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ISOLATION PROCEDURE AND SOME PROPERTIES OF MYELOPEROXIDASE FROM HUMAN LEUCOCYTES

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Summary

1. A rapid isolation procedure with a high yield for pure myeloperoxidase (donor:H_2O_2 oxidoreductase, EC 1.11.1.7) from normal human leucocytes is described. The enzyme was solubilized from leucocytes with the detergent, cetyltrimethylammonium bromide, and purified to apparent homogeneity. The yield of the enzyme was 17% with an absorbance ratio $A_{430\,nm}/A_{280\,nm}$ = 0.85.

2. The purified enzyme showed three isoenzyme bands after polyacrylamide gel electrophoresis; ultracentrifuge studies indicated one homogeneous band with a molecular weight of 144 000. After reduction of myeloperoxidase, sodium dodecyl sulfate gel electrophoresis resolved an intense band (63 000 daltons) and a weak band (81 000 daltons).

3. The carbohydrate content of the enzyme was at least 2.5%. Mannose, glucose and *N*-acetylglucosamine were present. The amino acid composition is reported.

4. The EPR spectrum exhibited a high-spin heme signal with rhombic symmetry (g_x = 6.92, g_y = 5.07 and g_z = 1.95). Upon acidification this signal was converted into a signal with more axial symmetry ($g_\perp$ = 5.89). At high pH (9.5) the EPR spectrum of the enzyme only shows low-spin ferric heme resonances. The circular dichroism spectra of ferric and ferrous myeloperoxidase in the visible and ultraviolet region show maxima and minima in ellipticity.

Introduction

Myeloperoxidase (donor:H_2O_2 oxidoreductase, EC 1.11.1.7) has been isolated from sources like pus [1,2], sputum [3], chloroma tumour tissue [4], leucocytes [5—8] and bone marrow [9]. However, pus, sputum, tumours and leukaemic leucocytes have the disadvantage that they are difficult to obtain in large quantities and that enzymes prepared from activated, degraded or abnormal cells might be modified in their protein structure and/or enzymic activity.

Moreover, myeloperoxidase isolated from tumour cells contains tightly bound porphyrins that are difficult to remove [4].

In some of the isolation procedures the enzyme is solubilized from cells by adding trypsin to the homogenate [10] or by degrading the cells by lysosomal hydrolases [7], but proteases may affect the peptide and subunit composition of the enzyme. An effective method for solubilizing myeloperoxidase from the leucocytes without use of proteolytic enzymes has been introduced by Desser et al. [11]. In this procedure the enzyme is extracted from granules with the detergent cetyltrimethylammonium bromide. We have used this positive soap in a rapid isolation procedure of myeloperoxidase from human leucocytes. A description of the isolation of large amounts of the enzyme and of some of its characteristic properties is given in this paper.

Materials

Buffy coat, the layer between plasma and red cells and containing the leucocytes, was collected by centrifugation of donor blood at $1000 \times g$ for 15 min. The buffy coat was washed twice with a hypotonic solution of 155 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA to lyse remaining red cells. After centrifugation at $800 \times g$ for 15 min the white cells were resuspended in 152 mM NaCl, 10 mM potassium phosphate (pH 7.3). The yield of leucocytes from 50 l human blood was $1-2 \cdot 10^{11}$ cells. A differential count showed the presence of 80–85% granulocytes in the leucocyte suspension. The white cells were stored in liquid N_2.

Cetyltrimethylammonium bromide was obtained from Fluka A.G. (Switzerland). Sulphonylpropyl-Sephadex (C-50), concanavalin A covalently bound to Sepharose 4-B and Sephadex G-100 were obtained from Pharmacia (Uppsala, Sweden), AcA-34 from LKB (Sweden) and D-10-camphorsulphonic acid from Baker Chemicals. All other chemicals were of analytical grade.

Methods

Absorption spectra were recorded on a Cary-17 recording spectrophotometer, circular dichroism spectra with a Cary-60 spectropolarimeter, equipped with a Cary-6002 CD attachment. The circular dichroism instrument was set to a spectral bandwidth of 1.5 nm and calibrated with aqueous D-10-camphorsulphonic acid (1 mg/ml). The optical cell used was of 1 cm path length. In the visible and ultraviolet region the data were expressed in terms of molar ellipticity, θ, i.e. degrees $\cdot$ cm^2 $\cdot$ $decimol^{-1}$ myeloperoxidase.

The concentration of myeloperoxidase was calculated with an absorption coefficient of 89 $mM^{-1} \cdot cm^{-1}$ at 430 nm [12]. The purity of the enzyme was expressed as the ratio of absorbance at 430 nm to that at 280 nm ($A_{430\,nm}/A_{280\,nm}$).

Polyacrylamide gel electrophoresis was carried out at pH 4.6 as described by Himmelhoch et al. [9]. SDS gel electrophoresis was performed as described by Weber et al. [13] and Maurer [14]. Standard proteins used for molecular weight estimates were: bovine serum albumin, 68000; catalase, 58000; ovalbumin, 45000; aldolase, 40000; chymotrypsin, 25000; myoglobin, 17000;

bovine cytochrome *c*, 12 000. Gels were stained with Coomassie Blue for protein and with the periodic acid-Schiff method [15] for carbohydrate. The staining for peroxidase activity was as follows: the gels were immersed in 1 mM *o*-dianisidine for 10—20 min and subsequently in 1 mM H_2O_2 for an additional 10—20 min.

Protein was determined by the method of Lowry et al. [16], with bovine serum albumin as standard, and amino acids by the method of Spackman et al. [17] using the Beckman Multichrom M amino acid analyzer. The quantitative analysis of monosaccharides present in myeloperoxidase was carried out essentially as described by Kamerling et al. [18].

Ultracentrifugal sedimentation analyses were performed in a MSE analytical ultracentrifuge Model MK 2 equipped with an ultraviolet scanning system. Sedimentation coefficients of myeloperoxidase were determined at 280 nm and 430 nm. The values of the coefficients were corrected for density and viscosity of the solution and the molecular weight was calculated [19] with the equation

$$\text{mol. wt.} = \frac{s_{20,w}}{D_{20,w}} \cdot \frac{R \cdot T}{(1 - v\rho)}$$

EPR spectra were examined with a Varian E-9 spectrometer equipped with a helium transfer system (Air Products Inc. Model LTD-3-100) with automatic temperature controller or with the low-temperature device described by Albracht [20]. Magnetic field and microwave power were measured as previously reported [21].

Purification of myeloperoxidase

The suspension of leucocytes (see Materials) was homogenized in 0.34 M sucrose/0.1 M potassium phosphate (pH 7.3) in a Potter-Elvehjem homogenizer with a motor-driven Teflon pestle. The homogenate (12 mg/ml protein) was centrifuged at 20000 × *g* (MSE-65 superspeed centrifuge) for 25 min and the supernatant was discarded. The pellet was homogenized in 0.5% cetyltrimethylammonium bromide, 0.1 M Na_2SO_4 and 0.1 M potassium phosphate (pH 7.3) in a 1-l Waring Blendor at 14000 rev./min for 45 s. After centrifugation at 20000 × *g* for 25 min, the clear green supernatant was saved; the pellet was rehomogenized in the buffer (with 1% cetyltrimethylammonium bromide). After centrifugation the supernatants were combined to a concentration of 3.8 mg/ml protein.

Myeloperoxidase was further purified by fractionation with solid $(NH_4)_2SO_4$ at various pH values. The first precipitation was carried out at pH 7.3, the pH being held constant by adding gradually 1 M K_2HPO_4. The precipitate between 52% and 70% saturation was dissolved in 0.1 M sodium acetate (pH 4.9). $(NH_4)_2SO_4$ fractionation was repeated at pH 4.9; the precipitate at 50—65% saturation was collected and dissolved in 0.1 M potassium phosphate (pH 7.3). To decrease the salt concentration the solution was dialysed overnight against 0.1 M potassium phosphate (pH 7.3).

A sulphopropyl-Sephadex (C-50) column (15 × 3 cm) was equilibrated with 0.1 M potassium phosphate (pH 7.3) and the dialysed myeloperoxidase applied. The enzyme was eluted with a continuous gradient of 0.1—1 M potassium phos-

phate (pH 7.3, flow rate 25 ml/h). The elution pattern (Fig. 1) shows that myeloperoxidase was eluted at 0.15 M phosphate. The enzyme fractions eluted between 190—210 ml were pooled and dialysed for 2 h against 0.1 M potassium phosphate (pH 7.3).

An AcA-34 resin column (70 × 3 cm) equilibrated with 0.1 M potassium phosphate (pH 7.3) was used (Fig. 2). Since the capacity of the column was rather small, 5 ml dialysed enzyme solution was applied. Elution was performed with 0.1 M potassium phosphate (20 ml/h). The fractions eluted between 230—250 ml ($A_{430\,nm}/A_{280\,nm} \geqslant 0.8$) were pooled.

Results

We have focused our attention on the isolation of myeloperoxidase from normal human leucocytes using detergent extraction. The starting leucocyte homogenate contained 3.5 μmol myeloperoxidase and, after extraction with cetyltrimethylammonium bromide, the solution contained 3.3 μmol myeloperoxidase: a yield of 94% (Table I). Purification of the enzyme was carried out by $(NH_4)_2SO_4$ fractionations at two pH values. The crude enzyme thus obtained had an absorbance ratio $A_{430\,nm}/A_{280\,nm}$ of 0.2 and contained 2.5 μmol myeloperoxidase (yield, 70%). Agner [3] reported that the isoelectric point of the enzyme was greater than pH 10 and, therefore, at pH 7.3 cationic exchangers were used. CM-cellulose 32, Amberlite CG-50, CM-Sephadex, hydroxylapatite and SP-Sephadex C-50 were tested and SP-Sephadex gave the best results. This material has a high capacity, tight binding at low ionic strength, rapid elution at higher ionic strength and gave high recovery (80%) of the enzyme. After elution of the SP-Sephadex column, about 60% of myeloperoxidase ($A_{430\,nm}/A_{280\,nm} = 0.5$) was recovered (Fig. 1). This fraction was further purified on AcA-34 and Sephadex G-100. The ratio $A_{430\,nm}/A_{280\,nm}$ increased in both cases to 0.82—0.87, but the recovery of myeloperoxidase was 35—40% using the AcA column and 30—35% using Sephadex. The total recovery of high purity enzyme from 50 l human blood was about 100 mg (Table I, a yield of 15—17%).

Affinity chromatography was also tested as the final purification step. For this purpose, the carbohydrate-binding resin, concanavalin A bound to Sepharose 4-B, was used. Myeloperoxidase was tightly bound to this material, but it

TABLE I

PURIFICATION OF MYELOPEROXIDASE FROM HUMAN LEUCOCYTES

Fraction	Protein (mg)	Enzyme (μmol)	Recovery (%)	A_{430nm}/A_{280nm}
Homogenate of 200 ml leucocytes	6000	3.5	100	<0.1
Cetyltrimethylammonium bromide extracts	2750	3.3	94	<0.1
$(NH_4)_2SO_4$ precipitate after dialysis	1100	2.5	70	0.2
Eluate from SP-Sephadex column	500	1.5	43	0.5
Eluate from AcA-34 column	100	0.6	17	0.85

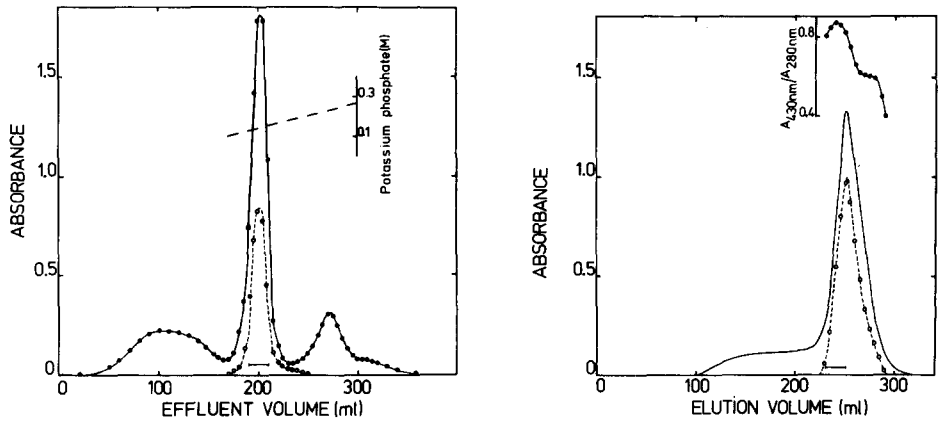

Fig. 1. Ion exchange chromatography of myeloperoxidase on SP-Sephadex C-50 at pH 7.3. Elution was carried out by increasing the potassium phosphate concentration as indicated in the figure. Flow rate, 25 ml · h^{-1}. Protein absorption was measured at 280 nm (———) and myeloperoxidase absorption at 430 nm (- - - - - -). |———|, fractions collected for further purification.

Fig. 2. Molecular filtration of purified myeloperoxidase on AcA-34 eluted with 0.1 M potassium phosphate (pH 7.3). Flow rate, 20 ml · h^{-1}. Protein was continuously measured at 280 nm (———) and myeloperoxidase at 430 nm (- - - - - -). The purity of the enzyme is given by the ratio $A_{430\,nm}/A_{280\,nm}$. |———|, fractions with a ratio above 0.80.

was impossible to elute the enzyme with 2 M α-methylglucose and/or high salt and detergent concentrations.

Myeloperoxidase has also been purified from pig leucocytes using the above-mentioned procedure. In this case, the concanavalin A column was used, since with 1—2 M α-methylglucose the enzyme was eluted with an $A_{430\,nm}/A_{280\,nm}$ ratio of 0.8. As pig leucocytes contain about 4 times less myeloperoxidase (unpublished data) the yield of the enzyme from pig blood was low.

The sedimentation coefficient ($s_{20,w}$) of human myeloperoxidase calculated from sedimentation velocity studies (not shown) is 7.6 S. The partial specific volume (calculated from the amino acid composition (Table II), by the method of Schachman [22]) is 0.729 ml/g. Using the value of $5.1 \cdot 10^{-7}$ $cm^2 \cdot s^{-1}$ for the diffusion coefficient ($D_{20,w}$) [23], the molecular weight of human myeloperoxidase was calculated at 144000, in agreement with other reports [5,7,11, 12,23]. From the last line of Table I, a molecular weight of 166000 for the purified enzyme may be calculated. This indirectly determined value is higher than that found in our ultracentrifuge studies. This discrepancy may be explained by an error in the absorption coefficient for the heme group which is based only on total iron analysis and not on heme iron (cf. ref. 23).

The amino acid composition of our preparation (Table II) is similar to that reported by others for the human [5], pig [23] and canine [2] enzyme.

To test the purity of the final preparation, myeloperoxidase (a cationic protein) was subjected to polyacrylamide gel electrophoresis and the gels stained for protein, carbohydrate and peroxidase activity. The gels showed the same pattern with all three staining procedures; one broad band with two minor bands located at the cathodic site (unpublished data). SDS gel electrophoresis of 2-mercaptoethanol-treated enzyme resolved one intense band (molecular

TABLE II

AMINO ACID COMPOSITION OF HUMAN MYELOPEROXIDASE

The amino acids were determined after hydrolysis of myeloperoxidase (A_{430nm}/A_{280nm} = 0.85) in 6N HCl at 110°C in vacuo. The analyses were carried out at 24, 48 and 72 h. The values in the table, corrected for losses during hydrolysis, are in terms of residues per 140 000 daltons.

Amino acid	Species			
	Human	Human * [5]	Pig * [23]	Canine [2]
Lys	39	33	41	24
His	14	12	16	14
Arg	107	105	139	122
Asp	136	153	145	152
Thr	58	69	52	61
Ser	61	63	52	74
Glu	129	111	99	108
Gly	80	78	81	100
Ala	75	78	78	68
Val	71	51	74	48
Met	31	36	22	22
Ile	48	48	37	48
Leu	121	129	136	128
Tyr	27	24	24	34
Phe	55	51	59	56

* Recalculated to a mol. wt. of 140 000.

weight 63000) and one weak band (molecular weight 81000 (unpublished data).

Since staining of myeloperoxidase for carbohydrate in polyacrylamide gels was positive and the enzyme was irreversibly bound on concanavalin-A-Sepharose we conclude that our preparation of human myeloperoxidase like other peroxidases [24,25] contains carbohydrate. This was confirmed by analysis of the carbohydrate composition, which showed the presence of 1.3% mannose, 0.6% glucose and 0.6% *N*-acetylglucosamine. Since an acetylation step was used in the assay, all glucosamines were also determined as *N*-acetylglucosamine.

The absorption spectra of oxidized and reduced myeloperoxidase (Fig. 3) are identical to those shown elsewhere [5,6,8,12]. The oxidized enzyme is characterized by absorption maxima at 428 nm and 565 nm with shoulders at 355, 500, 625 and 675 nm. Ferromyeloperoxidase, reduced by dithionite, shows maxima at 473 and 638 nm. The circular dichroism spectra of the enzyme are presented in Fig. 4. Oxidized myeloperoxidase exhibits negative ellipticity bands in the near ultraviolet at 282, 278, 268, 260 and 252 nm and in the far ultraviolet an intense negative band at 230 nm. In the Soret and visible region the oxidized enzyme is further characterized by a minimum at 355 nm, an intense negative band ($\theta = -7.55 \cdot 10^4$) at 412 nm and a broad negative band at 560 nm. Maxima are present at 450 and around 620 nm. The spectrum of the enzyme reduced by dithionite shows an ellipticity maximum at 480 and a negative band at 633 nm.

Fig. 5A shows the EPR spectrum at 15 K of isolated ferrimyeloperoxidase at pH 7.3. There is a well-resolved signal near $g = 6$, characteristic of high-spin

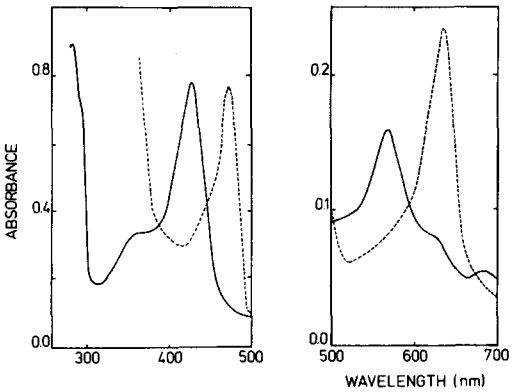

Fig. 3. Absorption spectra of oxidized (———) and reduced (- - - - -) myeloperoxidase (8.7 μM) in 100 mM potassium phosphate (pH 7.3). The protein was reduced with a slight excess of $Na_2S_2O_4$.

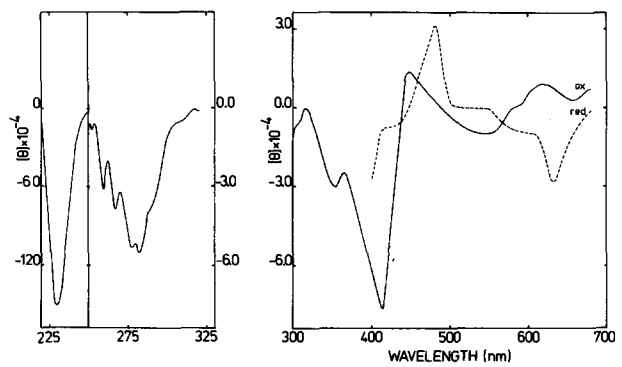

Fig. 4. Circular dichroism spectra of oxidized (———) and reduced (- - - - -) myeloperoxidase. 13.5 μM myeloperoxidase in 0.1 M potassium phosphate (pH 7.3).

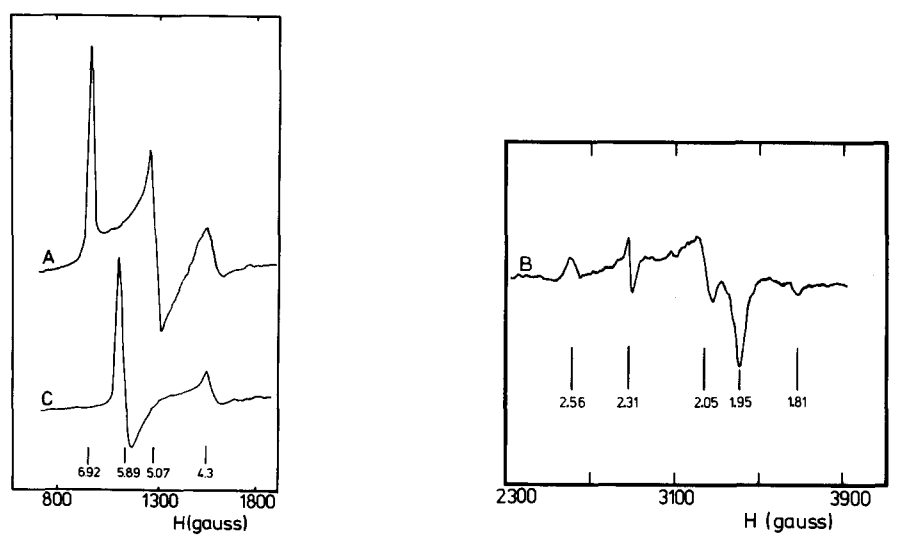

Fig. 5. EPR spectra of isolated myeloperoxidase. Concentrations of myeloperoxidase in A and B were 40 μM and 225 μM, respectively. Trace A and B, 0.1 M potassium phosphate (pH 7.3); trace C, 10 mM HCl (pH 2). The low-field region shown in A was taken at 5 X lower receiver gain than the record of B. The resonance at $g = 2.05$ is probably due to trace amounts of copper in this preparation. Conditions of EPR spectroscopy were: frequency, 9.319 GHz; microwave power, 10 mW; modulation amplitude, 10 G; scanning rate 500 G · min^{-1}; time constant, 1 s; temperature, 15 K.

ferric heme iron in an environment of rhombic symmetry ($g_x = 6.92, g_y = 5.07$ and $g_z = 1.95$). Additional weak intensity resonances were observed at higher field (Fig. 5B) one of which can be attributed to a minor amount of low-spin heme ($g_x = 2.56$, $g_y = 2.31$ and $g_z = 1.81$). At present the origin of this signal is not clear. At pH 9.5 myeloperoxidase shows only low-spin ferric heme resonances ($g_x = 2.84$, $g_y = 2.19, g_z = 1.67$) possibly representing an OH^- derivative of the enzyme (cf. ref. 26). The g values of these low-spin heme signals are different from those observed at neutral pH. The acid form (at pH 2.0) of the enzyme, first described by Odajima and Yamazaki [27], shows a high-spin heme signal (Fig. 5C) with a more axial symmetry ($g_\perp = 5.89$) than that observed in the neutral enzyme. The absorption spectrum of the acid enzyme has a Soret maximum at 410 nm, compared with 428 nm in the neutral and alkaline forms.

Discussion

Myeloperoxidase obtained from normal human leucocytes by detergent solubilization according to the method described in this study is not contaminated by other proteins as indicated by gel electrophoresis and ultracentrifugation, and the ratio $A_{430\,nm}/A_{280\,nm}$. The yield (100 mg) of purified enzyme is high compared with reports in the literature [6,7]. Most of the chemical and physical properties described here are in agreement with those of myeloperoxidase isolated from the same and other sources e.g. leukaemic granulocytes [7,8], infected dog uterus [1], rat chloroma tissue [4], guinea-pig bone marrow [9,11] and canine pus [2].

Most reports do not mention the presence of carbohydrate in the enzyme. Recently, however, Harrison et al. [2] demonstrated that myeloperoxidase isolated from canine pus contains 3.2—4% carbohydrate. The carbohydrate moiety of our preparation of human myeloperoxidase accounts for at least 2.5% of the total weight of the enzyme and consists of mannose, glucose and *N*-acetylglucosamine.

On polyacrylamide gels, one intense band and two minor bands are visible, all showing peroxidase activity and staining for protein and carbohydrate, indicating that our preparation consists of three myeloperoxidase isoenzymes. Felberg and Schultz [28] have reported that myeloperoxidase consists of six isoenzymes. In horseradish [25] and turnip [29] peroxidase also variable numbers of isoenzymes have been found.

The number of subunits revealed by SDS gel electrophoresis and the molecular weight of these components are still in dispute. Desser et al. [11] reported for the guinea-pig enzyme one subunit (65000*), Harrison et al. [2] two subunits (57000* and 10500) for canine myeloperoxidase whereas Olsson et al. [8] found four subunits (62000, 54000*, 38000 and 14000) in the enzyme isolated from leukaemic myeloid cells. In our preparation of myeloperoxidase only two polypeptides (81000 and 63000*) were observed upon treatment with 2-mercaptoethanol and SDS. It is clear that in all preparations from various species the most intensively stained subunit (*) has a molecular weight of about 60000. Whether the polypeptide with molecular weight of 81000 observed in our preparations is a contaminant remains to be established. The

total molecular weight of the two polypeptides is 144000 and thus it is conceivable that both subunits are integral part of the molecule. Since staining factors with Coomassie Blue of these two polypeptides may differ significantly, it is not yet possible to conclude that they are present in a 1 : 1 molar ratio.

The circular dichroism bands of oxidized and reduced myeloperoxidase correlate well with the absorption maxima of the enzyme. Some weak absorption bands have a large magnetic transition dipole moment and thus show intense circular dichroism whereas some strong absorption bands generate little or no optical activity. The bands at 282, 278, 260 and 252 nm can be attributed to aromatic amino acids and disulfides [30] and those at 350, 412, 450, 560 and 620 nm may be assigned to the heme group [31].

Is is interesting to note that myeloperoxidase, *b*-type cytochromes and catalase [32] show large negative peaks in the Soret region, but that horseradish peroxidase [31] and turnip peroxidase [29] show positive peaks in the Soret region.

Since optical and EPR spectra of the isolated enzyme are identical to those observed in resting granulocytes [33] it is concluded that the isolation procedure does not affect the active centre of the enzyme.

Finally, the large amounts of myeloperoxidase obtained in the rapid isolation procedure presented in this paper provides us with sufficient purified enzyme for studying the physical properties of myeloperoxidase in detail.

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References

1 Agner, K. (1958) Acta Chem. Scand. 12, 89—94
2 Harrison, J.E., Pabalan, S. and Schultz, J. (1977) Biochim. Biophys. Acta 493, 247—259
3 Agner, K. (1941) Acta Physiol. Scand. 2, Suppl. VIII
4 Newton, N., Morell, D.B. and Clarke, L. (1965) Biochim. Biophys. Acta 96, 463—475
5 Schultz, J. and Schmukler, H.W. (1964) Biochemistry 3, 1234—1238
6 Rohrer, G.F., von Wartburg, J.P. and Aebi, H. (1966) Biochem. Z. 344, 478—491
7 Zgliczyński, J.M., Stelmaszyńska, T., Ostrowski, W., Naskalski, J. and Sznajd, J. (1968) Eur. J. Biochem. 4, 540—547
8 Olsson, L., Olofsson, T. and Odeberg, H. (1972) Scand. J. Haematol. 9, 483—491
9 Himmelhoch, S.R., Evans, W.H., Mage, M.G. and Peterson, E.A. (1969) Biochemistry 8, 914—921
10 Maehly, A.C. (1955) Methods Enzymol. 2, 794—801
11 Desser, R.K., Himmelhoch, S.R., Evans, W.H., Januska, M., Mage, M. and Shelton, E. (1972) Arch. Biochem. Biophys. 148, 452—465
12 Ehrenberg, A. and Agner, K. (1958) Acta Chem. Scand. 12, 95—100
13 Weber, K., Pringle, J.R. and Osborn, M. (1972) Methods Enzymol. 26, 3—27
14 Maurer, H.R. (1971) in Disc Electrophoresis and Related Techniques of Polyacryl Amide Gel Electrophoresis, (Fishbeck, K., ed.), 2nd edn., W. De Gruijter, Berlin

15 Segrest, J.P. and Jackson, R.L. (1972) Methods Enzymol. 28, Part B, 54—63
16 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
17 Spackman, D.H., Stein, W.H. and Moore, S. (1958) Anal. Chem. 30, 1190—1206
18 Kamerling, J.P., Gerwig, G.J., Vliegenthart, J.F.G. and Clamp, J.R. (1975) Biochem. J., 151, 491—495
19 Svedberg, T. and Pederson, K.O. (1940) The Ultracentrifuge, Oxford University Press
20 Albracht, S.P.J. (1974) J. Magn. Resonance 13, 299—303
21 Wever, R., Van Drooge, J.H., Van Ark, G. and Van Gelder, B.F. (1974) Biochim. Biophys. Acta 347, 215—223
22 Schachman, H.K. (1957) Methods Enzymol. 4, 32—103
23 Odajima, T. and Yamazaki, I. (1972) Biochim. Biophys. Acta 284, 360—367
24 Morris, D.R. and Hager, L.P. (1966) J. Biol. Chem. 241, 1763—1768
25 Shannon, L.M., Kay, E. and Lew, J.Y. (1966) J. Biol. Chem. 241, 2166—2172
26 Tamura, M. and Hori, H. (1972) Biochim. Biophys. Acta 284, 20—29
27 Odajima, T. and Yamazaki, I. (1972) Biochim. Biophys. Acta 284, 368—374
28 Felberg, N.T. and Schultz, J. (1972) Arch. Biochem. Biophys. 148, 407—413
29 Job, D. and Dunford, H.B. (1977) Can. J. Biochem. 55, 804—811
30 Beychok, S. (1966) Science 154, 1288—1299
31 Strickland, E.H., Kay, E., Shannon, L.M. and Horwitz, J. (1968) J. Biol. Chem. 243, 3560—3565
32 Kajiyoshi, M.and Anan, F.K. (1977) J. Biochem. (Tokyo) 81, 1319—1325
33 Wever, R., Vulsma, T. and Bos, A. (1977) in Movement, Metabolsim and Bactericidal Mechanisms of Phagocytes (Rossi, F., Patriarca, P.L. and Romeo, D., eds.), pp. 201—206, Piccin Medical Books, Padua