BIOCHEMICAL LETTERS

The nature of the copper(II) components of caeruloplasmin

Caeruloplasmin (ferroxidase, EC 1.16.3.1) is a copper oxidase that occurs in vertebrate plasma and contains 5–6 copper atoms per protein molecule (Malmström, 1978; Mason, 1979). These have been reported to be composed of diamagnetic cuprous ions (50%) and two different types of cupric ion [Type 1 (blue) and Type 2 (non-blue)], which have distinct e.p.r. spectra (Andréasson & Vänngård,

1970). Most of the studies to date have been performed on samples of mammalian origin and, in human caeruloplasmin for example, differences have been reported in the amounts of the two types of copper(II) present (Veldsema & Van Gelder, 1973), although more recent papers seem to favour a situation in which there are two Type-1 and one Type-2 copper(II) ions per caeruloplasmin molecule (Herve *et al.*, 1978). Some further subdivision of these two types of copper has also been made. Andréasson & Vänngård (1970) report e.p.r. spectra with two distinct Type-2 components, whereas Herve *et al.* (1978) demonstrate the presence of two differing Type-1 components.

In order to compare a caeruloplasmin of marine origin with the mammalian enzyme, caeruloplasmin from plaice, *Pleuronectes platessa* (L), serum has now been studied both after purification by ethyleneimine affinity column chromatography and after further purification by Sephadex G-200 gel filtration (M. A. Syed & T. L. Coombs, unpublished work). The e.p.r. spectrum of the plaice caeruloplasmin derived from the ethyleneimine chromatography is shown in Fig. 1, curves (a) and (b). This is similar to the reported spectra of mammalian caeruloplasmins and both Type-1 and Type-2 copper(II) ions can be

Fig. 1. E.p.r. spectra of plaice serum caeruloplasmins (a) Spectrum at 77K of plaice caeruloplasmin purified by ethyleneimine column chromatography, 6.0 mg/ml in 0.05 M-sodium acetate buffer, pH 5.6.

(b) The parallel region of spectrum (a) expanded $\times 6$. (c) A computer simulation of spectrum (a) using two components (Type-1 and Type-2) with Gaussian lineshapes and the following parameters: Type-1, $g_{\parallel}=2.231$, $g_{\perp}=2.044$, $A_{\parallel}=7.4$ mT, $A_{\perp}=0.4$ mT, 45%; Type-2, $g_{\parallel}=2.260$, $g_{\perp}=2.048$, $A_{\parallel}=16.7$ mT, $A_{\perp}=0.5$ mT, 55%. It should be noted that similar agreement between the experimental and simulated spectra could be obtained with $A_{\perp} \leq 1.5$ mT and with the $g_{\perp}$ values of the two components interchanged. (d) Spectrum at 77K of plaice caeruloplasmin purified by Sephadex G-200 gel filtration in addition to ethyleneimine column chromatography, 4.3 mg/ml in 0.05 M-sodium acetate buffer, pH 5.6. (e) The parallel region of spectrum (d) expanded $\times 5$. (f) A computer simulation of spectrum (d) using a single component (Type-1) with Gaussian lineshape and the following parameters: $g_{\parallel}=2.231$, $g_{\perp}=2.044$, $A_{\parallel}=7.4$ mT, $A_{\perp}=0.4$ mT. It should be noted that spectra could be obtained with any value of $A_{\perp} \leq 0.75$ mT. The positions of the parallel peaks for the two types of copper are indicated by the bars at the base of the Figure.

seen clearly. A computer simulation of this spectrum, in which near-equal amounts of the two types of copper were assumed to be present, is shown in Fig. 1, curve (c). There is close agreement between curves (a) and (c). The spectrum from the sample derived from the Sephadex G-200 gel filtration is shown in Fig. 1, curves (d) and (e), together with a computer simulation, curve (f). These spectra show that the e.p.r.-detectable copper is entirely in the Type-1 form. Evidently the Type-2 copper has been removed as a result of the further Sephadex treatment.

Several physicochemical and enzymic criteria support the contention that treatment by gel filtration results in a further purification of the enzyme. For example, the specific enzymic activity increased from 41.9 to 68.1 units $\cdot$ min⁻¹ $\cdot$ mg⁻¹, in parallel with an increase in the copper:protein ratio of 1.31 to 2.22 μ g $\cdot$ mg⁻¹ and with a small increase in the A_{610}/A_{280} absorbance ratio. In addition, polyacrylamide-gel electrophoresis indicated a decrease of the three protein and the corresponding three copper-containing bands in the gel from the ethyleneimine chromatography sample to only one of each after the further purification step. This single band of protein coincident with copper also showed caeruloplasmin activity and corresponded precisely to the single band of caeruloplasmin activity found after gel electrophoresis of the original sample derived from the ethyleneimine chromatography.

It thus appears that in plaice caeruloplasmin the Type-2 copper(II) makes no contribution to the catalytic activity of the enzyme and probably is

associated with copper attached to a different contaminating protein, which was not separated at the affinity chromatography step, but which is subsequently removed by gel filtration. These results suggest that further work should now be carried out on the purification of mammalian caeruloplasmins to confirm the non-essentiality of Type-2 copper(II) in this metalloenzyme.

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M. Arif SYED and Thomas L. COOMBS
N.E.R.C. Institute of Marine Biochemistry,
St. Fittick's Road, Aberdeen, U.K.

Bernard A. GOODMAN and Donald B. McPHAIL
Macaulay Institute for Soil Research, Craigiebuckler,
Aberdeen, U.K.

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