THE OXIDATION OF CYSTEINE BY CERULOPLASMIN

Vincenzo ALBERGONI and Arnaldo CASSINI

Centre of the Italian National Research Council for the Physiology and Biochemistry of Hemocyanins, Institute of Animal Biology, University of Padova, Via Loredan, 10 - 35100 Padova, Italy

Received 12 May 1975

1. Introduction

D- and L-Penicillamine have been reported to inhibit the color development during the oxidation of *p*-phenylendiamine (PPD) by ceruloplasmin [1] When studying this inhibition, we have observed that the substance is itself oxidized by ceruloplasmin. This prompted us to investigate the activity of the protein on the oxidation of sulphydryl compounds and the action upon it of ferric ions eventually present in the system. In this communication the results obtained on D- and L-cysteine are reported. They show that cysteine is rapidly oxidized by ceruloplasmin at physiological pH values and that the reaction is iron independent.

2. Materials and methods

Bovine ceruloplasmin was prepared according to Stokes [2]. The final preparation was chromatographed on Chelex-100 (Bio-Rad) to eliminate contaminating metal ions. The protein had an absorbance ratio A_{610}/A_{280} of 0.042 which corresponds to a purity grade near to 100 per cent.

Oxygen uptake was measured in Warburg vessels with a Gilson respirometer. Spectrophotometric measurements were taken with a Zeiss PMQII spectrophotometer. Free SH groups were determined according to Ellman [3]. In all the experiments the molecular activity is defined as the number of molecules of the substrate transformed per minute by one molecule of ceruplasmin (mol. wt 160 000).

PPD · 2HCl (Merck) was washed with acetone; Desferal (Desferrioxamine mesylate) was purchased from Ciba Pharm. Co. All other reagents were of the highest analytical grade available. Water was twice deionized with a Millipore Milli-Q system.

3. Results

The oxidation of D- and L-cysteine by ceruloplasmin was studied at different pH values and in the presence of EDTA and of Desferal. As shown in fig.1, the optimum pH value is 7.3. Neither 0.24 mM EDTA nor 1.2 mM Desferal have any inhibitory effect on the oxidation rate.

The oxygen consumption has been correlated to

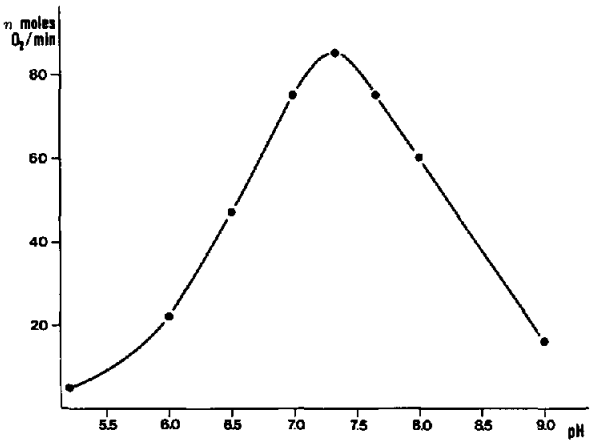


Fig.1. Oxidation of L-cysteine by ceruloplasmin at different pH values. The following buffers were used: 0.2 M acetate (pH 5.2); 0.1 M phosphate (pH 6.0-8.0); 0.1 M Tris–HCl (pH 9.0). The reaction vessels contained 6 mM L-cysteine, and 0.1 μM ceruloplasmin. t=37°C. The experiment was carried out also in the presence of EDTA (0.24 mM) and of Desferal (1.2 mM). See text.

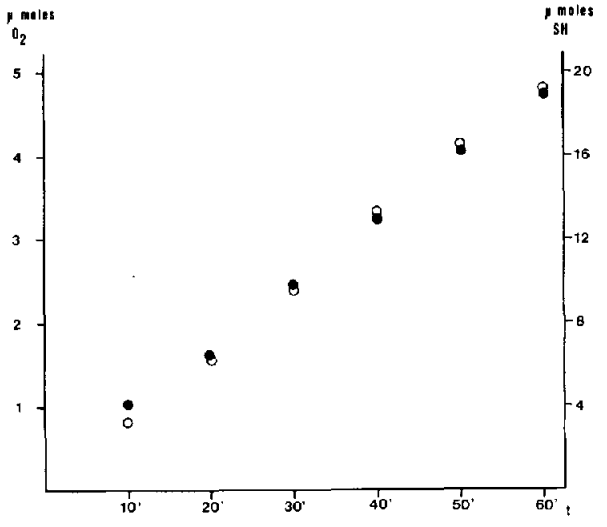
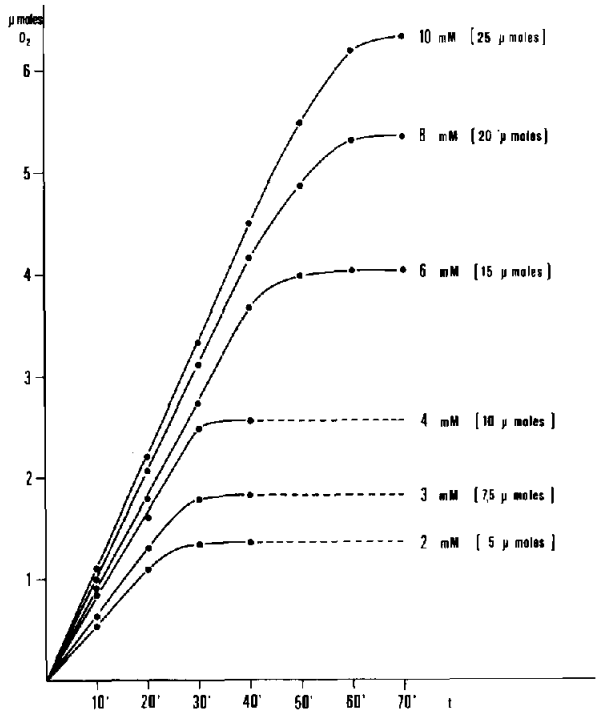


Fig.2. Relationship between oxygen consumption and sulphydryl groups oxidized. The reaction mixture contained: 8 mM L-cysteine, 0.15 μM ceruloplasmin, 0.1 M phosphate buffer pH 8.0. t=37°C. (•) Sulphydryl groups oxidized (○) Oxygen consumption.



the disappearance of free SH groups during the oxidation of cysteine. At intervals, samples of the fraction mixture were taken from the vessels and the amount of cysteine was determined colorimetrically. The results are graphed in fig.2. As it can be seen, the ratio between the sulphydryl groups oxidized and the oxygen consumed ranges from 3.89 to 4.06, in good agreement with the theoretical value of 4 SH/O_2.

The same correlation is observed during the oxidation of different amounts of substrate (fig.3): the ratio cysteine oxidized-oxygen consumed is always equal to 4. At optimum pH value, K_M is equal to 3.1 mM (fig.4) and the Q_{10} value, as determined between 27° and 37°C, is 3.6.

The molecular activity of ceruloplasmin towards cysteine is much larger than towards other substrates as iron (III), ascorbic acid or PPD (table 1). The difference of the optimal pH values found for these reactions (7.3 for cysteine and acidic for the others) cannot be underestimated.

4. Discussion

Ceruloplasmin has been shown to possess oxidase activity for a number of substrates in vitro as aromatic polyamines and polyphenols, enediols and a mixed group of reducing substances [4,5]. The molecular activity is relatively high only for synthetic substrates and on the acidic values of pH. The finding that the enzymic activity of ceruloplasmin is stimulated by iron [6,7], suggested that any substance which is oxidizable by Fe (III) is potentially oxidizable by a coupled iron–ceruloplasmin oxidation system [8–10]. It has also been found that the ascorbate oxidizing activity of ceruloplasmin is strongly inhibited by apotransferrin and by citrate which binds iron whereas it is enormously increased by Fe (II) [11,12]. In view of these facts, McDermott et al., [13] have reexamined the oxidase activity of ceruloplasmin and pointed out the central role of iron in the oxidation

Fig.3. Oxygen consumption at different concentrations of L-cysteine. The reaction system contained 0.1 M phosphate buffer pH 7.3 and 0.1 μM ceruloplasmin. Final vol 2.5 ml. t=37°C. The amount of L-cysteine used is indicated in brackets.

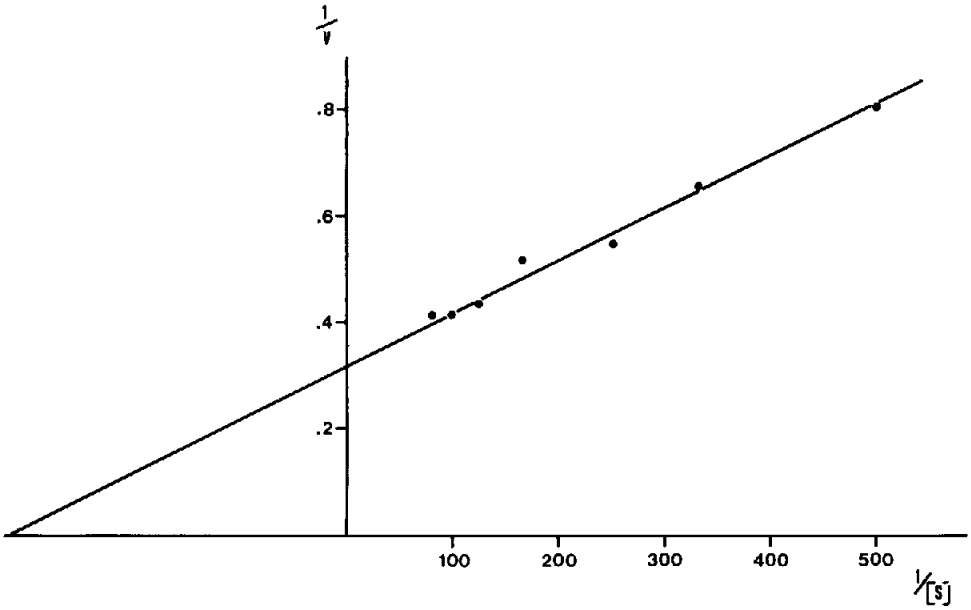


Fig.4. Ceruloplasmin activity at various L-cysteine concentrations. The results are plotted as a Lineweaver–Burk graph. General conditions were: 0.1 μM ceruloplasmin, 0.1 M phosphate buffer pH 7.3. t=37°C.

Table 1
Molecular activity of ceruloplasmin for L-cysteine and other substrates

Substrates	pH values and other conditions										
	5.9[a]	5.5[b]	5.3[c]	6.3[d]	5.2[e]	5.2[f]	5.2[g]	6.5[h]	5.5[i]	5.2[l]	7.3[m]
Fe (II)								555			
PPD		74			21	12	25		395		
DPD	67								246		
Ascorbic acid			12	267	15		18				
L-cysteine					1.5		26			6.8	1704
D-cysteine											1114

[a] Human ceruloplasmin, t° 5°C, 0.03 M acetate [12]
[b] Human ceruloplasmin, 17°C, 0.1 M acetate [12]
[c] Human ceruloplasmin, 30°C, 0.2 M acetate [12]
[d] Human ceruloplasmin, 30°C, 0.2 M acetate plus 0.2 mM Fe(II) [12]
[e] Human ceruloplasmin, 30°C, 0.2 M acetate [13]
[f] Human ceruloplasmin, 30°C, 0.2 M acetate plus 30 μM EDTA [13]
[g] Human ceruloplasmin, 30°C, 0.2 M acetate plus 30 μM EDTA and 34.6 μM Fe (III) [13]
[h] Human ceruloplasmin, 30°C, 0.1 M acetate plus apotransferrin 55 μM [12]
[i] Porcine ceruloplasmin, 37°C, 0.1 M acetate [12]
[l] Bovine ceruloplasmin, 37°C, 0.2 M acetate [this study]
[m] Bovine ceruloplasmin, 37°C, 0.1 M phosphate [this study]

of numerous substrates. According to these Authors, those substrates which reduce Fe (III) are not directly oxidized by the enzyme but function in an iron–ceruloplasmin coupled reaction and their oxidation is completely inhibited by iron chelators. Cysteine is considered among these substrates.

The results reported in this communication show that the oxidation of cysteine by ceruloplasmin is not affected at all by Desferal which is a specific chelator for Fe (III) and which has a high stability constant ($\log K = 31$). In our experimental conditions, the suggested coupled reaction therefore, seems to be unlike. We want also to emphasize that the optimum pH value of the oxidation reaction occurs in the physiological range. All previous studies have been carried out at low pH values which were optimal for the oxidation of PPD. Cysteine is not affected by ceruloplasmin at pH 5.2; in these conditions, however, its oxidation occurs if Fe (III) is added to the reaction system [13].

References

[1] Curzon, G. and Cumings, J. N. (1966) in: The Biochemistry of Copper (Peisach, J., Aisen, P. and Blumberg, W. E., eds.), pp. 545–557, Academic Press, New York.
[2] Stokes, R. P. (1967) Clin. Chim. Acta 15, 517–523.
[3] Ellman, G. L. (1959) Arch. Bioch. Biophys. 82, 70–77.
[4] Holmberg, C. G. and Laurell, C. B. (1948) Acta Chem. Scand. 2, 550–556.
[5] Holmberg, C. G. and Laurell, C. B. (1951) Acta Chem. Scand. 5, 476–480.
[6] Curzon, G. (1960) Biochem. J. 77, 66–73.
[7] Curzon, G. and O'Reilly, S. (1960) Biochem. Biophys. Res. Comm. 2, 284–286.
[8] Curzon, G. (1961) Biochem. J. 79, 656–663.
[9] Levine, W. G. and Peisach, J. (1963) Biochim. Biophys. Acta 77, 602–614.
[10] Peisach, J. and Levine, W. G. (1965) J. Biol. Chem. 240, 2284–2289.
[11] Osaki, J., McDermott, A. and Frieden, E. (1964) J. Biol. Chem. 239, PC 364–366.
[12] Osaki, J., McDermott, A., Johnson, D. A. and Frieden, E. (1966) in: The Biochemistry of Copper (Peisach, J., Aisen, P. and Blumberg, W. E., eds.), pp. 559–569. Academic Press, New York.
[13] McDermott, J. A., Huber, C. T., Osaki, S. and Frieden, E. (1968) Biochim. Biophys. Acta 151, 541–557.