Studies on mammalian intestinal peroxidase

Shioko KIMURA and Peter H. JELLINCK*

Department of Biochemistry, Queen's University, Kingston, Ontario K7L 3N6, Canada

(Received 26 January 1982/Accepted 14 April 1982)

A peroxidase, purified from rat small intestine to apparent homogeneity as judged by polyacrylamide-gel electrophoresis, exhibited an absorbance ratio (A_{412}/A_{280}) of 0.783. Its M_r ($44\,000 \pm 1000$) and spectral properties were similar to those of the pig intestinal enzyme. The velocity constant for the reaction between rat intestinal peroxidase and hydrogen peroxide was found to be $1.8 \times 10^7 \text{ M}^{-1} \cdot \text{s}^{-1}$. Benzhydroxamic acid inhibited the peroxidative oxidation of guaiacol by intestinal peroxidase from both species but the concentration required to cause half-inhibition of the enzyme from the rat was higher by one order of magnitude than for the pig enzyme. The amino acid composition of highly-purified pig intestinal peroxidase showed a relative abundance of basic amino acids (lysine and arginine) and was similar to that of lactoperoxidase, but not that of myeloperoxidase. The initial ten amino acid residues of this enzyme (the first reported partial sequence for a mammalian peroxidase) were also determined.

Ever since Rytömaa & Teir (1961) suggested that all animal peroxidases, with the exception of myeloperoxidase, were derived from eosinophil granulocytes, the properties of peroxidases from various tissues have been compared (Archer *et al.*, 1965; Morrison & Allen, 1963, 1966; Morrison *et al.*, 1965; Stelmaszyńska & Zgliczyński, 1971; Kimura & Yamazaki, 1978, 1979; Hosoya & Saito, 1981; Hosoya *et al.*, 1981; Wever *et al.*, 1981). Yet, it is still not fully established whether each peroxidase is synthesized by its intrinsic tissue or brought to it by eosinophil granulocytes.

Intestinal peroxidase was first purified from the small intestinal mucosa of the pig and shown by Stelmaszyńska & Zgliczyński (1971) to be similar to eosinophil peroxidase. However, the information on the properties of this enzyme was not sufficient to clarify its relationship to other peroxidases. It was therefore of interest to determine the properties of peroxidase isolated from rat small intestine and to compare them with those of a highly purified preparation of pig intestinal peroxidase with the view to extend these studies to other animal peroxidases from different tissues. In particular, we wished to resolve the problem about the origin of uterine peroxidase, which has been used extensively as a marker for oestrogen action (Lyttle & DeSombre, 1977; Jellinck *et al.*, 1979).

In this paper, a method for isolating and puri-

fying peroxidase from rat small intestine and some of its basic physicochemical and catalytic properties are described. We also report some molecular properties, including a partial sequence, of pig intestinal peroxidase, which should provide useful criteria for elucidating relationships among animal peroxidases.

Materials and methods

Materials

Benzhydroxamic acid was purchased from Aldrich and purified according to the method of Hauser & Renfrow (1943). Guaiacol was obtained from Eastman Kodak and Cetab, α -methyl mannoside, γ -globulin, myoglobin, α -chymotrypsinogen, ovalbumin, bovine serum albumin, lactoperoxidase and 3,3',5,5'-tetramethylbenzidine from Sigma. Bio-Rad Laboratories supplied gel electrophoresis standards for M_r determinations. Amberlite CG-50 (H) ion-exchange resin (type II, 200 mesh) was purchased from BDH and Sephadex G-100 and G-200 and concanavalin A–Sephadex 4B from Pharmacia. All other chemicals were the purest available commercially.

Tissue preparation

Female Holtzman rats (Canadian Breeding Laboratories, St. Constant, Quebec, Canada) weighing about 150 g were killed by cervical dislocation after anaesthesia with CO_2 . The small intestines were removed and cut longitudinally. Luminal contents were washed out gently with ice-cold saline, and the

Abbreviations used: Cetab, cetyltrimethylammonium bromide; SDS, sodium dodecyl sulphate.

* To whom reprint requests should be addressed.

intestines cut into small pieces with scissors and stored at -20°C for about 1 week. In the stability assay of peroxidase, the small-intestinal tissue was immediately homogenized in the appropriate volume of 10mM-Tris/HCl, pH 7.2, to give a 2.5% (w/v) homogenate using a Potter-Elvehjem homogenizer with a Teflon pestle, and centrifuged in a Beckman Model L ultracentrifuge (50 Ti rotor) at $40000g_{av}$ for 30 min at 4°C . After washing with the same buffer, the pellet was rehomogenized in 10mM-Tris/HCl, pH 7.2, containing either 0.5M- CaCl_2 or 1.2M- NH_4Cl , and centrifuged again at $40000g_{av}$ for 30 min to obtain a supernatant fraction.

Purification of rat intestinal peroxidase

All the steps were carried out at $0-4^{\circ}\text{C}$. Approx. 270g of frozen rat small intestinal sections were thawed and homogenized in 500ml of 0.25M-sucrose using a Dumore type KB-210 blender. After centrifugation at $13000g_{av}$ for 20 min, the precipitate was rehomogenized in 500ml of 50mM- Na_2HPO_4 containing 0.2% Cetab and kept frozen at -20°C for 24 h. After thawing, NH_4Cl was added to a concentration of 1.2M and mixed for 3 h. The sediment was removed by centrifugation for 30 min at $40000g_{av}$ to give a supernatant fraction. The

NH_4Cl extract was dialysed overnight against 20mM-sodium phosphate (pH 6.1) containing 0.2M- NH_4Cl (buffer A) with added 0.02% Cetab. The dialysate was centrifuged at $35000g_{av}$ for 40 min; most of the peroxidase activity was recovered in the sediment which was then resuspended by homogenization in 100ml of buffer A.

About 100g of CG-50 ion-exchange resin, previously equilibrated with buffer A, was added to the homogenate and the mixture was stirred for 6 h. The supernatant was then decanted and the procedure repeated twice more with fresh resin. The combined resin was washed with distilled water in batches until the supernatant was clear, and was then packed into a chromatographic column ($2.5\text{ cm} \times 25\text{ cm}$), followed by washing with buffer A. The resin was then eluted with 50mM-sodium phosphate (pH 7.1) containing 1.2M- NH_4Cl (buffer B) until no further peroxidase activity was removed. This solution (2730ml) was concentrated by ultrafiltration and then diluted 4-fold with distilled water before applying it to a column ($1.5\text{ cm} \times 20\text{ cm}$) of CG-50 equilibrated with buffer A. After washing with buffer A, the column was eluted with a linear gradient prepared from 600ml each of buffers A and B. The combined fractions (195 ml) containing peroxidase

Fig. 1. Gel filtration of rat intestinal peroxidase on Sephadex G-100

A_{280} (○) for protein, A_{415} (△) for peroxidase and A_{625} (▲) for Blue Dextran, and enzyme activity (●) were determined in every second fraction. Fractions underlined were combined for further purification.

activity were concentrated by ultrafiltration and applied to a column (1.5 cm $\times$ 85 cm) of Sephadex G-100 previously equilibrated with buffer B. Fractions (2.5 ml) containing peroxidase activity were combined (Fig. 1) and were passed through a column (1.5 cm $\times$ 6 cm) of concanavalin A-Sepharose equilibrated with buffer B. The column was washed with buffer B until the A_{280} of the eluate had decreased to nearly zero, and peroxidase was eluted with buffer B containing 0.5 M- α -methyl mannoside. Fractions (0.8 ml) were combined and dialysed against buffer B to remove α -methyl mannoside.

Pig intestinal peroxidase was prepared from the small intestine by the method of Stelmaszyńska & Zgliczyński (1971) with slight modifications. Since the molar absorbance values for rat and pig intestinal peroxidase were not known, their concentrations were estimated initially by using the value for lactoperoxidase: $\epsilon_{412} = 114 \text{ mm} \cdot \text{cm}^{-1}$ (Morrison *et al.*, 1957).

Assay of peroxidase

Peroxidase activity was determined at 25°C, using guaiacol as substrate (Himmelhoch *et al.*, 1969). The reaction mixture contained 13 mM-guaiacol and 0.33 mM- H_2O_2 in a total volume of 3.0 ml of 10 mM-Tris/HCl, pH 7.2, and 5–30 μl of the fraction containing peroxidase, depending on its activity. The linear increase in A_{470} resulting from the oxidation of guaiacol was measured by a Beckman model 25 recording spectrophotometer in a cuvette with a 1 cm light path. One enzyme unit is defined as the amount of enzyme required to produce an increase of 1 absorbance unit/min, and specific activity is expressed as the number of units per mg of protein. Protein was determined by the

method of Bradford (1976) with γ -globulin as a standard.

Polyacrylamide-gel electrophoresis

This was performed at pH 4.3 according to the method of Reisfeld *et al.* (1962) except that 0.02% Cetab was present both in the gel and in the electrode buffer. For detection of enzymic activity, the developed gel was immersed in 0.17 M-sodium acetate (pH 5.0) containing 1.9 mM-3,3',5,5'-tetramethylbenzidine (Thomas *et al.*, 1976) in the dark at 25°C for 2 h followed by the addition of H_2O_2 (29 mM). Before staining the gel with 10% Bio-Rad protein assay dye, the detergent was removed by using a modification of the method of Blakesley & Boezi (1977). Electrophoresis on polyacrylamide gel containing 0.1% SDS was performed as described by Laemmli (1970). Electrophoresis was carried out in a water-cooled vertical slab gel apparatus (model 220, Bio-Rad Laboratories) at a constant current of 20 mA/slab.

Amino acid analysis

Lyophilized pig intestinal peroxidase (1.0–1.2 mg) was hydrolysed *in vacuo* with constant boiling 6 M-HCl at 110°C for 48 h. Analyses were performed on a Beckman model 120C amino acid analyser modified for single column analysis. Cysteine and methionine were determined as cysteic acid and methionine sulphone respectively, following performic acid oxidation (Moore, 1963). Tryptophan was estimated spectrophotometrically as described by Spande & Witkop (1967).

Protein sequencing

The N-terminal end of pig intestinal peroxidase was sequenced using a Beckman 890C automatic

Fig. 2. Affinity chromatography of rat intestinal peroxidase on concanavalin A-Sepharose. A_{280} (O) and A_{415} (Δ) and enzyme activity ($\bullet$) were determined in every second fraction. The arrow indicates start of elution of peroxidase by 0.5 M- α -methyl mannoside. Fractions underlined were combined.

protein sequencer and a 0.33 M-Quadrol program with combined S_1 and S_2 wash. The protein (approx. 50 nmol) was added in 50% acetic acid and double coupling was performed for the first cycle only. Phenylthiohydantoin amino acids were analysed by reverse-phase high pressure liquid chromatography using a Du Pont-Zorbak Cyano column and a Beckman 334 h.p.l.c. system with a Beckman 160 μ V detector and an Altex model C-R1A integrator-plotter. The phenylthiohydantoin amino acids were eluted from the column using a slightly modified version of the method of Johnson *et al.* (1979). The repetitive yield was 93%.

Kinetic studies

The velocity constant k_1 for the reaction of rat intestinal peroxidase with H_2O_2 was determined at 25°C in triplicate with four different concentrations of enzyme by the steady state method of Maguire *et al.* (1971). The reaction mixture contained 78 or 90 mM-guaiacol, 26.3 μ M- H_2O_2 , and $(2.9-7.2) \times 10^{-9}$ M-rat intestinal peroxidase in 0.1 M-sodium phosphate, pH 6.3. In the inhibition studies with benzhydroxamic acid, the reaction mixture contained 2.9×10^{-9} M or 2.4×10^{-9} M of rat or pig intestinal peroxidase respectively, 90 μ M- H_2O_2 , and

various concentrations of guaiacol (up to 1.2 mM) and benzhydroxamic acid in 0.1 M-sodium phosphate, pH 6.3, or 10 mM-Tris/HCl, pH 7.2.

Results

Purification of rat intestinal peroxidase

Stelmaszyńska & Zgliczyński (1971) used 1 M- NH_4Cl to extract peroxidase from pig small intestine, whereas $CaCl_2$ was shown by Lyttle & DeSombre (1977) to be a superior extractant for rat uterine peroxidase even though it could not be stored in this medium without loss of activity (Olsen & Little, 1979; Keeping *et al.*, 1981). Table 1 shows that a 1.2 M- NH_4Cl extract of rat small intestine not only gave comparable peroxidase activity to that of a 0.5 M- $CaCl_2$ extract, but also that the activity was maintained on storage at -20°C. In addition, more activity remained after dialysis of 1.2 M- NH_4Cl extracts than 0.5 M- $CaCl_2$ extracts, although considerable losses of activity occurred in both cases during this process.

The final enzyme preparation was purified 3175-fold relative to the homogenate with a yield of 20.3% and 420-fold relative to the NH_4Cl extract with a yield of 4.0% (Table 2). The ratio of absorbance at the Soret maximum to that at 280 nm by the purified enzyme was 0.783.

Properties of rat and pig intestinal peroxidase

Gel electrophoresis. When purified rat and pig intestinal peroxidases were analysed by gel electrophoresis in 7.5% polyacrylamide gels at pH 4.3 (Reisfeld *et al.*, 1962), an additional band with lower mobility than the major band was observed in both enzyme preparations. To determine whether this additional band was an artifact, a concentrated solution of pig intestinal peroxidase was subjected to electrophoresis and each band (detectable without staining) was cut out, minced, and applied to a new slab gel. On electrophoresis, each band yielded the other component, leading to the conclusion that the second band was an artifact formed during electro-

Table 1. Effect of $CaCl_2$ and NH_4Cl on the solubilization and stability of rat intestinal peroxidase activity

Extracts of rat small intestine were prepared as described in the Materials and methods section. $CaCl_2$ and NH_4Cl extracts were stored at -20°C and assayed for peroxidase activity on the days indicated. Part of the extract on day zero was dialysed against 10 mM-Tris/HCl, pH 7.2. Peroxidase activity was determined after dialysis.

	Activity (units)			
	On days			After dialysis
Extractant	0	16	34	
0.5 M- $CaCl_2$	283	18.8	1.1	33.4
1.2 M- NH_4Cl	239	223	202	64.0

Table 2. Purification of peroxidase from rat small intestine

Purification step	Volume (ml)	Total protein (mg)	Total activity (units)	Specific activity (units/mg of protein)	A_{412}/A_{280}	Yield (%)		Purification (-fold)	
Homogenate	715	15 500	4250	0.274	—	100	—	1.0	—
NH_4Cl extract	645	10 400	21 300	2.05	—	500	100*	7.5	1.0*
Dialysis	150	1900	14 100	7.42	—	332	66.2	27.1	3.6
CG-50 (1)	2730	190	4000	21.0	—	94.1	18.8	76.6	10.2
CG-50 (2)	195	10.4	1160	112	0.275	27.3	5.4	409	54.6
Sephadex G-100	44.4	2.80	969	346	0.649	22.8	4.5	1263	169
Concanavalin A-Sepharose	12.5	0.99	861	870	0.783	20.3	4.0	3175	424

* Yield relative to the NH_4Cl extract.

phoresis and that the major and the minor zones were interconvertible. However, the formation of this second band could be prevented by the addition of 0.02% Cetab to both the gel and the electrode buffer, and it was shown by this method that the purified preparation of rat intestinal peroxidase was essentially homogeneous, with slightly lower mobility than pig intestinal peroxidase. Enzymic activity corresponded to the protein band with both peroxidases.

M_r determinations. When SDS/polyacrylamide-gel electrophoresis was used to determine the M_r of rat and pig intestinal peroxidase, values of 43 000 and 51 000 respectively were obtained (Fig. 3a). Similar values (45 000 and 49 000) were obtained after gel filtration through Sephadex G-200 (Fig. 3b). These molecular weights are comparable with those reported by Stelmaszyńska & Zgliczyński (1971) for pig intestinal peroxidase and by McNabb & Jellinck (1975), Lyttle & DeSombre (1977) and Olsen & Little (1981) for rat uterine peroxidase.

Amino acid composition and partial sequence determination of pig intestinal peroxidase. The amino acid composition of pig intestinal peroxidase and their individual ratios in the enzyme were determined (Table 3) and compared to the values obtained by others for lactoperoxidase (Carlström,

1969a) and myeloperoxidase (Schultz & Shmukler, 1964). The intestinal peroxidase shows greater similarity in composition to lactoperoxidase than to myeloperoxidase and had a relative abundance of arginine and lysine which accounts for its basic properties (Stelmaszyńska & Zgliczyński, 1971). No glucosamine or galactosamine was detected by the amino acid analyser, but the absorption of peroxidase to concanavalin A-Sepharose suggests that it contains carbohydrates, which could be mannose or glucose.

Sequence analysis of pig intestinal peroxidase resulted in the elucidation of the first ten amino acid residues of the enzyme:

The quantities of purified peroxidase available did not allow sequence studies beyond this point.

Spectral properties of rat intestinal peroxidase. The absorption spectra of native rat intestinal peroxidase and its cyanide and reduced cyanide complexes are shown in Fig. 4 and were found to be similar to those of pig intestinal peroxidase (Stelmaszyńska & Zgliczyński, 1971), lactoperoxidase

Fig. 3. M_r determination of rat and pig intestinal peroxidase

(a) The enzymes and marker proteins were subjected to electrophoresis (9% polyacrylamide gel containing 0.1% SDS) and stained with Coomassie Brilliant Blue. Abbreviations: Lys, lysozyme; STI, soya bean trypsin inhibitor; CA, carbonic anhydrase; Rat IPO, rat intestinal peroxidase; Pig IPO, pig intestinal peroxidase; BSA, bovine serum albumin; Phos B, phosphorylase B. (b) Gel filtration on Sephadex G-200 in buffer B at a flow rate of 1.5 ml/h. Void volume (V_0) of the column (1.5 cm $\times$ 90 cm) was determined by means of Blue Dextran. Proteins for elution volume (V_e) estimation were measured by their A_{280} , A_{410} or by their enzymic activities. Abbreviations: Mb, myoglobin; α -Chy, α -chymotrypsinogen; Oval, ovalbumin; LPO, lactoperoxidase; other abbreviations are as in (a).

Table 3. *Amino acid composition of pig intestinal peroxidase*

Amino acid	Pig intestinal peroxidase*		Lactoperoxidase§		Myeloperoxidase	
	Residues/mol	(%)	Residues/mol	(%)	Residues/mol	(%)
Lysine	21	5.4	34	5.6	22	4.1
Histidine	7	1.8	14	2.3	12	2.2
Arginine	38	9.8	37	6.1	140	25.9
Tryptophan	7†	1.8	15	2.5	26	4.8
Aspartic acid	41	10.6	71	11.6	51	9.4
Threonine	20	5.2	32	5.2	23	4.3
Serine	23	6.0	33	5.4	21	3.9
Glutamic acid	29	7.5	61	10.0	37	6.8
Proline	28	7.3	42	6.9	31	5.7
Glycine	25	6.5	40	6.5	26	4.8
Alanine	30	7.8	37	6.1	26	4.8
Half-cystine	10‡	2.6	16	2.6	13	2.4
Valine	19	4.9	28	4.6	17	3.1
Methionine	5‡	1.3	11	1.8	12	2.2
Isoleucine	17	4.4	27	4.4	16	3.0
Leucine	38	9.8	68	11.1	43	7.9
Tyrosine	8	2.1	15	2.5	8	1.5
Phenylalanine	20	5.2	30	4.9	17	3.1
Total amino acid residues	386		611		541	

* Residues per mol of enzyme were calculated on the basis of an M_r of 50000.

† Determined spectrophotometrically.

‡ Determined as cysteic acid and methionine sulphone.

§ Data from Carlström (1969a).

|| Data from Schultz & Shmukler (1964); values are in terms of mol of amino acid per g-atom of Fe.

Fig. 4. *Absorption spectra of rat intestinal peroxidase and its derivatives*

Free enzyme (—), 0.82 μ M; oxidized cyanide complex (---), free enzyme + 20 mM-KCN; reduced cyanide complex (.....), oxidized cyanide complex + 10 mM- $\text{Na}_2\text{S}_2\text{O}_4$. The spectra were measured in 0.1 M-sodium phosphate, pH 6.3.

(Morell, 1954), rat eosinophil peroxidase (Archer *et al.*, 1965) and rat uterine peroxidase (Olsen *et al.*, 1982). The absorption peaks of the oxidized and reduced forms of peroxidase as well as those of their cyanide complexes are given in Table 4. The Soret peak was changed from 412 nm to 415 nm by 1.2 M-NH₄Cl and this shift to a longer wavelength by Cl⁻ has been reported previously for pig intestinal peroxidase (Kimura & Yamazaki, 1978) and lacto-peroxidase (Morrison *et al.*, 1970; Kimura &

Yamazaki, 1978). Fig. 5 shows the absorption spectrum of the reduced form of rat intestinal peroxidase at pH 6.3 and its time-dependent change in the Soret region. A similar spectral change has been reported for pig intestinal peroxidase (Kimura *et al.*, 1981).

Kinetic studies. The velocity constant (*k*₁) of the reaction of rat intestinal peroxidase with H₂O₂ was measured using steady-state kinetics as described in the Materials and methods section and was found to

Table 4. Comparison of absorbance maxima of rat intestinal peroxidase with those of other peroxidases

Enzyme	Absorbance maxima of form:			
	Fe ³⁺	Fe ³⁺ -CN	Fe ²⁺	Fe ²⁺ -CN
Rat intestinal peroxidase	412, 500, 547, 593, 637	430, 553, 588	447, 563, 595 434	435, 535, 566
Pig intestinal peroxidase	414, 505, 545, 596, 640*	429, 555, 596, 650†	450, 565, 600‡ 436, 536, 565	435, 536, 569†
Lactoperoxidase	412, 500, 550, 600, 635§	432, 558, 595§	442, 565, 600§	435, 537.5, 571.5
Rat eosinophil peroxidase	415, 505¶ 640¶		445, 565, 595¶	
Rat uterine peroxidase	412, 500, 541** 640**	431, 555**	448, 562, 596**	435, 539, 569**

* Kimura & Yamazaki (1978).
† Stelmaszyńska & Zgliczyński (1971).
‡ Kimura *et al.* (1981).
§ Morell (1954).
|| Carlström (1969b).
¶ Archer *et al.* (1965).
** Olsen *et al.* (1982).

Fig. 5. Time-dependent change in the absorption spectrum of rat intestinal peroxidase after reduction. Free enzyme (----), 0.82 μ M in 0.1 M-sodium phosphate, pH 6.3. The spectra (1, 2 and 3) were measured at 25°C for 1 min, 20 min and 35 min respectively after the addition of 10 mM-Na₂S₂O₄.

Fig. 6. Effect of benzhydroxamic acid on the oxidation of guaiacol by rat and pig intestinal peroxidase (a) Rat intestinal peroxidase (2.9×10^{-9} M), H_2O_2 ($90 \mu\text{M}$) and various concentrations of guaiacol were incubated at 25°C in 0.1M -sodium phosphate, $\text{pH } 6.3$, in the presence of 0 ($\circ$), $1 \mu\text{M}$ (Δ), $10 \mu\text{M}$ ($\bullet$) and $100 \mu\text{M}$ ($\blacktriangle$) benzhydroxamic acid. (b) Pig intestinal peroxidase (2.4×10^{-9} M), was incubated as in (a) in the presence of 0 ($\circ$), $0.1 \mu\text{M}$ (Δ), $0.5 \mu\text{M}$ ($\bullet$), and $10 \mu\text{M}$ ($\blacktriangle$) benzhydroxamic acid. Peroxidase activity was measured as described in the Materials and methods section.

be $1.8 \times 10^7 \text{M}^{-1} \cdot \text{s}^{-1}$, a value comparable with that of pig intestinal peroxidase (Kimura & Yamazaki, 1978, 1979), lactoperoxidase (Chance, 1950; Maguire *et al.*, 1971; Kimura & Yamazaki, 1979), and uterine peroxidase (Hosoya & Saito, 1981). Fig. 6 shows that pig intestinal peroxidase activity was inhibited by benzhydroxamic acid. From these plots, it was calculated that the activity was reduced by about one-half in the presence of 0.25 – $0.54 \mu\text{M}$ -benzhydroxamic acid, which is consistent with our previous findings (Kimura & Yamazaki, 1979). On the other hand, rat intestinal peroxidase was found to have an inhibition constant (K_i) of 3.0 – $4.9 \mu\text{M}$, one order of magnitude higher than for the pig enzyme and differing from that of lactoperoxidase (Kimura & Yamazaki, 1979).

Discussion

Initially, we attempted to isolate and purify peroxidase from rat small intestines by using CaCl_2 as extractant according to the method of Lyttle & DeSombre (1977) for rat uterine peroxidase. However, NH_4Cl , as used originally by Stelmazyńska & Zgliczyński (1971) to extract peroxidase from pig small intestines, gave better results with respect to the stability of the enzyme, although some loss in activity was still observed (Table 1).

The purified rat intestinal peroxidase was shown by polyacrylamide-gel electrophoresis at $\text{pH } 4.3$ to be a basic protein with typical haemoprotein absorption spectra (Figs. 4 and 5). These properties were similar to those of pig intestinal peroxidase. The artifact that was produced from both peroxidases during polyacrylamide-gel electrophoresis has also been reported by Rombauts *et al.* (1967)

for lactoperoxidase. The kinetic value $k_1 = 1.8 \times 10^7 \text{M}^{-1} \cdot \text{s}^{-1}$ for rat intestinal peroxidase in the reaction with H_2O_2 indicates that this haemoprotein is a true peroxidase. The inhibition constant in the presence of benzhydroxamic acid increases in the order: lactoperoxidase (Kimura & Yamazaki, 1979) > rat intestinal peroxidase > pig intestinal peroxidase, which allows these differences to be used as criteria to distinguish between these enzymes.

Spectrophotometric and kinetic data (Archer *et al.*, 1965; Stelmazyńska & Zgliczyński, 1971; Kimura & Yamazaki, 1978, 1979; Hosoya & Saito, 1981; Wever *et al.*, 1981; Olsen *et al.*, 1982) have shown that pig intestinal peroxidase, lactoperoxidase, eosinophil peroxidase and uterine peroxidase are similar and do not resemble myeloperoxidase. The similarity in amino acid composition of pig intestinal peroxidase to that of lactoperoxidase and its dissimilarity to that of myeloperoxidase (Table 3) gives additional support to this concept and perhaps may be explained in terms of the origin of animal peroxidases. The *N*-terminal amino acid of lactoperoxidase has been reported to be leucine by Rombauts *et al.* (1967) and Sievers (1981). It is therefore of interest that in pig intestinal peroxidase, another non-polar aliphatic amino acid, valine, is present at the *N*-terminal end. The change in the reduced spectrum of rat intestinal peroxidase in the Soret region (Fig. 5) could only be followed readily between $\text{pH } 6.0$ and 7.0 , when the rate was slow. However, this spectral change was difficult to follow in the visible region because the concentration of the enzyme was low and the haem was gradually destroyed after adding $\text{Na}_2\text{S}_2\text{O}_4$. The resemblance of rat to pig intestinal peroxidase in basic properties, M_r values and absorption spectra

(Figs. 3–5) suggests that they belong to the same family of peroxidative enzymes.

On the basis of histochemical studies, it has been pointed out (Rytömaa, 1960; Rytömaa & Teir, 1961) that the high activities of peroxidase in some tissues such as the uterus and small intestine (Neufeld *et al.*, 1958) may be due to their high content of eosinophils, rich in peroxidase. It was suggested that, with the exception of myeloperoxidase, all animal peroxidases found in different tissues of the body are derived from eosinophil granulocytes. This proposal still remains to be resolved.

Recently, it has been suggested (Hosoya & Saito, 1981; Hosoya *et al.*, 1981) that peroxidase in rat uterine fluid and lactoperoxidase, which are soluble enzymes, differ from the peroxidases associated with membranes inside uterine cells or eosinophils and have about twice the M_r value of the latter. Rat intestinal peroxidase is also a particulate-bound protein with an M_r (Fig. 4) similar to that of rat uterine tissue peroxidase (McNabb & Jellinck, 1975; Lyttle & DeSombre, 1977; Hosoya & Saito, 1981; Olsen & Little, 1981). Thus, their further characterization should help to clarify the relationship between eosinophil and other membrane-bound peroxidases and the soluble peroxidases.

We thank Dr. T. G. Flynn for the amino acid analysis and sequence determination and for valuable comments. This work was supported by the Medical Research Council of Canada.

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