ACTA CHEMICA SCANDINAVICA 26 (1972) 2832–2836

Interaction of Ceruloplasmin with L- and D-Dopa

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The interaction between dopa and ceruloplasmin has been investigated. Both L-dopa and D-dopa are oxidized by ceruloplasmin in the absence of iron activation, the former being more rapidly oxidized than the latter. The apparent Michaelis constant is 1.8 mM for L-dopa and 2.6 mM for D-dopa. Both antipodes of dopa inhibited the rate of dopamine oxidation by the enzyme, but did not have any inhibitory effect on the ceruloplasmin catalyzed oxidation of PPD, indicating different binding sites for catecholamines and PPD on the enzyme molecule.

The enzymic nature of the copper containing serum protein, ceruloplasmin,* was reported by Holmberg and Laurell,[1,2] who showed that PPD,** dopa, adrenaline, ascorbate and other compounds were oxidized by ceruloplasmin.

Curzon and O'Reilly[3] discovered that iron ions have an activating effect on the ceruloplasmin catalyzed reaction *via* a coupled iron-ceruloplasmin oxidation system. McDermott *et al.*,[4] using an oxygen electrode for enzymic activity measurements, showed that several compounds like ascorbate, dopa, hydroquinone, catechol, and cysteine could not be oxidized by ceruloplasmin in the absence of iron ions, and these were consequently not true substrates.

In the present communication the interaction between dopa and ceruloplasmin was investigated in the absence of iron activation. Both L- and D-dopa were able to act as substrate for ceruloplasmin, although the rate of oxidation was rather low.

EXPERIMENTAL

Materials. Human ceruloplasmin was obtained from AB Kabi, Stockholm. The enzyme was crystallized according to the method of Deutsch,[5] and the ratio between the absorptions at 610 mμ and 280 mμ was 0.040. Enzyme concentration was calculated from the extinction at 610 mμ, using $\varepsilon_{610} = 1.09 \times 10^4$ M^{-1} cm^{-1}.[5] Dopa, dopamine, tyrosinase

* The systematic name of ferroxidase (E.C. 1. 12. 3) has been proposed by Osaki *et al.* in *J. Biol. Chem.* **241** (1966) 2746.

** Abbreviations used: PPD, *p*-phenylenediamine; dopa, 3,4-dihydroxyphenylalanine; dopamine, 3-hydroxytyramine; Desferal, deferoxamine. B-methane sulfonate.

and PPD were purchased from Sigma Chemical Company and Desferal from Ciba Pharmaceutical Company. All aqueous solutions were prepared in deionized, glass distilled water.

Methods. The system for determining the molar absorption of dopachrome at 305 mμ contained 0.027 M potassium phosphate buffer, pH 6.8, 5.0 mM KCl, 0.1 mg tyrosinase (total volume 3.6 ml) and dopa (29 – 86 μM) (Fig. 1).

The ceruloplasmin catalyzed oxidation of dopa to dopachrome was followed spectrophotometrically at 305 mμ. The enzyme activity (V) was calculated from the initial, linear part of the curve. The reaction mixture contained 1.3 μM ceruloplasmin, 0.5 mM Desferal in 0.2 M sodium acetate buffer, pH 5.4. The L- and D-dopa concentration ranged from 1.0 mM to 7.0 mM (Fig. 3). Desferal was added in order to prevent the activating effect of trace amounts of iron ions. The concentration of Desferal used did not affect the enzyme activity. Stock solutions of dopa (10 mM) were made immediately before use.

When dopamine was used as substrate for ceruloplasmin, the formation of dopaminochrome was followed spectrophotometrically at 305 mμ in the absence and presence of L- and D-dopa (0.65 – 5.0 mM) (Fig. 4). The reaction mixture contained 1.0 μM ceruloplasmin, 4.0 mM dopamine and 0.5 mM Desferal in 0.2 M sodium acetate buffer, pH 5.4. It was necessary to correct for some dopachrome formation in these experiments.

In another set of experiments dopamine was replaced by PPD (0.2 – 2.0 mM). The reaction mixture contained 0.75 μM ceruloplasmin, 0.5 mM Desferal in 0.2 M sodium acetate buffer, pH 5.4. PPD was oxidized to a purple product, and the activity was measured spectrophotometrically at 525 mμ. The dopa concentration ranged from 0.15 mM to 5.0 mM. A Beckman DK-1 recording spectrophotometer, equipped with a thermo cell (1 cm light path) was used and the temperature was kept at 30° (± 0.2°) in all experiments.

RESULTS

Addition of ceruloplasmin to solutions containing L- and D-dopa results in the formation of a brownish colored product, dopachrome, even in the presence of large amounts of the strong iron chelating agent, Desferal. The dopachrome spectrum is characterized by absorption maxima at 305 mμ[6] and 480 mμ, the former being considerably higher than the latter, and was used for studying the rate of chrome formation in the kinetic experiments.

The molar absorption at 305 mμ was determined according to Osaki,[7] using tyrosinase for catalyzing the oxidation of L-dopa and D-dopa and recording the time course of chrome formation. The final absorbance readings were plotted against substrate concentration as shown in Fig. 1. A molar absorp-

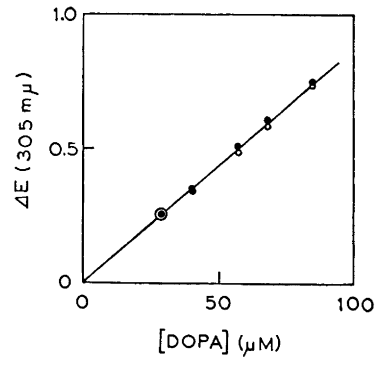

Fig. 1. Final absorbance readings at 305 mμ plotted against the initial concentration of L-dopa (○) and D-dopa (●).

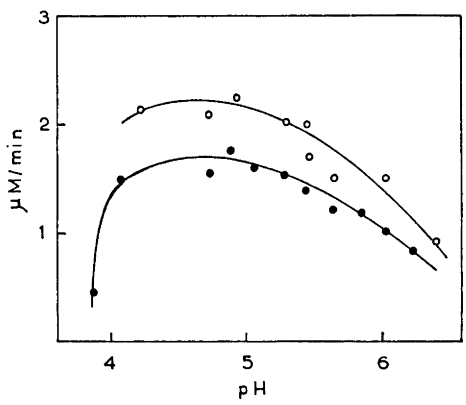

Fig. 2. pH activity curve of the ceruloplasmin catalyzed oxidation of L-dopa (○) and D-dopa (●). The reaction mixture contained 1.3 μM ceruloplasmin, 10 mM dopa, 0.5 mM Desferal in 0.15 M sodium acetate buffer at 30°.

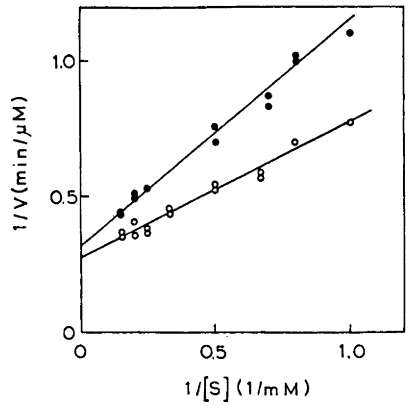

Fig. 3. Effect of L-dopa (○) and D-dopa (●) on the ceruloplasmin oxidase activity.

tion of 8.7×10^3 M^{-1} cm^{-1} was adopted for dopachrome at 305 mμ when both L-dopa and D-dopa acted as substrate. Because of its greater efficiency as a catalyzing agent, tyrosinase was preferred to ceruloplasmin in these experiments.

The oxidation of dopa at different conditions is presented in Figs. 2 and 3. It is clearly seen that L-dopa is more rapidly oxidized by ceruloplasmin than D-dopa over a wide range of pH and substrate concentrations. For both substrates a pH optimum at approximately 4.6 was observed. The activity slowly drops at higher pH (Fig. 2). Straight lines were obtained when the reciprocal activity (1/V) was plotted against the reciprocal substrate concentration (1/[S]) (Fig. 3). The apparent Michaelis' constant, K_m, was 1.8 mM and 2.6 mM for L-dopa and D-dopa, respectively, and the catalytic constant 2.8 min^{-1} for the former and 2.4 min^{-1} for the latter. The rate of the ceruloplasmin catalyzed oxidation of L- and D-dopa is slow compared to the enzymic oxidation of other substrates like Fe(II),[8] PPD,[9] and dopamine.[10] In order to investigate the enzyme affinity for L- and D-dopa the inhibitory effect of these slow reacting molecules on the enzymic oxidation of dopamine was measured. As shown in Fig. 4 no significant difference in the degree of inhibition could be detected.

When increasing amounts of either L-dopa or D-dopa were assayed with PPD, no inhibition of the ceruloplasmin catalyzed oxidation of PPD was found (Fig. 4). The lack of inhibitory effect was observed for several PPD concentrations ranging from 0.2 mM to 2.0 mM, excluding the possibility that large PPD concentrations prevent the binding of dopa to ceruloplasmin. The initial rate of PPD oxidation in the absence of dopa could not be satisfactorily

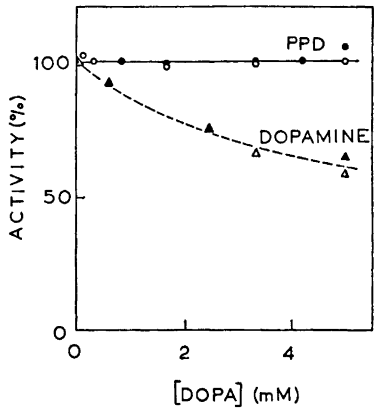

Fig. 4. Initial rate of the enzyme catalyzed oxidation of PPD (solid line) and dopamine (broken line) in the presence of L-dopa (○, △) and D-dopa (●, ▲).

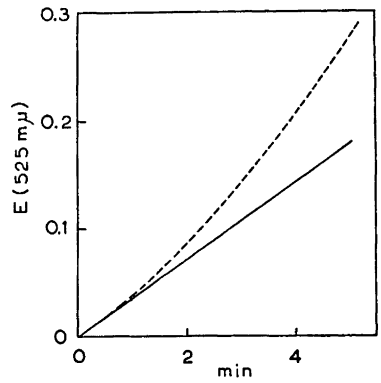

Fig. 5. Recorded time course of the formation of the purple oxidation product of PPD in the absence (broken line) and presence of L-dopa or D-dopa (solid line). The reaction mixture contained 0.75 μM ceruloplasmin, 2.0 mM PPD, 0.5 mM Desferal in 0.2 M sodium acetate buffer, pH 5.4, at 30°. Dopa concentration ranged from 0.15 mM to 5.0 mM.

measured, since the absorbance at 525 mμ did not increase linearly with time during the first minutes as shown in Fig. 5. However, in the presence of L- or D-dopa (0.15 – 5.0 mM) perfectly straight lines were initially obtained.

DISCUSSION

The experiments demonstrate that L- and D-dopa can act as substrate for ceruloplasmin, when the activating effect of ionic iron is prevented by addition of the iron chelating agent, Desferal. The formation of dopachrome involves two oxidative steps,[10] the first being the ceruloplasmin catalyzed oxidation of dopa to dopaquinone. During the process electrons are transferred from substrate to enzyme bound cupric ions, reducing them to cuprous. Molecular oxygen reoxidizes the latter to cupric ions.

The finding that dopa reacts more slowly with ceruloplasmin than other catecholamines, like dopamine and noradrenaline,[10] is in accordance with the observation of Walaas *et al.*,[11] who showed that the blue color of ceruloplasmin is more rapidly reduced by dopamine and isopropylnoradrenaline than dopa, suggesting that the COOH group in dopa to a large extent hinders the interaction with ceruloplasmin.

The greater oxidation velocity exhibited with L-dopa as compared to D-dopa is similar to that found for other copper enzymes, like mushroom, mammalian and *Neurospora* tyrosinases.[12] Whether this is a common property for polyphenol oxidases is not clear. A striking difference between ceruloplasmin

and tyrosinase interaction with catecholamines is that dopamine and noradrenaline are more slowly oxidized by tyrosinase than L-dopa, while the opposite effect was observed with ceruloplasmin.

The investigation of the effect of L-dopa and D-dopa on the enzymic oxidation of dopamine showed that both antipodes inhibited the reaction. This was to be expected since the structural similarity between the three compounds would imply a binding to the same site on the enzyme molecule. Ceruloplasmin bound copper probably participate in the binding of catecholamines since copper readily enters into various types of chelation reactions and because the blue color of the enzyme, due to cupric ions, is rapidly reduced by catecholamines.[11] Ceruloplasmin appears to have equal affinity for L- and D-dopa (Fig. 4). The lack of inhibitory effect of dopa on the initial rate of PPD oxidation (Fig. 4) was surprising and suggests that PPD and catecholamines bind to different sites on the enzyme molecule.

The stabilizing effect of dopa on the formation of the colored oxidation product of PPD (Fig. 5) is not readily explained since the reason for the observed curvature is unknown. According to Peisach and Levine [9] PPD is first oxidized to a free radical. The second step involves the loss of another electron from the free radical, either through a disproportionation process or by reaction with enzyme to form a diradical species which react with PPD to form the purple product. This complex reaction mechanism may give rise to the type of curve observed (Fig. 5), but other effects like enzyme activation by some intermediate product(s) may take place. In the latter case a binding of dopa to the enzyme could prevent the activating effect.

Acknowledgements. The author thanks Professor O. Walaas and Dosent E. Walaas for their help and interest, Mrs. A. Horn for technical assistance and AB Kabi for the generous gift of ceruloplasmin.

REFERENCES

1. Holmberg, C. G. and Laurell, C. B. *Acta Chem. Scand.* **2** (1948) 550.
2. Holmberg, C. G. and Laurell, C. B. *Acta Chem. Scand.* **5** (1951) 476.
3. Curzon, G. and O'Reilly, S. *Biochem. Biophys. Res. Commun.* **2** (1960) 284.
4. McDermott, J. A., Huber, C. T., Osaki, S. and Frieden, E. *Biochim. Biophys. Acta* **151** (1968) 541.
5. Deutsch, H. F. *Arch. Biochem. Biophys.* **89** (1960) 225.
6. Yasunobu, K. T., Peterson, W. H. and Mason, H. S. *J. Biol. Chem.* **234** (1959) 3291.
7. Osaki, S. *Arch. Biochem. Biophys.* **100** (1963) 378.
8. Osaki, S. and Walaas, O. *J. Biol. Chem.* **242** (1967) 2653.
9. Peisach, J. and Levine, W. G. *Biochim. Biophys. Acta* **77** (1963) 615.
10. Løvstad, R. *Acta Chem. Scand.* **25** (1971) 3144.
11. Walaas, E., Walaas, O. and Løvstad, R. In Peisach, J., Aisen, P. and Blumberg, W. E., Eds., *The Biochemistry of Copper*, Academic, New York and London 1966, p. 537.
12. Harrison, W. H., Whisler, W. W. and Ko, S. *J. Biol. Chem.* **242** (1967) 1660.

Received November 16, 1971.