DOES CAERULOPLASMIN DISMUTE SUPEROXIDE? NO

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Received 4 June 1980

1. Introduction

Caeruloplasmin (ferroxidase, EC 1.16.3.1) is a copper containing plasma α_2-glycoprotein. Three biological functions have been ascribed to caeruloplasmin: (a) ferroxidase activity; (b) copper transport and storage; and (c) maintenance of copper homeostasis in the tissues. In [1] another function for caeruloplasmin has been proposed. These workers have demonstrated that caeruloplasmin inhibits the reduction of ferricytochrome *c* and of nitroblue tetrazolium by superoxide produced by the aerobic action of xanthine oxidase on hypoxanthine. Consequently it was proposed that caeruloplasmin may perform the function of scavenging any superoxide that leaks in the plasma where the levels of superoxide are extremely low.

Here we report that caeruloplasmin does not catalyse the disproportionation of superoxide anion radicals generated by pulse radiolysis. However, the reduction of the type 1 copper(II) in caeruloplasmin is observed.

2. Materials and methods

Human caeruloplasmin was prepared from Cohn fraction IV of plasma (supplied by the Blood Products Lab., Lister Institute, Elstree, Herts) by the method in [2]. Purified protein displayed an A_{610}/A_{280} ratio of >0.030 and was found to be electrophoretically homogeneous on 7.5% acrylamide gels run in 0.05 M glycine/NaOH buffer (pH 9.5). Concentrations of caeruloplasmin were calculated using the extinction coefficient at 610 nm, $E^{1\%}_{1\,cm} = 0.68$ [3]. Caeruloplasmin was reduced by treatment with ascorbic acid. Copper–zinc superoxide dismutase was prepared from bovine erythrocytes. Pulse radiolysis experiments were carried out using the facilities available at Brunel University, Uxbridge [4]. Superoxide radicals were generated by irradiating an air-equilibrated solution of 50 mM phosphate buffer (pH 7.8) usually containing 100 mM sodium formate as hydroxyl radical scavenger.

3. Results and discussion

The spontaneous disproportionation of superoxide and its reaction with caeruloplasmin and copper–zinc superoxide dismutase are shown in fig.1. The rate constants for the disappearance of superoxide were found to be $0.16 \times 10^5\ M^{-1} . s^{-1}$ for superoxide alone, $3.04 \times 10^5\ M^{-1} . s^{-1}$ for superoxide in the presence of caeruloplasmin and $1.8 \times 10^9\ M^{-1} . s^{-1}$ for superoxide in the presence of superoxide dismutase. The rate of disappearance of superoxide is not markedly increased by the presence of caeruloplasmin. We found no evidence for any accelerated disproportionation comparable to that caused by superoxide dismutase. Since an inhibition of the rate of reduction of ferricytochrome *c* has been observed [1] we investigated the

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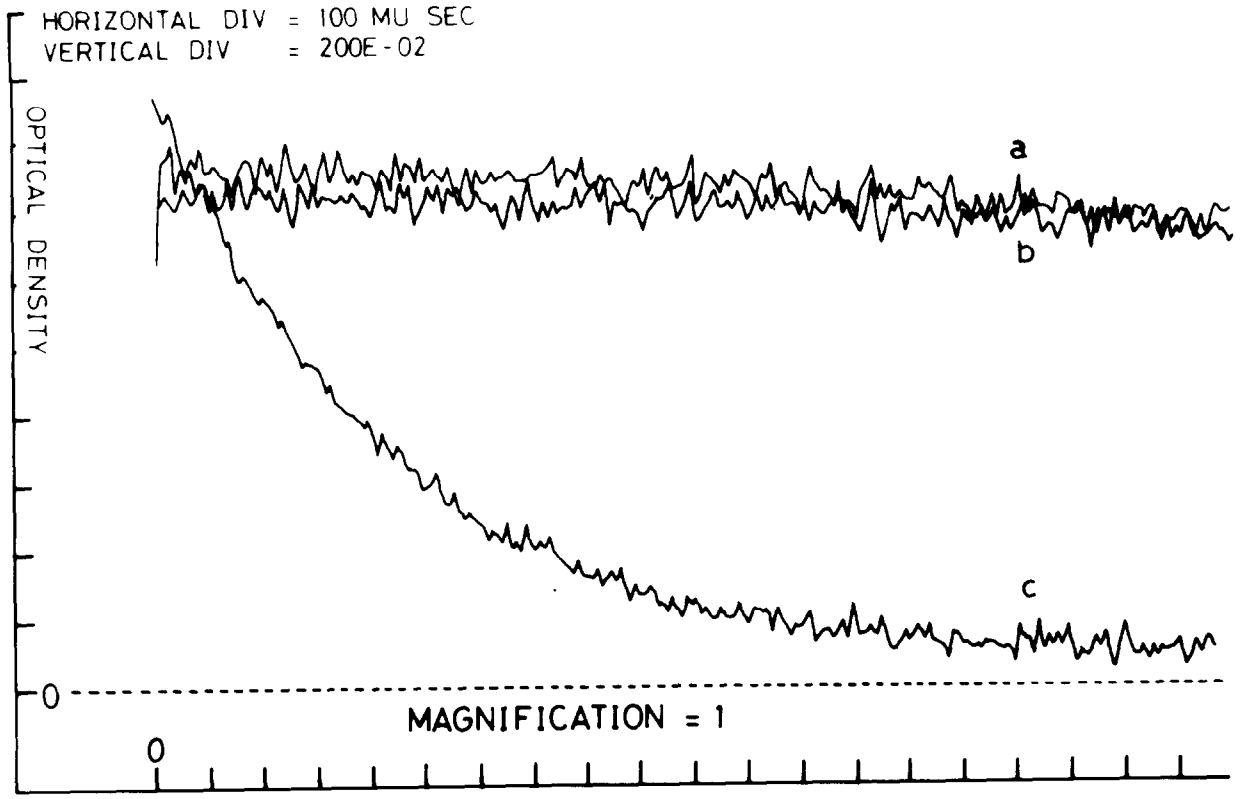

Fig.1. Pulse radiolysis assay. The decay of superoxide was followed at 250 nm: (a) natural decay of superoxide; (b) decay of superoxide in the presence of caeruloplasmin; (c) decay of superoxide in the presence of superoxide dismutase.

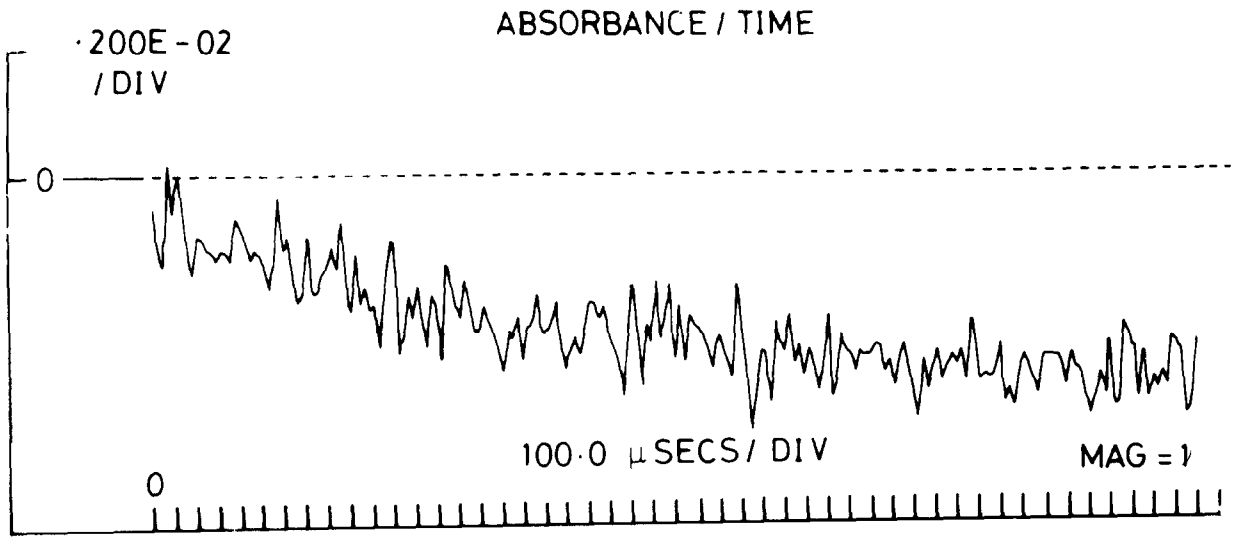

Fig.2. Time-course of the reduction of A_{610}.

reaction between superoxide and caeruloplasmin. No change in A_{330} was observed when superoxide was reacted with caeruloplasmin indicating that there was no reaction between type 3 copper of caeruloplasmin with which the 330 nm band is associated. However, a decrease in A_{610} was observed (fig.2) indicating that reduction of the type 1 copper had taken place. The rate constant for the decrease in A_{610} was found to be $1.8 \times 10^6\ M^{-1} . s^{-1}$. The reduction of the type 1 copper may be caused not only by O_2^- but also by CO_2^- radicals present in the pulse radiolysis system; formate radicals have been demonstrated to react with the type 1 copper of human caeruloplasmin and laccase [5] and superoxide has been shown to reduce the type 1 copper(II) in laccase [6]. When reduced caeruloplasmin was used, no increase in A_{610} was observed indicating that no re-oxidation of the type 1 copper had occurred in contrast to the reaction of superoxide with superoxide dismutase in which re-oxidation of the copper(I) in reduced copper–zinc superoxide dismutase takes place [7]. We also found no increase in A_{420} as observed during stopped-flow investigations of the re-oxidation with air of ascorbate-reduced caeruloplasmin [8]. For a proper dismutation reaction to be established, evidence for the acceleration of the production of hydrogen peroxide as observed with superoxide dismutase [9] must be observed. No increase in hydrogen peroxide production was found during the inhibition of the reduction of cytochrome *c* by caeruloplasmin [1]. It therefore appears that caeruloplasmin does not dismute superoxide and does not mimic the function attributed to superoxide dismutase in [10].

At a lower formate concentration (~50 mM) where hydroxyl radicals may also be present, we have observed spectral changes at 300–500 nm. The spectral changes vanished completely in the presence of high formate concentration (~200 mM). It therefore appears, that, as proposed [11], caeruloplasmin can react with hydroxyl radicals. The mechanism for this reaction is under investigation.

Acknowledgements

J. V. B. and W. H. B. thank the Wellcome Trust for a Research Fellowships. H. A. O. H. thanks the Science Research Council and the Medical Research Council for support. This is a contribution from the Oxford Enzyme Group, of which H. A. O. H. is a member. R. L. W. thanks the Cancer Research Campaign and the Medical Research Council for support.

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