Characterization of the Major Hydroperoxide-reducing Activity of Human Plasma

PURIFICATION AND PROPERTIES OF A SELENIUM-DEPENDENT GLUTATHIONE PEROXIDASE

(Received for publication, May 26, 1987)

Krishna Rao Maddipati and Lawrence J. Marnett‡

From the Department of Chemistry, Wayne State University, Detroit, Michigan 48202

We have recently characterized the major hydroperoxide-reducing enzyme of human plasma as a glutathione peroxidase (Maddipati, K. R., Gasparski, C., and Marnett, L. J. (1987) Arch. Biochem. Biophys. 254, 9–17). We now report the purification and kinetic characterization of this enzyme. The purification steps involved ammonium sulfate precipitation, hydrophobic interaction chromatography on phenyl-Sepharose, anion exchange chromatography, and gel filtration. The purified peroxidase has a specific activity of 26–29 nmol/min/mg with hydrogen peroxide as substrate. The human plasma glutathione peroxidase is a tetramer of identical subunits of 21.5 kDa molecular mass as determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and is different from human erythrocyte glutathione peroxidase. The plasma peroxidase is a selenoprotein containing one selenium per subunit. Unlike several other glutathione peroxidases this enzyme exhibits saturation kinetics with respect to glutathione ($K_m$ for glutathione = 4.3 mM). The peroxidase exhibits high affinity for hydroperoxides with $K_m$ values ranging from 2.3 μM for 13-hydroperoxy-9,11-octadecadienoic acid to 13.3 μM for hydrogen peroxide at saturating glutathione concentration. These kinetic parameters are suggestive of the potential of human plasma glutathione peroxidase as an important regulator of plasma hydroperoxide levels.

Fatty acid hydroperoxides are the primary products of enzymatic and nonenzymatic oxygenation of unsaturated fatty acids. They are intermediates in the biosynthesis of prostaglandins, leukotrienes, and a host of biologically active molecules. Organic hydroperoxides are relatively stable but react with transition metal complexes and metalloproteins to generate highly toxic free radicals (1). Another important toxicological aspect of hydroperoxides is their role in the modulation of enzyme activities of the arachidonic acid cascade. Regulation of the vascular prostacyclin/thromboxane ratio is considered an important factor in the development of several vascular diseases (2). Prostacyclin and thromboxane synthases that are responsible for the synthesis of these important physiological substances are differentially inactivated by fatty acid hydroperoxides. Whereas thromboxane synthase is relatively insensitive to fatty acid hydroperoxides, prostacyclin synthase is inactivated at hydroperoxide concentrations as low as 0.5 μM (3, 4). The emergence of the concept of "peroxide tone" of tissues (5) as a result of this selectivity in inactivation gained support in recent years from the observation that atherosclerotic lesions contain elevated levels of lipid hydroperoxides (6, 7).

The major metabolic fate of fatty acid hydroperoxides, apart from their conversion to physiological end products, is reduction by peroxidases to the corresponding alcohols. We recently reported the characterization of the major hydroperoxide-reducing activity of human plasma, showed that it is dependent on reduced glutathione, and demonstrated that it is a glutathione peroxidase (8). This glutathione peroxidase activity appears to account for all the hydroperoxide-reducing activity of human plasma. Considering the potential deleterious effects of fatty acid hydroperoxides, this peroxidase activity may play an important protective role against hydroperoxide pathology. We have undertaken the purification of this glutathione peroxidase from human plasma to study its physical and kinetic properties and as first step in elucidation of its involvement in the control of circulating fatty acid hydroperoxide levels. Our results are described herein.

MATERIALS AND METHODS

Fresh frozen human plasma was obtained from a local American Red Cross center. Glutathione, NADPH, glutathione reductase from baker's yeast, phenyl-Sepharose CL-4B, DEAE-Sepharadex A-50, protein standards for SDS-PAGE and gel filtration chromatography, human erythrocyte glutathione peroxidase were purchased from Sigma. Sephadex G-200 was obtained from Pharmacia LKB Biotechnology Inc., and 2,3-diaminonaphthalene hydrochloride was from Aldrich. Selenious acid was purchased from Alfa Products. 13-OOH-18:2 and 15-OOH-20:4 were prepared from linoleic and arachidonic acids, respectively, using soybean lipoxygenase according to the method of Funk et al. (9). PPHP was prepared according to the method of Marnett (11) using hydrogen peroxide as substrate. In brief, the enzyme was incubated in 0.1 M Tris-HCl containing 5 mM EDTA, 1 mM glutathione, 0.2 mM NADPH, 1 mM sodium azide, and 1 IU of glutathione peroxidase. Assay of Glutathione Peroxidase—Glutathione peroxidase activity was assayed by the coupled assay described by Paglia and Valentine (11) using hydrogen peroxide as substrate. In brief, the enzyme was incubated in 0.1 M Tris-HCl containing 5 mM EDTA, 1 mM glutathione, 0.2 mM NADPH, 1 mM sodium azide, and 1 IU of glutathione peroxidase. The specificity of the assay was determined by the ability of the enzyme to reduce hydrogen peroxide in the presence of various substrates.

1 The abbreviations used are: SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; 13-OOH-18:2, 13-hydroperoxy-9,11-octadeccadienoic acid; 15-OOH-20:4, 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid; PPHP, 5-phenyl-1-pentenyl hydroperoxide; HPLC, high performance liquid chromatography.
Purification of Human Erythrocyte Glutathione Peroxidase—Commercially available human erythrocyte glutathione peroxidase contains considerable impurities. To compare human plasma glutathione peroxidase with erythrocyte enzyme, the commercial erythrocyte peroxidase was purified by HPLC gel filtration on a TSK G4000SW column using the conditions described above. Each peak eluted from the column was tested for peroxidase activity by the coupled assay described above.

Molecular Mass Determination—The molecular mass of the purified human plasma peroxidase was determined by gel filtration. This was done using a conventional Sephacryl S-200 column and by HPLC gel filtration on a TSK G4000SW column. In both methods buffer C was used as the mobile phase. Gel filtration on Sephacryl S-200 was performed on a 1.6 x 80-cm column at a flow rate of 10 ml/h. Protein samples were applied to the column in 1 ml of buffer C containing 5% glycerol. The eluent was collected in 1.5-ml fractions and their 280 nm absorbances and peroxidase activities determined. The elution volume for each protein was determined separately. HPLC gel filtration on TSK G4000SW (7.5 x 300 mm) was carried out by connecting two columns in series on a Varian model 5000 liquid chromatograph. All protein samples were dissolved in buffer C and injected separately. The injection volume was 50 µl. The column was eluted at 1 ml/min flow rate and the eluent was monitored at 280 nm. In addition to UV absorbance, the peroxidase peaks were confirmed by activity measurement.

Subunit Molecular Mass Determination—SDS-PAGE was performed according to the procedure of Laemmli using a 12% acrylamide gel (13). The mixture of molecular mass standards in SDS-7 (Sigma) was used for molecular weight determination. All the samples were heat-denatured (5 min at 80 °C) in the presence of β-mercaptoethanol (2.5%) and SDS (1%). The gel was stained by Coomassie Blue.

**RESULTS**

Purification of Peroxidase from Human Plasma—Table I presents a summary of the purification of glutathione peroxidase activity from human plasma. Precipitation of peroxidase was found to be optimal at 20–30% saturation of ammonium sulfate, although there was a loss of about 50% of the activity. Recovery of peroxidase was greater at higher ammonium sulfate concentrations, but precipitation of extraneous protein negated the advantage of higher recovery. Hydrophobic interaction chromatography on phenyl-Sepharose proved to be a

**FIG. 1. Hydrophobic interaction chromatography on phenyl-Sepharose CL-4B.** The profile shows only the gradient elution of the column. Details are given under "Materials and Methods."

**TABLE 1**

| Purification step | Total activity | Protein | Specific activity | Recovery | Purification |
|-------------------|----------------|---------|------------------|----------|--------------|
| Plasma (NH₄)₂SO₄ | 152 units | 80.3 g | 0.002 units/mg | 100 | 1 |
| (20–30%) | 80 | 9.6 | 0.008 | 52 | 4 |
| DEAE-Sepharose | 60 | 0.039 | 1.554 | 40 | 800 |
| DEAE-Sepharose (A-50) | 41 | 0.0085 | 4.76 | 26 | 2,500 |
| Gel filtration (G-200) | 29 | 0.0011 | 26.5 | 19 | 13,900 |
key step that eliminated most of the extraneous protein. Extensive washing of the gel with buffer A before application of the gradient was essential for the level of purification achieved. The peroxidase activity did not elute as a sharp peak (Fig. 1) because the gel was mixed with protein and then loaded on the column. The peroxidase eluted as a single peak on DEAE-Sephadex (Fig. 2) suggesting the absence of multiple forms of the enzyme contrary to human erythrocyte glutathione peroxidase (16). The final purification step of gel filtration on Sephadex G-200 eliminated the major high molecular weight impurity (Fig. 3), as well as some low molecular weight proteins, and yielded an electrophoretically homogeneous protein (Fig. 4A). The enzyme also yielded a single peak on a TSK G4000SW HPLC gel filtration column (data not shown).

Partial Purification of Human Erythrocyte Glutathione Peroxidase—Commercially available erythrocyte glutathione peroxidase contained approximately five proteins as judged by SDS-PAGE, and the fractions containing only the peroxidase were pooled. Other details are given under “Materials and Methods.”

Partial Purification of Human Erythrocyte Glutathione Peroxidase—Commercially available erythrocyte glutathione peroxidase contained approximately five proteins as judged by SDS-PAGE, and the fractions containing only the peroxidase were pooled. Other details are given under “Materials and Methods.”

FIG. 2. Anion exchange chromatography on DEAE-Sephadex A-50. The profile shows only the gradient elution of the column. Details are given under “Materials and Methods.”

FIG. 3. Gel filtration on Sephadex G-200. One to two milliliter fractions were collected. Fractions in between the two protein peaks were analyzed by SDS-PAGE, and the fractions containing only the peroxidase were pooled. Other details are given under “Materials and Methods.”

![Graph](http://example.com/graph1.png)

![Graph](http://example.com/graph2.png)

![Graph](http://example.com/graph3.png)

![Graph](http://example.com/graph4.png)

![Graph](http://example.com/graph5.png)
Molecular Mass and Subunit Composition—The molecular mass of the purified plasma glutathione peroxidase was determined by gel filtration on a Sephacryl S-200 column and by HPLC on a TSK G4000SW column. Using standards ranging from 150 to 29 kDa, the molecular mass of the plasma peroxidase was estimated to be about 73 kDa by conventional gel filtration, but 94 kDa by HPLC gel filtration (Fig. 5). The subunit molecular mass was estimated to be 21.5 kDa by SDS-PAGE on a 12% acrylamide gel. The relative mobilities of the standards on 12% acrylamide gel were plotted, and the molecular masses of the subunits of human erythrocyte and plasma peroxidases were calculated from the graph (Fig. 4B). The subunit of human erythrocyte glutathione peroxidase has about 800 Da lower molecular mass (Fig. 5A). This difference in the molecular mass, calculated based on their difference in electrophoretic mobility, was highly consistent (800 ± 31 Da, n = 6). Based on eletrophoretic and chromatographic behavior under denaturing and nondenaturing conditions, respectively, human plasma glutathione peroxidase appears to be a tetramer of identical subunits of about 21.5 kDa molecular mass.

Selenium Determination of Plasma Peroxidase—The selenium content of the peroxidase was estimated fluorimetrically, at three different protein concentrations simultaneously with a set of standards ranging from 0 to 150 ng of selenium. The selenium content was 3.7 ± 0.2 ng/µg protein (1.03 nmol of selenium/nmol of subunit) and was linear with protein concentration (r = 1.00, Fig. 6). The enzyme is competitively inhibited (with respect to glutathione) by β-mercaptosuccinic acid (I50 = 33 µM), which is a specific inhibitor for selenium-dependent glutathione peroxidases (17).

Kinetics of Plasma Glutathione Peroxidase—The purified peroxidase exhibited a specific activity of 26–29 units/mg (n = 8). Saturation of the peroxidase with glutathione was attempted using 13-OOH-18:2 (4 µM) as the hydroperoxide substrate (Fig. 7). At concentrations of glutathione above 5 mM the peroxidase appeared to be inhibited slightly. Data points below 5 mM glutathione were used to construct a double-reciprocal plot (inset of Fig. 7). The Km value for glutathione calculated from the double-reciprocal plot is 4.3 mM. Substrate saturation curves were obtained for hydroperoxides, viz. 13-OOH-18:2, 15-OOH-20:4, PPHP, and H2O2 at constant glutathione concentrations of 1 and 5 mM using the coupled assay of Paglia and Valentine (11) as described under “Materials and Methods.” Methanol at 1% (vehicle for organic hydroperoxides) did not affect the rate of peroxidase activity when H2O2 was used as a substrate (data not shown). Also, 200 µM Tween 20, used to maintain homogeneous assay mixtures in the presence of organic hydroperoxides, did not affect the rate of glutathione oxidation with H2O2 as substrate. Lineweaver-Burk plots of the data from the linear portion of the substrate saturation curves are given in Fig. 8. Kinetic constants derived from the double-reciprocal plots are given in Table II. At 1 mM concentration of glutathione, commercially available human erythrocyte glutathione peroxidase gave Km values of 12 and 10 µM for hydrogen peroxide and PPHP, respectively.

![Fig. 7. Substrate saturation of human plasma glutathione peroxidase with respect to glutathione. The concentration of 13-OOH-18:2 used as the hydroperoxide substrate was 4 µM. All the values are averages of triplicate determinations with standard deviations less than 1%. Details of the assay are given under "Materials and Methods." Inset, Lineweaver-Burk plot of the kinetic data obtained from the saturation of plasma peroxidase with glutathione. Data obtained from the above experiment with glutathione concentrations from 0.25 to 2 mM were used to obtain the double-reciprocal plot. The Km value calculated for glutathione from the plot is 4.3 mM.](image1)

![Fig. 8. Lineweaver-Burk plots of the kinetic data obtained for various hydroperoxides with human plasma glutathione peroxidase. The concentrations of peroxidase and glutathione used in the kinetic studies are 5.9 nm and 5 mM, respectively. All values are averages of triplicate determinations. The standard deviations are less than 1%. Details of the assay are given under "Materials and Methods." Open squares, 13-OOH-18:2; closed squares, 15-OOH-20:4; open triangles, PPHP; closed triangles, H2O2.](image2)
Human Plasma Glutathione Peroxidase

**Table II**

| Hydroperoxide | K_m (μM GSH) | kcat (μM GSH) | kcat/K_m (×10^{-5} M^{-1} s^{-1}) |
|---------------|--------------|---------------|----------------------------------|
| 13-OOH-18:2   | 0.66         | 2.3           | 1.4 × 10^5                       |
| 15-OOH-20:4   | 2.1          | 4.5           | 1.2 × 10^5                       |
| PPHP          | 2.6          | 11.2          | 7.4 × 10^5                       |
| H_2O_2        | 3.3          | 13.2          | 5.7 × 10^5                       |

*The kinetic constants k_cat and k_cat/K_m are calculated from the data obtained with 5 mM glutathione.

In a recent report, we published the characterization of a glutathione-dependent peroxidase activity in human plasma that appeared to be different from the well-characterized and purified human erythrocyte glutathione peroxidase (8). To complete the characterization of the human plasma glutathione peroxidase and to study its physical and kinetic properties, we undertook the purification of the peroxidase. Purification of the enzyme from human plasma was achieved in four steps with an overall yield of about 19%. Precipitation of the peroxidase at relatively low salt concentration in the first step prompted us to employ hydrophobic interaction chromatography. This step eliminated most of the impurities and dramatically increased the specific activity in the initial stage of the purification. Another important aspect of the purification was careful fraction collection in the gel filtration step. The purified peroxidase was electrophoretically homogeneous. Using the recovery and purification data in Table I, one can calculate that this glutathione peroxidase accounts for about 0.007% of the total human plasma protein.

Glutathione peroxidase from human tissues such as erythrocytes and placenta have been purified and characterized (16, 18). When we recently characterized the glutathione peroxidase activity as the only peroxidase of human plasma (8), a major concern was the possible contamination of plasma with glutathione peroxidase from erythrocytes. Although several biochemical and immunological differences were observed between these two peroxidase activities, the argument that the two activities are due to different proteins was equivocal. Like glutathione peroxidases from other sources, the human plasma glutathione peroxidase appears to be a tetramer of identical subunits of about 21.5 kDa subunits. It is difficult to assign an exact molecular mass to the native enzyme because of different results obtained with conventional and HPLC gel filtration methods. The molecular mass calculated for a tetrameric structure should be 86 kDa, which lies in between the molecular masses obtained by conventional gel filtration on Sephacryl S-200 column (73 kDa) and HPLC gel filtration on TSK G4000SW column (94 kDa). From SDS-PAGE it is clear that the subunits of human plasma and erythrocyte peroxidases are electrophoretically different by about 800 Da (±30 Da, n = 6) (Fig. 4). The molecular mass of the human erythrocyte peroxidase subunit is reported to be 23 kDa based on SDS-urea-polyacrylamide gel electrophoresis (16); however, the molecular mass calculated from our electrophoretic experiments is 20.6 kDa. The difference in the electrophoretic mobility of the subunits of plasma and erythrocyte peroxidases supports the suggestion that these two peroxidases are different.

Human erythrocyte and plasma glutathione peroxidases are also kinetically different. The K_m values for H_2O_2 and PPHP are 3.3 and 2.6 μM, respectively, for plasma peroxidase, and 12 and 10 μM, respectively, for erythrocyte peroxidase at 1 mM glutathione. In our earlier experiments with whole plasma, we obtained a much higher K_m value for PPHP (54 μM) at the same concentration of glutathione (8). This is probably due to the presence of large amounts of extraneous proteins in plasma (e.g., albumin) that bind to organic hydroperoxides. However, the K_m value for the same substrate with erythrocyte peroxidase was also higher in our earlier experiments (24 μM) than in the present experiments (10 μM). This difference is probably due to a limitation of the direct HPLC assay, employed in our earlier studies, where the initial rate was estimated based on the product formed at the end of 1 min of the reaction. It is difficult to follow the initial rates continuously in the HPLC assay, unlike the coupled assay presently employed. That the two peroxidases are different is also substantiated by immunological experiments of Takahashi et al. (19) who showed that rabbit anti-human erythrocyte glutathione peroxidase does not cross-react with purified human plasma glutathione peroxidase.

It appears there is no significant lysis of erythrocytes during plasma preparation because only a single peak of peroxidase activity is obtained at every step of the purification protocol. Furthermore, none of the erythrocyte peroxidase is detected when fractions are analyzed by SDS-PAGE. The plasma glutathione peroxidase does not cross-react with purified human plasma glutathione peroxidase.

For glutathione peroxidases from bovine and ovine erythrocytes and rat and hamster livers show no saturation with respect to glutathione, so true K_m values cannot be obtained for glutathione (20, 21). For human erythrocyte glutathione peroxidase, a K_m value of 4.3 mM was reported for glutathione, but no data were presented showing the saturation of the peroxidase with glutathione. The plasma glutathione peroxidase definitely shows saturation with respect to glutathione. The K_m value obtained from the double-reciprocal plot is 4.3 mM, which is identical to the reported value for human erythrocyte glutathione peroxidase. The activity of plasma glutathione peroxidase appears to decrease with increasing concentrations of glutathione beyond the saturation point of 5 mM. Because of this, all the kinetic experiments with various hydroperoxides were carried out at 5 mM glutathione. However, to compare the kinetic constants of plasma peroxidase with those of glutathione peroxidases from other sources reported in literature, we also determined the "K_m" values at 1 mM glutathione. The apparent K_m values for human plasma glutathione peroxidase are approximately 10-fold lower than the corresponding values for bovine, ovine, and human erythrocyte peroxidases with hydrogen peroxide as substrate under similar conditions (20). With organic hydroperoxides, the apparent K_m values for plasma peroxidase are as much as 30-fold lower than other peroxidases. The plasma glutathione peroxidase appears to be a very efficient enzyme for the reduction of organic hydroperoxides. In fact, the values of k_cat/K_m are very close to the diffusion-controlled limit for a bimolecular reaction. At 5 mM concentrations of glutathione, the K_m values for all the hydroperoxides tested are in the low micromolar range (Table II). This makes the human plasma glutathione peroxidase a very effective hydroperoxide scavenger,
envelope even at relatively low concentrations of glutathiones. Recent estimates of organic hydroperoxide concentrations of human plasma are about 0.5 μM and H₂O₂ is estimated to be approximately 5 μM (22, 23). The low Kₐ values for organic hydroperoxides, especially for 13-hydroperoxycodacidi

dienic acid (2.3 μM), may indicate a possible physiological role for this enzyme in the control of circulating fatty acid hydroperoxides. It is also interesting to note that the cholesterol ester of 13-hydroperoxycodacidi
dienic acid was recently identified as a major hydroperoxide of atherosclerotic lesions (7). Furthermore, esters of hydroperoxy fatty acids to cholesterol appear to be the major circulating forms of organic hydroperoxides (23). Estenified hydroperoxides are very poor substrates for glutathione peroxidases (24). However, Ursini et al. (25) have purified a selenium-dependent glutathione peroxidase from pig heart that accepts both esterified and non-esterified fatty acid hydroperoxides as substrates. Studies are in progress to determine the versatility of plasma glutathione peroxidase in the reduction of various hydroperoxides of physiological relevance.

It is known that the steady-state levels of glutathione in human plasma are in the range of 0.3 μM (26). Although it is difficult to assess the physiological significance of the plasma peroxidase activity at this low concentration of glutathione, inter-organ transport of glutathione may play an important role in the modulation of the peroxidase activity. γ-Glutamyl transpeptidase has been shown to actively degrade extracellular glutathione, whereas liver constantly releases this ubiquitous tripeptide into plasma (27). It has been demonstrated that liver glutathione is transported in substantial amounts to hepatic vein plasma in rats, where the level of glutathione is found to be as high as 26 μM (28). Arterial glutathione levels can be increased severalfold by selectively inhibiting γ-glutamyl transpeptidase. This suggests that γ-glutamyl transpeptidase is an important regulator of plasma glutathione levels, thereby indirectly regulating the plasma peroxidase activity. Thus, a detailed knowledge of glutathione transport and its regulation appears to be essential to ascertain the physiological function of the plasma glutathione peroxidase.

Acknowledgment—We are grateful to Dr. Harvey Cohen for communication of the results of his purification of human plasma glutathione peroxidase prior to publication.

REFERENCES
1. Marnett, L. J., Weller, P., and Battista, J. R. (1986) in Cyt
ochrome P-450 (Oriz de Montellano, P. R., ed) pp. 29–76, Plenum Press, New York
2. Moncada, S., and Vane, J. R. (1979) N. Engl. J. Med. 300, 1142–1147
3. Ham, E. A., Egan, R. W., Sodeman, D. D., Gale, P. H., and Kuehl, F. A., Jr. (1979) J. Biol. Chem. 254, 2191–2194
4. Moncada, S., and Vane, J. R. (1977) in Biochemical Aspects of Prostaglandins and Thromboxanes (Kharasch, N., and Fried, J., eds) pp. 155–177, Academic Press, New York
5. Lauds, W. E. M., Cook, H. W., and Rome, L. H. (1976) in Advances in Prostaglandins and Thromboxane Research (Samuelsson, B., and Paoletti, R., eds) Vol. 1, pp. 7–77, Raven Press, New York
6. Harland, H., Gilbert, J., and Brooks, C. (1973) Biochim. Biophys. Acta 316, 378–385
7. Mowri, H., Chinen, H., Ohkuma, S., and Takano, T. (1986) Biochem. Int. 12, 347–352
8. Maddipati, K. R., Gasparski, C., and Marnett, L. J. (1987) Arch. Biochem. Biophys. 254, 9–17
9. Funk, M. O., Isaac, R., and Porter, N. A. (1976) Lipids 11, 113–117
10. Weller, P. E., Markey, C. M., and Marnett, L. J. (1985) Arch. Biochem. Biophys. 243, 633–643
11. Paglia, D. E., and Valentine, W. N. (1967) J. Lab. Clin. Med. 70, 158–169
12. Scope, R. K. (1982) Protein Purification, pp. 261–263, Springer-Verlag, New York
13. Laemmli, U. K. (1970) Nature 227, 680–685
14. Watkins, J. H. (1986) Anal. Chem. 38, 92–97
15. Bayfield, R. F., and Romalis, L. F. (1986) Anal. Biochem. 144, 569–576
16. Awasthi, Y. C., Beutler, E., and Srivastava, S. K. (1975) J. Biol. Chem. 250, 5144–5149
17. Chaudiere, J., Wilhelmson, E. C., and Tappe, A. L. (1984) J. Biol. Chem. 259, 1043–1050
18. Awasthi, Y. C., Doo, D. D., Lal, A. K., and Srivastava, S. K. (1979) Biochem. J. 177, 471–476
19. Takahashi, K., Avisser, N., Whitin, J., and Cohen, H. (1987) Arch. Biochem. Biophys. 269, 677–686
20. Wendel, A. (1980) in Enzymatic Basis of Detoxication (Jacoby, W. B., ed) Vol. 1, pp. 233–355, Academic Press, New York
21. Chaudiere, J., and Tappe, A. L. (1983) Arch. Biochem. Biophys. 226, 448–457
22. Warso, M. A., and Lands, W. E. M. (1984) Clin. Physiol. Biochem. 2, 70–77
23. Yamamoto, Y., Brodsky, M. H., Baker, J. C., and Ames, B. N. (1987) Anal. Biochem. 160, 7–13
24. Grossmann, A., and Wendel, A. (1983) Eur. J. Biochem. 135, 549–553
25. Ursini, F., Maiorino, M., and Gregolin, C. (1985) Biochim. Biophys. Acta 839, 62–70
26. Lath, L. H., and Jones, D. P. (1985) Arch. Biochem. Biophys. 240, 583–592
27. Meister, A., and Anderson, M. E. (1983) Annu. Rev. Biochem. 52, 711–760
28. Anderson, M. E., Bridges, R. J., and Meister, A. (1980) Biochem. Biophys. Res. Commun. 96, 848–853