Evidence for the Presence of a Myeloperoxidase-like Green Heme Peroxidase in Human Spleen

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Myeloperoxidase [1], isolated from leukocytes of a variety of species [2–5], contains an unusual green heme as its prosthetic group. The enzyme has been shown to possess an $\alpha_2\beta_2$ structure, with two heavy subunits (M_r = 57,500) and two light subunits (M_r = 10,500) [1]. Myeloperoxidase contains two spectroscopically equivalent hemes per molecule, at least one of which is covalently attached to one of the heavy subunits [1, 3, 6]. The nature of the heme has not been unequivocally established, but a formylheme structure related to that of heme *a* has been proposed [6, 7].

We have recently described the preparation and some properties of a greeen heme peroxidase from bovine spleen [8]; it is very similar to myeloperoxidase in its spectroscopic properties, but differs in its observed substrate specificity and molecular weight (M_r = 57,000). These results suggested that the green heme protein might be related in some way to the heavy heme-containing subunit of myeloperoxidase. The green heme protein is apparently distinct from hemi-myeloperoxidase [9], which has been prepared by reduction cleavage of myeloperoxidase and contains one each of the heavy and light subunits (M_r = 78,000). We present herein evidence for the presence of an apparently similar green heme peroxidase in human spleen.

Experimental

A 1.5 kg spleen, removed from an adult female patient with malignant lymphoma and provided courtesy of Dr. N. V. Dimitrov, M. D. (Department of Medicine, Michigan State University), was sectioned, and ~0.5 kg was utilized for pathological studies. The remaining 1.0 kg was stored on ice for ~2 hours prior to extraction.

After homogenization with 2.0 l of 0.25 M KCl, pH 3.5, in a Waring blendor at medium speed for one minute and at high speed for two minutes, the spleen extract was adjusted to pH 3.5 with 6 M HCl and stirred at 20 °C for 20 hours. The homogenate was centrifuged at 9000 × g for 10 minutes at 20 °C, and the supernatant filtered through glass wool to remove small waxy particles. The resulting filtrate was made 0.1 M in ascorbic acid and adjusted to pH 5.5 with 12% NaOH. The solution was then treated with 4 g/l of cellulose phosphate (P-11, Whatman). The P-11 was filtered, washed twice with 50 ml of H_2O, resuspended in 100 ml of 2.0 M KCl, and stirred for one hour. The P-11 was filtered and washed with 25 ml of 2.0 M KCl resulting in a pale yellow green filtrate. The cloudy solution was centrifuged at 9000 × g for 20 minutes at 20 °C for clarification to avoid plugging the chromatography column in the succeeding step. (All subsequent operations were carried out at 5–10 °C in a cold cabinet.)

The clear filtrate was diluted to 0.15 M KCl and loaded onto a carboxymethyl-cellulose (CM-52, Whatman) column (1.0 × 10 cm) at 1.5 ml/min. The column was washed with 30 ml of 0.2 M KCl, 0.05 M sodium acetate, pH 5.0 and eluted with a 0.15–1.0 M KCl gradient in 0.05 M sodium acetate buffer, pH 5.0. The KCl concentration was monitored by measuring the conductivity of the samples and comparing to a set of KCl/buffer standards. The green fractions with $A_{434}/A_{280} \geq 0.10$ were pooled, concentrated to 2.0 ml by ultrafiltration, loaded onto a Sephadex G-75 column (1.5 × 85 cm), and eluted with 0.2 M KCl, 0.05 M sodium acetate, pH 5.0. The green fractions with $A_{434}/A_{280} \geq 0.4$ were combined and loaded onto the CM-52 column used earlier. After elution with 30 ml of 0.2 M KCl, 0.05 M sodium acetate, pH 5.0, the column was sectioned, the top 5 mm extracted with 5.0 ml of 2.0 M KCl by shaking, and the extract decanted after brief centrifugation. The ratio A_{434}/A_{280} was 0.73.

Electronic spectra were obtained on a Cary 219 spectrophotometer; activity data using I^- as substrate were obtained using a Beckman DU spectrophotometer equipped with a Gilford 252 D accessory. Experimental conditions were identical to those described previously for the bovine spleen enzyme [8]. Reduction and adduct formation with CN^- and NO and molecular weight estimation were performed as previously described [8].

Results

The purification of the green heme protein from human spleen is summarized in Table I. It involves

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TABLE I. Purification of Green Heme Protein from Human Spleen

Step	Total Protein[a] (mg)	Total Enzyme[b] (mg)	A_{434}/A_{280}	Recovery (%)
Acid extract	18,500	–	–	–
P-11	360	12.5	0.028	100
CM-52	21.4	5.9	0.22	47
CM-52 rechromatography	6.0	3.8	0.50	30
G-75	3.2	2.2	0.57	18
CM-52 column sectioning	1.4	1.2	0.73	10

[a]Estimated by assuming that an absorption at 280 nm of 1.0 is equivalent to 1.0 mg protein/ml. [b]Assumes that A_{434}/A_{280} = 0.80 for homogeneous enzyme (value observed for bovine spleen enzyme (Ref. [8])).

TABLE II. Summary of Visible Absorption Data for Green Heme Peroxidase from Human and Bovine Spleen.

	Absorption Maxima (nm)					
	α		β		γ	
Form	Human	Bovine	Human	Bovine	Human	Bovine
Native (Fe^{3+})	572	574	500	500	434	434
Native (Fe^{3+}) + CN^-	630	632	–	–	450	452
Reduced (Fe^{2+})	632	636	592	590	468	473
Reduced (Fe^{2+}) + CN^-	610	613	520	522	460	461
Reduced (Fe^{2+}) + NO	625	626	–	–	450	452

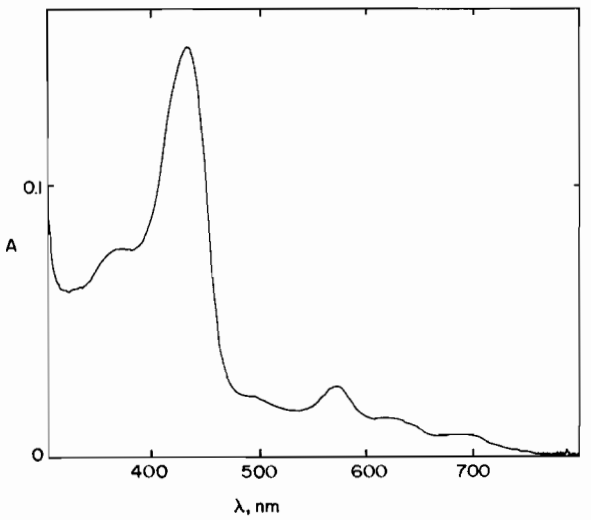

Fig. 1. Visible absorption spectrum of the native (Fe^{3+}) human green heme peroxidase from spleen.

extraction of spleen tissue at low pH, followed by batch adsorption on cellulose phosphate and sequential cation exchange and gel filtration chromatography, with a final adsorption on and elution from carboxymethyl cellulose. This procedure involves only minor modifications of that used for bovine spleen green heme protein [8]. The final purity achieved with the human protein was apparently lower than that of the bovine protein, as judged by the A_{434}/A_{280} ratio of 0.73 *vs.* 0.80.

Figure 1 shows the visible absorption spectrum for the native (as isolated) human enzyme; principal features include a large peak at 434 nm (γ band) with shoulders at 370 nm and at 500 nm (β band) and a peak at 572 nm (α band). Both the relative positions and relative intensities of peaks in the human enzyme spectrum are essentially identical to those of the bovine spleen enzyme. Table II summarizes the visible absorption data for the beef spleen and human spleen enzymes and their CN^- and NO adducts. Except for the reduced forms, all spectra of the two enzymes have maxima agreeing within 3 nm. The spectra of the reduced forms of the two enzymes are similar, but the Soret (γ) band of the human enzyme is at 5 nm shorter wavelength than the corresponding band of the bovine enzyme.

Experiments using I^- as substrate show that the human spleen green heme protein also exhibits peroxidase activity, utilizing hydrogen peroxide to form I_3^- from I^-. The specific activity of the enzyme is difficult to ascertain since the enzyme is not homogeneous; it appears to be in the same range as for the bovine enzyme, assuming similar molar absorptivities for the Soret peaks. The method of Porath [10] was used to estimate the molecular weight, and gave a value of M_r = 58,000. This is in good agreement with the values of M_r = 56,000 and 57,000 deter-

mined for the molecular weight of the bovine enzyme using gel electrophoresis and gel permeation chromatography [8].

Discussion

The isolation procedure for and physical properties of the green heme peroxidase from human spleen are essentially identical to those reported for the corresponding protein from bovine spleen [8]. The yield of human enzyme is substantially lower than that typically achieved with bovine spleen (about 3 mg/kg *vs.* 12 mg/kg), however, possibly accounting for the lower degree of purification obtained. In particular, the visible absorption spectra of the bovine and human green heme peroxidases are virtually identical [*cf.* Table II], and are very similar to that of myeloperoxidase [11], strongly suggesting the presence of closely related chromophores in all three proteins.

The similarity in molecular weight between the spleen enzymes and the heavy subunit of myeloperoxidase, together with the recent report of reductive cleavage of myeloperoxidase to hemi-myeloperoxidase [9], suggests that the spleen enzymes may indeed be closely related to myeloperoxidase, possibly as degradation products or as precursors. Our observations (J. C. Davis and B. A. Averill, unpublished results) that the levels of both the green heme protein and the copurifying purple acid phosphatase [12, 13] vary tremendously in spleens from cows with varying nutritional status and health suggest that levels of the green heme peroxidase may be correlated with the activity of the immune system. Immunochemical studies are in progress to examine the extent of cross reactivity between the human and bovine green peroxidases and myeloperoxidase, as well as to develop a more convenient and accurate way of assessing the content of the green heme peroxidases in spleen tissue so that more precise correlations with medical histories may be obtained.

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