Biochem. J. (1973) **135**, 379–381
Printed in Great Britain

Superoxide Dismutase: a Contaminant of Bovine Catalase

By BARRY HALLIWELL
Botany School, University of Oxford, South Parks Road, Oxford OX1 3RA, U.K.

(*Received* 9 *July* 1973)

Some commercial samples of bovine catalase contain superoxide dismutase activity. Therefore the inhibition of a reaction on the addition of a catalase preparation need not necessarily mean that H_2O_2 is responsible for the reaction.

The superoxide radical ($O_2^{\cdot-}$) is known to be involved in several biochemical reactions. It is an intermediate in the reduction of O_2 by various metalloproteins (for a review see Fridovich, 1973). It is formed on illumination of solutions of flavins (Massey *et al.*, 1969; Ballou *et al.*, 1969). It may also be involved in haemoglobin autoxidation (Misra & Fridovich, 1972), photochemical reactions in chloroplasts (Asada & Kiso, 1973*a*,*b*; Allen & Hall, 1973) and various hydroxylation reactions (Strobel & Coon, 1971; Kumar *et al.*, 1972; Bhatnagar & Liu, 1972).

Proteins catalysing the dismutation of $O_2^{\cdot-}$ radicals to O_2 and H_2O_2 have been demonstrated in a wide range of living organisms, including animals, plants and bacteria (Fridovich, 1973). The enzymes from certain animal tissues were found to be identical with proteins previously isolated on the basis of their copper content, such as erythrocuprein and hepatocuprein. The availability of proteins with superoxide dismutase activity has facilitated investigations of the role of $O_2^{\cdot-}$ in various biochemical systems, including ethylene synthesis (Beauchamp & Fridovich, 1970), destruction of bacteria by leucocytes (Babior *et al.*, 1973), activation of tryptophan oxygenase (Brady *et al.*, 1971) and lipid peroxidation in membranes (Fee & Teitelbaum, 1972; Zimmermann *et al.*, 1973). Many papers in the literature have described an inhibitory effect of catalase on such systems (see, e.g., Rose & Gyorgy, 1950, 1952; Neubert *et al.*, 1962), from which it was concluded that H_2O_2 was responsible for the observed reactions. However, the purity of the catalase preparations used in these experiments is often not made clear. It is shown in the present paper that commercial samples of catalase may be heavily contaminated with superoxide dismutase activity.

Materials and methods

Catalase. From the Sigma Chemical Co. (Kingston-upon-Thames, Surrey, U.K.) was purchased Sigma bovine liver catalase (stock no. C10; 'purified powder'; specific activity 3300 units/mg). This was dissolved in 30mM-K_2HPO_4–KH_2PO_4 buffer, pH 7.8, immediately before use. This preparation is referred to below as Sigma catalase A.

Also from the Sigma Chemical Co. was purchased Sigma bovine liver catalase (stock no. C100; aqueous suspension; 30000 units/mg of protein. This is referred to below as Sigma catalase B.

From the Boehringer Corp. (London W.5, U.K.) was purchased bovine liver catalase (stock no. 7043357; crystalline suspension; 39000 units/mg of protein). This preparation is referred to below as Boehringer catalase.

Other reagents. Xanthine oxidase was obtained from the Boehringer Corp. Cytochrome *c* and Nitro-Blue Tetrazolium were obtained from the Sigma Chemical Co. All other reagents were the highest purity available from BDH Chemicals Ltd. (Poole, Dorset, U.K.).

Assay of superoxide dismutase. This activity was assayed by its ability to inhibit reactions dependent on the generation of $O_2^{\cdot-}$ by a xanthine–xanthine oxidase system. The reactions used were the conversion of Nitro-Blue Tetrazolium into formazan, assayed spectrophotometrically at 560nm (Beauchamp & Fridovich, 1971), and the reduction of cytochrome *c*, followed at 550nm (McCord & Fridovich, 1969). Both assays were carried out at 20°C in a Hilger–Watts H-700 Uvispek spectrophotometer.

Results

Reduction of Nitro-Blue Tetrazolium. In the absence of added catalase, a rapid rate of formazan production was observed in the presence of xanthine and xanthine oxidase (Fig. 1). Addition of Sigma catalase A severely decreased the rate of this reaction. Indeed, addition of 0.1mg of this preparation, containing 330 units of catalase activity, almost completely abolished formazan production. However, the same amount of catalase heated at 100°C for 2min had very little inhibitory effect. It is therefore likely that the preparation is contaminated with superoxide dismutase, since the activity of this enzyme is abolished by heating.

Reduction of cytochrome c. Sigma catalase A also

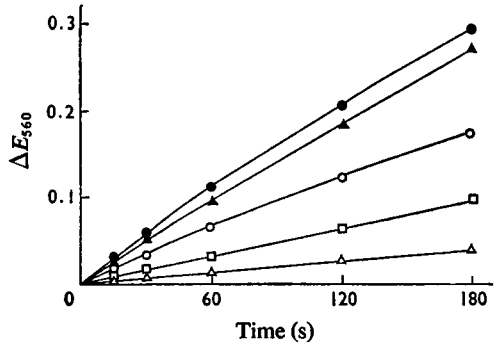

Fig. 1. *Effect of catalase preparations on the reduction of Nitro-Blue Tetrazolium by* $O_2^{\cdot-}$

The assay was carried out as described in the text under 'Materials and methods'. ●, No added catalase; ○, 0.005mg of Sigma catalase A (16 units) added; □, 0.01mg of Sigma catalase A (33 units) added; △, 0.1mg of Sigma catalase A (330 units) added; ▲, 0.1mg of Sigma catalase A previously heated at 100°C for 2min added.

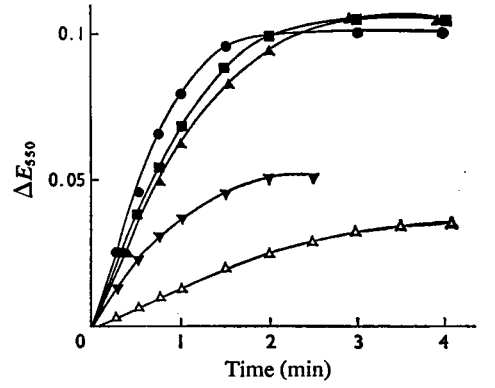

Fig. 2. *Effect of catalase preparations on the reduction of cytochrome c by* $O_2^{\cdot-}$

●, No added catalase; △, 0.1mg of Sigma catalase A (330 units) added; ▲, 0.1mg of Sigma catalase A previously heated at 100°C for 2min added; ■, 2mg of Boehringer catalase (78000 units) added; ▼, 0.5mg of Sigma catalase B (15000 units) added.

decreased the rate of cytochrome *c* reduction by the xanthine–xanthine oxidase system (Fig. 2), and again this inhibitory effect was almost completely abolished by heating the catalase before use. These observations confirm those reported above.

This assay was also used to examine the superoxide dismutase activity of more-purified commercial catalase. Boehringer catalase had little, if any, dismutase activity (Fig. 2). However, Sigma catalase B contained some dismutase activity, although much less than Sigma catalase A. For example, 0.25mg of Sigma catalase B, containing 7500 units of catalase activity, were needed to produce the same degree of inhibition as 0.005mg of Sigma catalase A (containing only 16 units of catalase activity). These results were essentially confirmed with the Nitro-Blue Tetrazolium assay procedure.

Discussion

The results obtained clearly show that catalase of high purity must be used in investigations of the role of H_2O_2 in systems where $O_2^{\cdot-}$ might be involved. Catalase samples of low specific activity are likely to be contaminated with superoxide dismutase activity, since an enzyme of this type is present in bovine liver (Weser *et al.*, 1972). It is often not clear from published papers to what extent the catalase used had been purified, and it is possible that many claims of the inhibition of reactions by catalase alone, especially those in earlier papers, might have been due to contaminating superoxide dismutase activity. Bovine erythrocytes also contain a superoxide dismutase activity (McCord & Fridovich, 1969), and preparations of carbonic anhydrase from this source may be contaminated with this enzyme (McCord & Fridovich, 1968).

I am very grateful to Professor F. R. Whatley for advice on the preparation of this manuscript and to the Science Research Council for a Research Studentship.

Allen, J. F. & Hall, D. O. (1973) *Biochem. Biophys. Res. Commun.* **52**, 856–862
Asada, K. & Kiso, K. (1973*a*) *Eur. J. Biochem.* **33**, 253–257
Asada, K. & Kiso, K. (1973*b*) *Agr. Biol. Chem.* **37**, 453–454
Babior, B. M., Kipnes, R. S. & Curnutte, J. T. (1973) *J. Clin. Invest.* **52**, 741–744
Ballou, D., Palmer, G. & Massey, V. (1969) *Biochem. Biophys. Res. Commun.* **36**, 898–904
Beauchamp, C. & Fridovich, I. (1970) *J. Biol. Chem.* **245**, 4641–4646
Beauchamp, C. & Fridovich, I. (1971) *Anal. Biochem.* **44**, 276–287
Bhatnagar, R. S. & Liu, T. Z. (1972) *FEBS Lett.* **26**, 32–34
Brady, F. O., Forman, H. J. & Feigelson, P. (1971) *J. Biol. Chem.* **246**, 7119–7124
Fee, J. A. & Teitelbaum, H. D. (1972) *Biochem. Biophys. Res. Commun.* **49**, 150–158
Fridovich, I. (1973) *Biochem. Soc. Trans.* **1**, 48–50
Kumar, R. P., Ravindranath, S. D., Vaidyanathan, C. S. & Rao, N. A. (1972) *Biochem. Biophys. Res. Commun.* **48**, 1049–1054
Massey, V., Strickland, S., Mayhew, S. G., Howell, L. G., Engel, P. C., Matthews, R. G., Schuman, M. & Sullivan, P. A. (1969) *Biochem. Biophys. Res. Commun.* **36**, 891–897

McCord, J. M. & Fridovich, I. (1968) *J. Biol. Chem.* **243**, 5753–5760
McCord, J. M. & Fridovich, I. (1969) *J. Biol. Chem.* **244**, 6049–6055
Misra, C. & Fridovich, I. (1972) *J. Biol. Chem.* **247**, 6960–6967
Neubert, D., Wojtczak, A. B. & Lehninger, A. L. (1962) *Proc. Nat. Acad. Sci. U.S.* **48**, 1651–1658
Rose, C. S. & Gyorgy, P. (1950) *Blood* **5**, 1062–1069
Rose, C. S. & Gyorgy, P. (1952) *Amer. J. Physiol.* **168**, 414–418
Strobel, H. W. & Coon, M. J. (1971) *J. Biol. Chem.* **246**, 7826–7829
Weser, U., Prinz, R., Schallies, A., Fretzdorff, A., Krauss, P., Voelter, W. & Voetsch, W. (1972) *Hoppe-Seyler's Z. Physiol. Chem.* **353**, 1821–1831
Zimmermann, R., Flohé, L., Weser, U. & Hartmann, H. J. (1973) *FEBS Lett.* **29**, 117–120