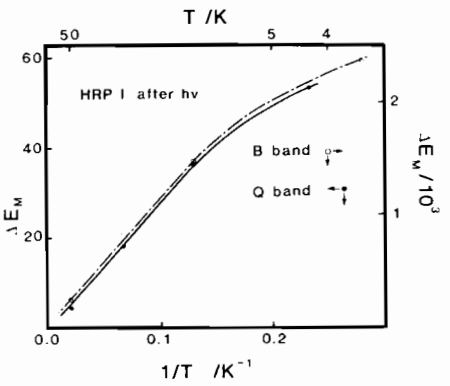

Fig. 2. The temperature dependence of the MCD peak-to-trough intensity for the B or Soret band at 416 nm (right hand ordinate scale), and the Q or α band at 580 nm (left hand ordinate scale) of the photolysed HRP compound I. The signal intensities are expressed in units of $L \cdot mol^{-1} \cdot cm^{-1} \cdot T^{-1}$. The experimental conditions were the same as in Fig. 1.

relationship between the intensity and the inverse of the absolute temperature suggests that the ground state in the iron porphyrin species which is formed photochemically is orbitally degenerate. The non-linearity of this relationship at very low temperatures and the high magnetic field (4.58 T) used in this study most likely arises from simple Boltzmann saturation effects.

Various structures have been suggested for the product of the photolysis of HRP compound I [2, 4, 6, 7]. The close similarity between the optical absorption and MCD spectra of the photolysis product and HRP compound II suggests that the heme retains the S = 1 Fe(IV) porphyrin electronic structure following photolysis and that the porphyrin is reduced from the 17 π-electron cation radical of compound I to the stable 18 π electron configuration as in compound II [8, 9]. In addition, the strong temperature dependence of the MCD intensity observed in the spectra of the photochemical product indicates that significant coupling takes place between the paramagnetic iron and the diamagnetic porphyrin π system.

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O5

The Copper of Dopamine β-Monooxygenase: High Accessibility and Rapid Exchange

TORE SKOTLAND, TORGEIR FLATMARK

Department of Biochemistry, University of Bergen, Årstadveien 19, N-5000 Bergen, Norway

and TORBJØRN LJONES

Department of Chemistry, College of Arts and Sciences, University of Trondheim, N-7055 Dragvoll, Norway

Dopamine β-monooxygenase (dopamine β-hydroxylase; EC 1.14.17.1) catalyzes the reaction:

Dopamine + ascorbate + O_2 →

noradrenaline + dehydroascorbate + H_2O

($RH + 2e^- + 2H^+ + O_2 \rightarrow ROH + H_2O$)

The purified water-soluble enzyme from bovine adrenal medulla contains 4 copper atoms per enzyme tetramer of 290,000 daltons. These copper atoms are essential for enzymic activity, and they most probably participate in both electron transfer and binding of O_2 [1].

The copper in dopamine β-monooxygenase can be classified as type 2 copper according to its EPR spectrum, with a large hyperfine splitting and the low absorption in the visible spectrum (ϵ = 40 $M_{Cu}^{-1} \cdot cm^{-1}$ at 680 nm, the maximum of the Cu(II)-band) [1]. This enzyme-bound copper is, however, different from type 2 copper in the blue oxidases by other criteria, especially by showing a high accessibility of the copper sites. Thus, we have shown that the copper atoms of dopamine β-monooxygenase can be rapidly removed by chelators at non-denaturing conditions both in the reduced and oxidized states, and the inactive apoenzyme is reactivated in less than 2s by addition of $CuSO_4$ [2, 3].

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.

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O6

Zinc in Aminolevulinic Acid Dehydratase

R. SOMMER, M. COX and D. BEYERSMANN

Universität Bremen, F.R.G.

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

L. AVIGLIANO, G. MARCOZZI

Ist. Chimica Biologica, Fac. Medicina, Università di Roma, Rome Italy

M. BOLOGNESI, G. GATTI and A. CODA

Dip. Genetica, Sez. Cristallografia, Università di Pavia, Pavia, Italy

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.