Abilities of peroxidases to catalyse peroxidase–oxidase oxidation of thiols

Björn E. SVENSSON

Research and Development Laboratories, Astra Alab AB, S-15185 Södertälje, Sweden

The abilities of various peroxidases to catalyse the peroxidase–oxidase oxidation of seven aminothiols were studied. Cysteamine and cysteine esters were found to be peroxidase–oxidase substrates for eosinophil peroxidase and myeloperoxidase, whereas other thiols tested were inactive or poorly active with these peroxidases. With lactoperoxidase and horseradish peroxidase, all the tested thiols were inactive or poorly active as peroxidase–oxidase substrates. These studies suggest that a main reason for thiols being poor peroxidase–oxidase substrates is because these thiols are poor peroxidatic substrates.

INTRODUCTION

Peroxidases (donor:H₂O₂ oxidoreductases, EC 1.11.1.7) are widely distributed in Nature, where they perform various physiological functions (reviewed by Dunford, 1982). In animals, haem-containing peroxidases have been found in various body fluids and tissues, for example myeloperoxidase (MPO) and eosinophil peroxidase (EPO) in blood (mainly latent in granulocytes), lactoperoxidase (LPO) in milk, saliva and tears, and thyroid peroxidase in thyroid.

The granulocyte peroxidases (MPO and EPO) have been implicated in antimicrobial host defence (Klebanoff & Clark, 1978; Migler *et al.*, 1978) as well as in deleterious injuries to host structures (Clark, 1983; Agosti *et al.*, 1987). Hence selective modification of the activities of these peroxidases would be of pharmacological interest. Peroxidases are, however, characterized by a poor specificity for oxidizable organic and inorganic substrates (Mason, 1957; Saunders *et al.*, 1964). Moreover, differences in catalytic properties between the various peroxidases have been observed with only a few substrates. For example, thyroid peroxidase has been found to catalyse the bivalent oxidation of a limited number of tyrosine analogues, whereas LPO and HRP catalyse the univalent oxidation of the same compounds (Nakamura *et al.*, 1985). MPO, EPO and chloroperoxidase, but not LPO and HRP, have been found to oxidize chloride (Agner, 1972; Stelmaszyńska & Zgliczyński, 1974; Harrison & Schultz, 1976; Morrison & Schonbaum, 1976; Wever *et al.*, 1981; Griffin, 1983).

Peroxidase–oxidase reactions are characterized by peroxidase-catalysed oxidation of compounds without H₂O₂ addition (Yamazaki & Yokota, 1973). Thiols such as cysteine and glutathione have been reported to be HRP–oxidase substrates (Olsen & Davis, 1976; Harman *et al.*, 1984; Wefers *et al.*, 1985). The thiols cysteamine and cysteine esters, but not cysteine and glutathione, have been reported to be MPO–oxidase substrates (Svensson, 1988a; Svensson & Lindvall, 1988). In the present paper the abilities of EPO, LPO, HRP and MPO to catalyse the peroxidase–oxidase oxidation of various thiols were compared under identical conditions.

EXPERIMENTAL

Materials

Cysteamine hydrochloride, cystamine dihydrochloride, L-cysteine and L-glutathione were bought from Fluka (Buchs, Switzerland), and L-cysteine methyl ester hydrochloride, L-cysteine ethyl ester hydrochloride, D-penicillamine, Nitro Blue Tetrazolium and DTPA were bought from Sigma (St. Louis, MO, U.S.A.). N-Acetylcysteine (99.4%) was manufactured by Astra (Södertälje, Sweden).

Purification of human EPO [dialysed against distilled water; 1290 kat/mol, $A_{414}/A_{280} = 0.74$; $\epsilon_{414} = 1.10 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Bolscher *et al.*, 1984)] and MPO [$\epsilon_{430} = 1.78 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Agner, 1958)] have previously been described (Svensson *et al.*, 1987). Cation-exchange peak IV from chronic-myeloid-leukaemia MPO (dialysed; 677 kat/mol; $A_{430}/A_{480} = 0.83$) was used in Table 1 and Fig. 1 (below) and cation-exchange peak II from normal MPO (without dialysis; 734 kat/mol; $A_{430}/A_{280} = 0.82$) was used in Table 2 (below). Bovine LPO [dialysed; 670 kat/mol; $A_{412}/A_{280} = 0.91$; $\epsilon_{412} = 1.10 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Morrison *et al.*, 1957)], HRP [dialysed; 590 kat/mol; $A_{403}/A_{275} = 3.3$; $\epsilon_{403} = 1.00 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Sawada & Yamazaki, 1973)], bovine catalase [H₂O₂:H₂O₂ oxidoreductase; EC 1.11.1.6; dialysed; 31 000 units/mg (Svensson & Lindvall, 1988)], human superoxide dismutase (O₂^{•-}:O₂^{•-} oxidoreductase; EC 1.15.1.1; 2900 units/mg as assayed by the manufacturer) and human haemoglobin [type IV; dialysed; $\epsilon_{406} = 1.20 \times 10^5 \text{ M}^{-1} \cdot \text{cm}^{-1}$ (Lemberg & Legge, 1949)] were bought from Sigma. The MPO contamination of the EPO preparation was less than 0.5% as analysed by radioimmunoassay, which was kindly carried out by Dr. P. Venge, Department of Clinical Chemistry, University Hospital, Uppsala, Sweden (Carlson *et al.*, 1985). After dialysis the haemoglobin was mainly in its methaemoglobin form, as judged by its visible-light-absorption spectrum.

Methods

O₂ consumption was measured at 37 °C with a Clark-type oxygen electrode (Svensson & Lindvall, 1988), and

Abbreviations used: MPO, myeloperoxidase; EPO, eosinophil peroxidase; LPO, lactoperoxidase; HRP, horseradish peroxidase; DTPA, diethylenetriaminepenta-acetic acid; O₂^{•-}, superoxide radical.

Table 1. Abilities of EPO, LPO, HRP and MPO to catalyse peroxidase-oxidase oxidation of thiols

Incubation mixtures contained 400 μM -thiol/91 nM-EPO, 91 nM-LPO, 100 nM-HRP or 56 nM-MPO, 200 μM -DTPA and 50 mM-potassium phosphate buffer, pH 7.0. Without peroxidase the initial O_2 consumption rate was less than 0.3 $\mu\text{M}/\text{min}$. Mean values and ranges for a number of experiments (n) are shown.

Thiol	Initial O_2 consumption rate ($\mu\text{M}/\text{min}$)											
	EPO			LPO			HRP			MPO*		
	Mean	(Range)	(n)	Mean	(Range)	(n)	Mean	(Range)	(n)	Mean	(Range)	(n)
Cysteamine	5.5	(4.4–6.7)	(4)	1.2	(1.0–1.4)	(4)	0.1	(0–0.4)	(4)	4.2	(3.4–5.2)	(8)
Cysteine	0.8	(0.3–1.2)	(4)	0.7	(0.5–1.0)	(4)	0	(0–0.1)	(4)	0.3	(0–0.5)	(2)
<i>N</i> -Acetylcysteine	0	(0)	(3)	0	(0)	(3)	0	(0)	(3)	0.1	(0–0.4)	(3)
Cysteine methyl ester	5.6	(4.5–7.1)	(4)	0.6	(0–1.1)	(4)	1.3	(0.7–1.7)	(4)	11.0	(10.7–11.3)	(3)
Cysteine ethyl ester	4.4	(3.5–5.3)	(4)	0.5	(0.4–0.5)	(4)	1.7	(1.5–1.8)	(4)	7.2	(6.0–8.0)	(3)
Penicillamine	0.3	(0.3–0.4)	(3)	0.1	(0–0.4)	(3)	0.5	(0.4–0.6)	(3)	0.2	(0.1–0.3)	(2)
Glutathione	0	(0)	(3)	0	(0)	(3)	0	(0)	(3)	0.1	(0.1–0.2)	(2)

* Values taken from Svensson (1988a).

the initial O_2 consumption rate was calculated by assuming that the initial O_2 concentration was 200 μM (Lessler & Brierley, 1969). Cysteamine oxidation was measured by u.v. spectroscopy at 37 °C by recording the increase at 270 nm or the decrease at 235 nm (Svensson & Lindvall, 1988), and the initial cysteamine oxidation rate was calculated by using $\epsilon_{270} = 91 \text{ M}^{-1} \cdot \text{cm}^{-1}$ [difference in the molar absorption coefficient between cysteamine in its oxidized form (cystamine) and reduced form] or $\epsilon_{235} = 283 \text{ M}^{-1} \cdot \text{cm}^{-1}$. Nitro Blue Tetrazolium reduction was measured as previously described (Svensson, 1988a).

Visible-light-absorption spectra were scanned (1200 nm/min) at about 30 °C in a cell with 5 cm light path in a Beckman DU-7HS spectrophotometer. The concentrations of the peroxidases and of methaemoglobin were calculated by using the molar absorption coefficients of their Soret bands. Specific enzyme activities of the peroxidases were determined with guaiacol as substrate in 0.1 M-potassium phosphate buffer, pH 8.0, as described by Himmelhoch *et al.* (1969). Enzyme activity was expressed as mol of H_2O_2 consumed/s (= 1 kat) by using a molar absorption coefficient (ϵ_{470}) of $26.6 \text{ mM}^{-1} \cdot \text{cm}^{-1}$ for tetraguaiacol (Chance & Maehly, 1955).

RESULTS

Abilities of EPO, LPO, HRP and MPO to catalyse peroxidase-oxidase oxidation of thiols

Seven thiols were compared as EPO-oxidase substrates. Only cysteamine and the cysteine esters were found to be good peroxidase-oxidase substrates as measured by the initial O_2 consumption rates (Table 1). This structure-activity relationship found with EPO is the same as that previously found with MPO, and it has been found that a main reason for the thiols being poor MPO-oxidase substrates is that these thiols are poor peroxidatic substrates for MPO (Svensson, 1988a).

When LPO was used as peroxidase enzyme, all thiols were found to be less effective as peroxidase-oxidase substrates. With HRP only did the cysteine esters result in some O_2 consumption. These results indicate that differences exist also between peroxidases in their relative abilities to catalyse peroxidase-oxidase oxidation of thiols.

Reasons for the different abilities of peroxidases to catalyse peroxidase-oxidase oxidation of cysteamine

In order to investigate whether the differences between the peroxidases found in Table 1 were related to an inhibition by excess enzyme (Yamazaki & Piette, 1963; Yokota & Yamazaki, 1977; Svensson & Lindvall, 1988), the peroxidase-oxidase oxidation of cysteamine by varied concentrations of the peroxidases was studied.

Cysteamine was not oxidized by HRP over the peroxidase concentration range 25–400 nM (Fig. 1). By contrast, both EPO and LPO were found to catalyse the oxidation of cysteamine over the same concentration range. Moreover, the dependence on peroxidase concentration indicates that both the EPO- and LPO-mediated oxidations are less sensitive than the MPO-mediated oxidation to excess peroxidase. Analysis of the u.v.-absorption spectrum of cysteamine showed that the changes observed were consistent with disulphide (cystamine) formation (results not shown). Thus inhibition by excess peroxidase cannot explain the differences in activities of the peroxidases.

Under the conditions described in Fig. 1 and with 91–100 nM-peroxidase haem, addition of 16 μg of catalase/ml abolished peroxidase-oxidase oxidation of cysteamine (< 1 $\mu\text{M}/\text{min}$). After substitution of haemoglobin (83–1330 nM-haem) for peroxidase, no changes in the u.v.-absorption spectrum of cysteamine were observed (< 0.5 $\mu\text{M}/\text{min}$). The inability of haemoglobin to catalyse the peroxidase-oxidase oxidation of cysteamine as well as the inhibition by catalase are consistent with this reaction requiring a peroxidatic oxidation step (Svensson & Lindvall, 1988).

Possible differences between the peroxidases in their abilities to catalyse the peroxidatic oxidation (i.e. with added H_2O_2) of cysteamine were examined. These studies were carried out with 100 μM -cysteamine, a concentration at which the peroxidase-oxidase oxidation of cysteamine is small. Both EPO and MPO catalysed peroxidatic oxidation of cysteamine, as measured by the initial oxidation rate and Nitro Blue Tetrazolium reduction (Table 2). These findings were confirmed by studying changes in the u.v.-absorption spectrum of cysteamine during the reactions (Svensson & Lindvall, 1988). These changes were consistent with disulphide (cystamine)

Fig. 1. Influence of peroxidase concentration on peroxidase-oxidase oxidation of cysteamine

Incubation mixtures contained 400 μM -cysteamine hydrochloride, EPO (LPO, HRP or MPO) at indicated haem concentrations, 200 μM -DTPA and 50 mM-potassium phosphate buffer, pH 7.0. Cysteamine oxidation was measured at 270 nm. Mean values and ranges for duplicate experiments are shown.

formation (results not shown). Although the initial oxidation rate indicated that cysteamine oxidation was not catalysed by LPO, some Nitro Blue Tetrazolium reduction was observed. A slight oxidation was confirmed by examining the u.v.-absorption changes, which indicated a minute cystamine formation after 5 min incubation (results not shown). With HRP no cysteamine oxidation was observed with any of the methods.

Changes in the oxidation states of the various peroxidases

Diluted HRP and MPO in buffered solutions have been found previously to exist in their compound II oxidation states (Smith *et al.*, 1982; Svensson, 1988b). When LPO was added to the phosphate buffer, it was found also to be in its compound II oxidation state (Fig. 2b, ----) when this spectrum was compared with the previously reported LPO spectra [Kimura & Yamazaki (1979) and references cited therein]. Addition of HRP to the buffer yielded a spectrum that is a mixture of its compound I and its compound II spectrum (Fig. 2c, ----) [Yokota & Yamazaki (1977) and references cited therein]. The corresponding spectrum obtained with EPO may be the compound II spectrum of EPO (Fig. 2a,

Table 2. Abilities of EPO, LPO, HRP and MPO to catalyse peroxidatic oxidation of cysteamine

Incubation mixtures contained 100 μM -cysteamine hydrochloride, 91 nM-EPO, 91 nM-LPO, 100 nM-HRP or 56 nM-MPO, 50 μM - H_2O_2 , 25 μM -Nitro Blue Tetrazolium (in the assay of Nitro Blue Tetrazolium reduction), 200 μM -DTPA and 50 mM-potassium phosphate buffer, pH 7.0. Reactions were started by the addition of 20 μl portions of cysteamine, and absorption changes at 235 nm (cysteamine oxidation) and 560 nm (Nitro Blue Tetrazolium reduction) were recorded. On omission of peroxidase or H_2O_2 the initial cysteamine oxidation rate was $< 1 \mu\text{M}/\text{min}$ and Nitro Blue Tetrazolium reduction was $< 15 \times 10^{-4} \Delta A_{560}/2.5 \text{ min}$. Nitro Blue Tetrazolium reduction was inhibited by the addition of 1 μg of superoxide dismutase/ml ($\Delta A_{560}/2.5 \text{ min} < 6 \times 10^{-4}$), which indicates that Nitro Blue Tetrazolium reduction measures $\text{O}_2^{\cdot -}$ formation. Mean values and ranges for triplicate experiments are shown.

Peroxidase	Initial cysteamine oxidation rate ($\mu\text{M}/\text{min}$)		Nitro Blue Tetrazolium reduction ($10^4 \times \Delta A_{560}/2.5 \text{ min}$)	
	Mean	(Range)	Mean	(Range)
EPO	9	(8–12)	362	344–373
LPO	1	(1)	62	(57–70)
HRP	0	(0)	5	(0–11)
MPO	17	(16–18)	494	(488–504)

----), although, as far as can be ascertained, the visible-light-absorption spectra of oxidized EPO have not previously been reported [Svensson *et al.* (1987) and references cited therein]. EPO compound II formation is supported by the close resemblance with the compound II spectrum of LPO. It cannot, however, be excluded that the spectrum is the compound I spectrum or a mixture of the two spectra. For comparison the ferric-peroxidase spectra (obtained in the presence of catalase) are shown (.....).

On addition of cysteamine to LPO, the compound II spectrum was changed into a typical compound III spectrum (Fig. 2b, —). Also, in the presence of 16 μg of catalase/ml, a LPO compound III spectrum was obtained on addition of cysteamine (results not shown). The spectral change observed on addition of cysteamine to EPO (Fig. 2a, —) was similar to that found with LPO. The same spectral change was found in the presence of 16 μg of catalase/ml (results not shown). This spectrum was different from those of native and reduced EPO and of EPO in buffer. Thus these results indicate that, similarly to MPO (Svensson, 1988b), compound III is formed during the EPO- and LPO-oxidase oxidation of cysteamine. In contrast with this result, the compound III spectrum was not observed on addition of cysteamine to HRP, but instead a typical compound II spectrum was obtained (Fig. 2c, —). In the presence of 16 μg of catalase/ml, no changes in the visible-light-absorption spectrum of HRP were observed on addition of cysteamine, i.e. HRP remained in its ferric oxidation state (results not shown).

Fig. 2. Changes in the visible-light-absorption spectra of EPO, LPO and HRP induced by cysteamine

400 μ M-Cysteamine hydrochloride was added to 91 nM-EPO (or 91 nM-LPO or 200 nM-HRP)/200 μ M-DTPA/50 mM-potassium phosphate buffer, pH 7.0, and the absorption spectrum scanned after 0.5 min (—). Scans before cysteamine addition with (....., i.e. ferric peroxidase) and without (----, i.e. compound I and/or compound II) 16 μ g of catalase/ml are included as references. Typical results are shown.

DISCUSSION

On the basis of previous studies with MPO and various thiols it has been suggested that the mechanism for the propagation and amplification of the MPO-oxidase oxidation of aminothiols consists of two interdependent processes, i.e. H_2O_2 -mediated peroxidatic oxidation of thiol, yielding a thiyl radical, and the further non-enzymic oxidation of the thiyl radical to the corresponding disulphide, with concomitant O_2 consumption and H_2O_2

regeneration (Svensson, 1988a). A minute autoxidation of thiol, even in the presence of metal-ion chelator, has been detected by compound III formation, but this autoxidation does not seem to be necessary for initiation, because dilute MPO has been found to exist in its compound II oxidation state [the preceding paper (Svensson, 1988b)].

In the present paper it was found that even EPO- and LPO-oxidase oxidation of cysteamine involves a per-

oxidative oxidation step, and that dilute EPO and LPO exist in their compound II oxidation states. Therefore it is proposed that even the EPO- and LPO-oxidase oxidation of thiols occurs according to the scheme of reactions previously proposed for the MPO-oxidase oxidation of thiols (Svensson, 1988a,b).

A possible explanation for the different abilities of the peroxidases to catalyse the peroxidase-oxidase oxidation of cysteamine and cysteine esters (Table 1) may be the differential ability of the peroxidases to catalyse peroxidative oxidation of the thiols (Table 2). In the case of HRP this explanation is also supported by the finding that compound II accumulates on addition of cysteamine to HRP (Fig. 2c). The bimolecular rate constant for the reaction of cysteamine with MPO compound II has been determined to be about $2 \times 10^3 \text{ M}^{-1} \cdot \text{s}^{-1}$ (Svensson, 1988b). By using the results in Fig. 1, the corresponding rate constant for the reaction of cysteamine with HRP compound II was calculated to be less than $100 \text{ M}^{-1} \cdot \text{s}^{-1}$.

Cysteine and glutathione have previously been found to be HRP-oxidase substrates at higher concentrations of thiol and enzyme (Olsen & Davies, 1976; Harman *et al.*, 1984; Wefers *et al.*, 1985), but in the present study these thiols were found to be poor substrates with all peroxidases studied (Table 1). A possible explanation for this anomaly may be that these thiols have only been reported to be peroxidative substrates at higher thiol concentrations (Turkall & Tsan, 1982; Ross *et al.*, 1985; Harman *et al.*, 1986; Nakamura *et al.*, 1986; Svensson, 1988a). However, with the thiols that were inactive with all the peroxidases, it cannot be excluded that an inability to regenerate sufficient H_2O_2 during their non-enzymic oxidation to disulphides might also contribute (Svensson, 1988a).

The scanning of the visible-light-absorption spectra of EPO and LPO (Figs. 2a and 2b) and MPO (Svensson, 1988b) during the peroxidase-oxidase oxidation of cysteamine indicates that compound III is formed during these reactions. Previous studies have indicated that the inhibition of cysteamine oxidation by excess MPO is due to compound III formation through the reaction of $\text{O}_2^{\cdot -}$ with ferric MPO (Svensson, 1988b) and thus competing with the essential reaction of $\text{O}_2^{\cdot -}$ with cysteamine (Svensson & Lindvall, 1988). MPO is unique among peroxidases in that it contains two iron centres, whereas other haem-containing peroxidases contain only one. If the haem groups of MPO are assumed to be equivalent and to function independently (Andrews & Krinsky, 1981; Babcock *et al.*, 1985), then it is consistent to reason that MPO compound III formation can hamper the peroxidase-oxidase reaction even at low MPO concentration, since the adjacent haem group of MPO might compete for the generated $\text{O}_2^{\cdot -}$ even at low MPO concentration. Thus a possible explanation for the lower degree of inhibition by excess EPO and LPO (as compared with MPO) might be that EPO and LPO contain one haem group, whereas MPO contains two.

In this paper it was shown that differences exist between the granulocyte peroxidase (i.e. MPO and EPO), LPO and HRP with respect to peroxidase-oxidase oxidation of some aminothiols. These results suggest that it might be feasible to selectively modify the activities of the granulocyte peroxidases.

I thank Sven Lindvall for his generous and encouraging support and a critical review of this paper before its submission.

I also thank Dr. Desmond Lake-Bakaar for linguistic corrections and Ms. Inga Zander and Ms. Ruth Lundin for professional typing of the manuscript.

REFERENCES

- Agner, K. (1958) *Acta Chem. Scand.* **12**, 89–94
- Agner, K. (1972) in *Structure and Function of Oxidation-Reduction Enzymes* (Åkeson, Å. & Ehrenberg, A., eds.), pp. 329–335, Pergamon Press, Oxford
- Agosti, J. M., Altman, L. C., Ayars, G. H., Loegering, D. A., Gleich, G. J. & Klebanoff, S. J. (1987) *J. Allergy Clin. Immunol.* **79**, 496–504
- Andrews, P. C. & Krinsky, N. I. (1981) *J. Biol. Chem.* **256**, 4211–4218
- Babcock, G. T., Ingle, R. T., Oertling, W. A., Davis, J. C., Averill, B. A., Hulse, C. L., Stufkens, D. J., Bolscher, B. G. J. M. & Wever, R. (1985) *Biochim. Biophys. Acta* **828**, 58–66
- Bolscher, B., Plat, H. & Wever, R. (1984) *Biochim. Biophys. Acta* **784**, 177–186
- Carlson, M. G. C., Petterson, C. G. B. & Venge, P. (1985) *J. Immunol.* **134**, 1875–1879
- Chance, B. & Maehly, A. C. (1955) *Methods Enzymol.* **2**, 764–775
- Clark, R. A. (1983) *Adv. Inflammation Res.* **5**, 107–146
- Dunford, H. B. (1982) *Adv. Inorg. Biochem.* **4**, 41–68
- Griffin, B. W. (1983) *Biochem. Biophys. Res. Commun.* **116**, 873–879
- Harman, L. S., Mottley, C. & Mason, R. P. (1984) *J. Biol. Chem.* **259**, 5606–5611
- Harman, L. S., Carver, D. K., Schreiber, J. & Mason, R. P. (1986) *J. Biol. Chem.* **261**, 1642–1648
- Harrison, J. E. & Schultz, J. (1976) *J. Biol. Chem.* **251**, 1371–1374
- Himmelhoch, S. R., Evans, W. H., Mage, M. G. & Peterson, E. A. (1969) *Biochemistry* **8**, 914–921
- Kimura, S. & Yamazaki, I. (1979) *Arch. Biochem. Biophys.* **198**, 580–588
- Klebanoff, S. J. & Clark, R. A. (1978) *The Neutrophil: Function and Clinical Disorders*, North-Holland, Amsterdam
- Lemberg, R. & Legge, J. W. (1949) *Hematin Compounds and Bile Pigments: Their Constitution, Metabolism and Function*, p. 748, Interscience, New York
- Lessler, M. A. & Brierley, G. P. (1969) *Methods Biochem. Anal.* **17**, 1–29
- Mason, H. S. (1957) *Adv. Enzymol. Relat. Areas Mol. Biol.* **19**, 79–233
- Migler, R., DeChatelet, L. R. & Bass, D. A. (1978) *Blood* **51**, 445–456
- Morrison, M. & Schonbaum, G. R. (1976) *Annu. Rev. Biochem.* **45**, 861–888
- Morrison, M., Hamilton, H. B. & Stotz, E. (1957) *J. Biol. Chem.* **228**, 767–776
- Nakamura, M., Yamazaki, I., Kotani, T. & Ohtaki, S. (1985) *J. Biol. Chem.* **260**, 13546–13552
- Nakamura, M., Yamazaki, I., Ohtaki, S. & Nakamura, S. (1986) *J. Biol. Chem.* **261**, 13923–13927
- Olsen, J. & Davies, L. (1976) *Biochim. Biophys. Acta* **445**, 324–329
- Ross, D., Norbeck, K. & Moldéus, P. (1985) *J. Biol. Chem.* **260**, 15028–15032
- Saunders, B. C., Holmes-Siedle, A. G. & Sart, B. P. (1964) *Peroxidase*, Butterworths, London
- Sawada, Y. & Yamazaki, I. (1973) *Biochim. Biophys. Acta* **327**, 257–265
- Smith, A. M., Morrison, W. L. & Milham, P. J. (1982) *Biochemistry* **21**, 4414–4419

- Stelmaszyńska, T. & Zgliczyński, J. M. (1974) *Eur. J. Biochim.* **45**, 305–312
- Svensson, B. E. (1988a) *Biochem. J.* **253**, 441–449
- Svensson, B. E. (1988b) *Biochem. J.* **256**, 751–755
- Svensson, B. E. & Lindvall, S. (1988) *Biochem. J.* **249**, 521–530
- Svensson, B. E., Domeij, K., Lindvall, S. & Rydell, G. (1987) *Biochem. J.* **242**, 673–680
- Turkall, K. M. & Tsan, M. (1982) *J. Reticuloendothelial Soc.* **31**, 353–360
- Wefers, H., Riechman, E. & Sies, H. (1985) *J. Free Radical Biol. Med.* **1**, 311–318
- Wever, R., Plat, H. & Hamers, M. N. (1981) *FEBS Lett.* **123**, 327–331
- Yamazaki, I. & Piette, L. H. (1963) *Biochim. Biophys. Acta* **77**, 47–64
- Yamazaki, I. & Yokota, K. (1973) *Mol. Cell. Biochem.* **2**, 39–52
- Yokota, K. & Yamazaki, I. (1977) *Biochemistry* **16**, 1913–1920

Received 26 February 1988/24 June 1988; accepted 30 June 1988