Spectral properties of the oestrogen-induced rat uterus peroxidase II and some of its derivatives

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1. The visible absorption spectrum of peroxidase II, isolated from the uterine tissue of oestradiol-treated rats, and some of its derivatives were recorded. The spectral properties of this enzyme are very similar to eosinophile peroxidase and lactoperoxidase, suggesting that these enzymes may have a similar form of haem as prosthetic group. 2. The uterine peroxidase is modified upon interaction with H_2O_2 and the difference spectrum of this modified enzyme is similar to that of complex II of lactoperoxidase. The modified enzyme was found to revert spontaneously to the native enzyme at rates which depended on the concentration of free enzyme and H_2O_2 .

The oestrogen-induced peroxidase found in rat uterus has attracted considerable interest because of its proposed involvement in oestrogen metabolism, notably the inactivation of oestradiol (Klebanoff, 1965; Brökelmann, 1969; Lytle & Jellinck, 1972; Keeping & Jellinck, 1978). A further factor has been the suggestion that such activity is a possible criterion for distinguishing between hormone-dependent and hormone-independent mammary cancer (Anderson *et al.*, 1975; Duffy & Duffy, 1977; Jellinck *et al.*, 1979). The oestrogen-induced peroxidase activity in the uterus was originally thought to derive from invading eosinophile leucocytes (Rytömaa & Teir, 1961; Klebanoff, 1965). More recent studies suggest that peroxidase activity is indeed produced in the uterine endometrial cells after oestrogen stimulation (Brökelmann & Fawcett, 1969; McNabb & Jellinck, 1974).

We are now characterizing this activity. In a previous study, it was shown that the peroxidase activity of uterine extracts of rats previously given low doses of oestradiol can be separated into two electrophoretically distinct forms of classical peroxidase with M_r values of approx. 92 000 (peroxidase I) and approx. 40 000 (peroxidase II) (Olsen & Little, 1979). We have subsequently purified peroxidase II to >95% purity (Olsen & Little, 1981) and in the present study report some spectral properties of this enzyme and compare it with certain other haem peroxidases, in particular lactoperoxidase and eosinophile peroxidase.

Materials and methods

Female Wistar rats (160–180 g) were given daily intramuscular injections of $5 \mu\text{g}$ of oestradiol-17 β in

0.2 ml of sesame oil for 3 days; 24 h after the last injection, the animals were decapitated and the uteri were removed and adhering fat was dissected away.

Except for some minor modifications, uterine peroxidase was isolated as previously described (Olsen & Little, 1981). By omitting the first gel-filtration step and using Ultrogel AcA 44 (LKB Produkter, Bromma, Sweden) as matrix in the second gel filtration, the final recovery of enzyme activity was twice that reported previously and the final specific activity was 5760 units/mg of protein. The yield of >95% electrophoretically homogeneous peroxidase was approx. $5 \mu\text{g}$ per uterus from 50 oestradiol-treated rats.

For the present spectrophotometric work, the enzyme was concentrated to approx. 0.1 mg/ml by binding to a small (0.5 ml) column of immobilized concanavalin A (Pharmacia, Uppsala, Sweden) and eluted as described by Olsen & Little (1981). The concentrated enzyme was dialysed against 0.2 M-potassium phosphate buffer (pH 7.4). The material was finally centrifuged (10 000 g for 10 min) to remove insoluble material.

Spectrophotometry was performed in a Cary model 219 spectrophotometer, using $350 \mu\text{l}$ microcuvettes (1 cm light-path), at a scanning rate of 6 nm/min.

Unless otherwise stated 0.2 M-potassium phosphate buffer, pH 7.4, was used as solvent in this study. For the measurement of the spectra of reduced peroxidase a portion of 1 M-sodium dithionite (dissolved in anaerobic buffer) was added to give a final concentration of approx. 3 mM.

Saturation with CO was obtained by bubbling the gas through the solution immediately before the

spectral analysis. The final concentration of potassium cyanide used was approx. 20 mM. The pyridine haemochromogen spectrum was determined by using 0.1 M-NaOH and 25% (v/v) pyridine as described by Paul *et al.* (1953).

The kinetics of formation and decomposition of the peroxidase II-H₂O₂ complex were obtained at room temperature with an Aminco-Chance dual-wavelength spectrophotometer supplied with a Kontron potentiometric recorder (model 1100); for wavelength settings, see the Results section.

Enzyme concentrations were calculated from the A_{412} by using $\epsilon = 112\,200 \text{ litre} \cdot \text{mol}^{-1} \cdot \text{cm}^{-1}$ as for lactoperoxidase (Carlström, 1969).

All chemicals were of an analytical grade.

Results

Spectral properties of uterine peroxidase II and some of its derivatives

The visible-region spectra of the native peroxidase, the reduced enzyme and certain complexes with ligands are shown in Figs. 1 and 2. The ratio A_{412}/A_{280} for the native enzyme in 0.2 M-potassium phosphate buffer was 1.0.

The absorption maxima of the free enzyme, in both native and reduced form, and with some ligands (Table 1) are very similar to those observed for bovine lactoperoxidase (Carlström, 1969). The

Fig. 1. Absorption spectra of native uterine peroxidase II (—), reduced peroxidase (dithionite) (----) and CO-reduced peroxidase (.....)

The concentration of enzyme was $1.6 \mu\text{M}$. Left ordinate refers to Soret region absorbance peak, right ordinate to remainder of spectral traces.

Fig. 2. Absorption spectra of native uterine peroxidase II (—), its CN⁻ compound (----) and CN⁻-reduced peroxidase (.....)

The concentration of enzyme was $1.8 \mu\text{M}$. Left ordinate refers to Soret region absorbance peak, right ordinate to other spectral traces.

known spectral absorption maxima for eosinophile peroxidase from human (Wever *et al.*, 1981) and rats (Archer *et al.*, 1965) are also included in Table 1. The spectrum of the reduced enzyme and its CO complex must, however, be considered with some caution due to the time-dependence of the absorbance at the wavelength maximum. This instability has also been reported for lactoperoxidase, where it seems to be more prominent at lower pH (Carlström, 1969).

When the pyridine haemochromogen spectrum was taken under conditions optimal for proteins with protohaem as the prosthetic group (Paul *et al.*, 1953), an α -band was initially obtained at 564 nm (spectrum not shown). Due to the very limited supply of enzyme, a more detailed characterization of the prosthetic group has not yet been obtained.

Enzyme-substrate complex of uterine peroxidase II

As shown in Fig. 3(a) a rapid spectral change is observed when H₂O₂ is added to the free enzyme

Table 1. Comparison of spectral properties of rat uterine peroxidase II, lactoperoxidase and eosinophile peroxidase

	λ (nm)			
	Rat uterine peroxidase II	Bovine lactoperoxidase*	Human eosinophile peroxidase†	Rat eosinophile peroxidase‡
Peroxidase (Fe^{3+})	412	412	413	415
	500	501	500	505
	541	541	550	
	640	631	640	640
Peroxidase (Fe^{2+})	448	446	449	445
	562	565	563	565
	596	596	597	595
CO-Peroxidase (Fe^{2+})	435	424, 5	435	
CN ⁻ -Peroxidase (Fe^{3+})	431	430		
	555	555, 5		
CN ⁻ -Peroxidase (Fe^{2+})	435	435		
	539	537, 5		
	569	571, 5		
Pyridine haemochrome (α -band)	564	563		567

* Carlström (1969).
† Wever *et al.* (1981).
‡ Archer *et al.* (1965).

Fig. 3. Formation and decomposition of the secondary complex of uterine peroxidase II and H_2O_2 as measured by the wavelength pair 431 nm/420.5 nm. The concentration of enzyme was 484 nM (a) and 27 nM (b). Abbreviation: T, transmission.

(E), and the difference spectrum for the compound thus formed (Fig. 4) revealed a maximum at about 341 nm and an isosbestic point at about 420.5 nm. By using this wavelength pair, it is seen (Fig. 3) that a maximal increase in absorbance was reached approx. 30 s after the addition of H_2O_2 . This behaviour resembles the formation of the secondary complex of lactoperoxidase (Chance, 1949, 1950).

Fig. 4. Absorption spectrum of native uterine peroxidase II (●—●), and the difference spectrum of the secondary complex with H_2O_2 versus native enzyme (○—○). The concentrations of enzyme and H_2O_2 were 27 nM and 2.1 μM respectively.

However, the stability of the spectral change was very dependent on the enzyme concentration. At a high concentration of enzyme ($0.48\mu\text{M}$) the peroxidase- H_2O_2 complex decomposes rather rapidly ($t_{1/2}$ approx. 5.2 min; Fig. 3a), whereas it is relatively stable at a low concentration of enzyme (27 nm) relative to that of H_2O_2 (Fig. 3b). In both cases, however, the addition of iodide, which is a good substrate for the uterine peroxidase (Olsen & Little, 1979), induces a rapid reversal of the spectral change characteristic of the modified/oxidized enzyme, i.e. conversion into native enzyme.

Discussion

The ratio A_{412}/A_{280} for the enzyme preparation used in the present work was 1.0. This value is higher than that reported previously (Olsen & Little, 1981), even though the two enzyme samples had similar specific activities. However, we have observed that the use of the immobilized concanavalin A column to concentrate the enzyme removes from the sample some non-protein material with a peak absorbance at around 260 nm. This latter material contributes to the A_{280} of the enzyme solution (R. L. Olsen, unpublished work).

Although the present spectral studies on uterine peroxidase II must be considered preliminary, due to the very limited amount of pure enzyme available, they are of considerable interest in the general characterization of this peroxidase.

The prosthetic group of the present enzyme has spectral properties that distinguish it clearly from thyroid peroxidase (Hosoya & Morrison, 1967), myeloperoxidase (Agner, 1958; Schultz & Shmukler, 1964). However, similarities appear to exist between uterine peroxidase II and lactoperoxidase and eosinophile peroxidase. In addition, the properties of the H_2O_2 -modified form of lactoperoxidase and of uterine peroxidase are similar (Fig. 3). Both lactoperoxidase and uterine peroxidase are suggested to be secreted enzymes with antimicrobial function (Klebanoff & Smith, 1970; Anderson *et al.*, 1975). Furthermore peroxidases with an immunological resemblance to bovine lactoperoxidase have been observed in several different glands of the cow (Morrison *et al.*, 1965). It should, however, be noted that there are at least two differences between lactoperoxidase and peroxidase II from uterine tissue. Lactoperoxidase is much more readily soluble and has mol.wt. approx. 78 000 (Rombauts *et al.*, 1967), which is about twice that of the uterine enzyme. Possibly, lactoperoxidase and uterine peroxidase II have the same haem-form as prosthetic group.

Limited spectral characterization of eosinophile peroxidase from both man and rat has been carried out (Archer *et al.*, 1965; Wever *et al.*, 1981) and the

prosthetic group of this enzyme appears similar to that of uterine peroxidase II, although detailed differences in the positions of the various absorbance maxima are apparent. Very recently it has been shown that the 40 000-mol.wt. peroxidase from rat uterine tissue (peroxidase II) and rat eosinophile peroxidase have similar molecular weights and electrophoretic mobilities (Hosoya & Saito, 1981). It is therefore reasonable to conclude that the origin of rat uterine tissue peroxidase activities has not yet been definitively established and that further work is needed in this field.

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