changes as a function of pH and buffer media, while the spectroscopic properties and the activity towards ascorbate remain unchanged.

Although the information available at present is not sufficient to fully elucidate the sequence of redox events which take place within the protein, there exists some evidence that the three classes of copper ions fulfil different functions. Type-1 copper is the primary site of electron acceptance; type-2 and type-3 coppers are implicated respectively in ascorbate and O_2 binding [4]. Ascorbate oxidase is thus an ideal model enzyme for the study of biochemistry and biophysics of vegetal copper proteins.