A COUPLED IRON-CAERULOPLASMIN OXIDATION SYSTEM

G. Curzon and S. O'Reilly*

Department of Chemical Pathology
Institute of Neurology
The National Hospital
London, W. C. 1

March 31, 1960

Caeruloplasmin, the blue cuproprotein of plasma, has oxidase properties (Holmberg and Laurell, 1951), although the physiological substrate (if any) is unknown. During investigation of the effects of ions on the oxidase activity (Curzon, 1960), it was found that metal ions in low concentration had marked effects. In particular, ferrous iron apparently enhances activity against N, N-dimethyl-p-phenylenediamine dihydrochloride (DPD). This communication describes experiments which indicate a coupled iron-caeruloplasmin oxidation system to be involved.

Experimental and Results

Purified caeruloplasmin was made by the method of Curzon and Vallet (1960). Oxidase activity was determined using DPD as substrate shown spectrographically to be negligibly contaminated with metal. Sodium acetate was freed from trace metals by dithizone and 8-hydroxyquinoline extractions. Acetic acid was redistilled. Ion-exchange purified water was used throughout. To 2 ml. of metal-free 0.2 M, pH 5.5 acetate buffer + 1 ml. of substances to be tested + 1 ml. of 1.67×10^{-3} M DPD at 25° was added 1 ml. of caeruloplasmin solution. After 15 minutes, reaction was stopped by 2.0 ml. of 3×10^{-4} M sodium azide and the optical density of the red free radical oxidation product measured against a suitable blank at 550 mμ with 1 cm. path.

Fe^{++} and Fe^{+++} solutions were made immediately before use from stock 10^{-2} M ferrous ammonium sulphate stored at -25° and stock M ferric ammonium sulphate in 5×10^{-3} M HCl.

The Effect of Iron on Caeruloplasmin Oxidase Activity. Both Fe^{++} and Fe^{+++} apapparently increase the oxidase activity of caeruloplasmin (Table 1). Activity is increased more by Fe^{++} than by Fe^{+++} at the same concentration.

Oxidation of Fe^{++} by Caeruloplasmin. Partially purified caeruloplasmin was used in this experiment (Fraction V, Curzon and Vallet, 1960). To 3 ml. caeruloplasmin in pH 5.5 buffer having $E_{1\,cm.}$ (605 mμ) = 0.33 was added 0.075 ml. 10^{-2} g. atom/l. μ Fe^{++} at room temperature. This was approximately 1 atom Fe^{++}/atom caeruloplasmin Cu. The blue colour of the caeruloplasmin rapidly disappeared, started to reappear within

* Permanent address: Dept. of Neurology, U.C. Medical Center, San Francisco.

Table 1

The Effects of Fe^{++} and Fe^{+++} on Caeruloplasmin Oxidase Activity

Caeruloplasmin copper in the incubation mixture was 5×10^{-7} M.

Fe in incubation mixture g. atoms/1.	$E_{1\,cm.}$ (550 mu) after 15 min. 25°.
-	0.188
Fe^{++} 4×10^{-7}	0.273
Fe^{++} 4×10^{-6}	0.445
Fe^{++} 10^{-5}	0.602
Fe^{+++} 4×10^{-7}	0.221
Fe^{+++} 4×10^{-6}	0.325
Fe^{+++} 10^{-5}	0.376

40 sec., and completely returned in 300 sec. On adding more Fe^{++} the cycle was repeated. Using 1:10 phenanthroline to determine Fe^{++}, it was shown that 3 ml. of the above caeruloplasmin solution caused 80% disappearance of 0.20 ml. 10^{-2} g. atom/l. Fe^{++} in 60 sec. There was no detectable disappearance in the absence of caeruloplasmin. Oxidation of Fe^{++} to Fe^{+++} by caeruloplasmin was directly demonstrated by thiocyanate (in the absence of acetate buffer which interferes with the colour reaction).

Oxidation of DPD by Fe^{+++}. Under the conditions of oxidase activity determination, Fe^{+++} is able to oxidize the substrate to the red free radical product directly, although the amount of oxidation occurring is comparatively slight. Thus 10^{-5} and 10^{-4} g. atom Fe^{+++}/l. in the incubation mixture but with water instead of caeruloplasmin, resulted in $E_{1\,cm.}$ (550 mμ) of 0.020 and 0.362, respectively. When 1 ml. 10^{-2} g. atom/l. Fe^{++} was subsequently added the red colour rapidly disappeared. Thus the following equilibrium is indicated:

$$DPD + Fe^{+++} \rightleftharpoons \overset{*}{DPD} + Fe^{++}$$

Discussion

Michaelis, Schubert, and Granick (1939) showed that bromine oxidizes DPD in two successive univalent steps. The first step which is reversible, gives rise to the red free radical oxidation product determined above as a measure of oxidase activity. It has now been shown that Fe^{+++} can also take part in the reversible oxidation of DPD and that in the presence of caeruloplasmin the Fe^{++} formed is reoxidized to Fe^{+++}. Thus, as well

as the direct oxidation of DPD by caeruloplasmin a coupled iron-caeruloplasmin-DPD oxidation system occurs which gives rise to an apparant activation of caeurloplasmin by iron:

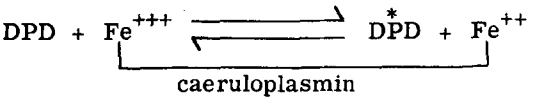

Fig. 1

The finding that Fe^{+++} enhances activity less than Fe^{++} may perhaps be explicable in terms of the second irreversible stage of DPD oxidation, Fe^{+++} hydrolysis effects or the strong inhibitory effect of other trivalent cations (Curzon, 1960).

It is apparent from Fig. 1 that the DPD-caeruloplasmin system is affected by very low concentrations of Fe^{++}. Thus, unless care is taken to use pure materials the coupled oxidation system may interfere significantly in caeruloplasmin oxidase determinations. Also some of the conflicting results in the literature on the activity of caeruloplasmin against 5-hydroxytryptamine and adrenaline (Holmberg and Laurell, 1951; Martin, Eriksen, and Benditt, 1958; Geller, Eiduson, and Yuwiler, 1959; Curzon and Vallet, 1960) may to some extent be explicable in terms of coupled iron-caeruloplasmin oxidation systems. The possibility of coupled oxidation systems involving caeruloplasmin and physiologically occurring iron compounds is also of some interest.

We thank the Research Advisory Committee of the Institute of Neurology (G. C.) and the Parkinson's Disease Foundation, New York (S. O'R.) for financial support.

References

Curzon, G., (1960) Biochem. J. In press.

Curzon, G. and Vallet, L., (1960) Biochem. J., 74, 279.

Geller, E., Eiduson, S. and Yuwiler, A., (1959) J. Neurochem., 5, 73.

Holmberg, C. G. and Laurell, C. B., (1951) Acta chem. scand., 5, 476.

Martin, G., Eriksen, N. and Benditt, E. P., (1958) Fed. Proc., 17, 447.

Michaelis, L., Schubert, M. D. and Granick, S., (1939) J. Amer. chem. Soc., 61, 1981