MYELOPEROXIDASE X: COMPARISON OF NORMAL HUMAN LEUCOCYTE MYELOPEROXIDASE PREPARED WITH AND WITHOUT THE USE OF TRYPSIN

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In 1958 myeloperoxidase (MPO), the peroxidase of the neutrophile, was separated into two components (Agner, 1958). By means of two-directional electrophoresis on acrylamide gel (Felberg and Schultz, 1968) and free-flow electrophoresis in urea (Schultz, et al., 1967) it was shown that MPO is indeed quite heterogeneous. Recently Himmelhoch, et al., (1969) observed only two forms of MPO obtained from guinea pig bone marrow. They suggested that the multi-component system observed by us was due to the use of trypsin for solubilizing the MPO (Schultz and Shmukler, 1964). If trypsin catalyzed the cleavage of several bonds distant from the catalytic center of the MPO, one might isolate a variety of molecular species, each possessing full enzymic activity but migrating differently on electrophoresis. In order to test this possibility MPO was prepared without the use of trypsin and compared with MPO prepared with trypsin. In addition, the highly purified preparation obtained without using trypsin was treated with the proteolytic enzyme to give a third preparation. The three preparations gave the same multi-component system when examined by means of electrophoresis on acrylamide gel.

METHODS AND MATERIALS

Over a three-week period, 800 ml of "buffy coat" prepared from 606 pints of whole blood, were processed by a modification of the methods of

Schultz and Shmukler, 1964, and Zgliczynski, et al., 1968. The "buffy coat" was mixed with an equal volume of 10 mM potassium phosphate buffer, pH 7.0, containing 1 mM di-sodium EDTA, and centrifuged at 32,000 x g for 30 minutes. The supernate was discarded and the packed cells diluted to the original volume with water. The suspension was homogenized in a Waring Blendor for four 3-minute periods. Solid NaCl was added to the homogenate to a concentration of 6%. After 15 hours, the homogenate was centrifuged and an equal volume of ethanol was added to the supernate. The suspension was centrifuged and the MPO was extracted from the precipitate by 0.1 M potassium phosphate buffer, pH 7.0. The extract was dialyzed against 50 mM potassium phosphate buffer, pH 7.0 and purified on XE-64 (according to Schultz and Shmukler, 1964) to an RZ (A_{430nm}/A_{280nm}) of 0.58. The MPO was further purified on CM-Sephadex according to Felberg, 1969. MPO was applied to a 1.5 x 48 cm column of CM-Sephadex C-50, in 0.1 M sodium phosphate buffer, pH 7.0 and eluted with a pH gradient from 0.1 M sodium phosphate buffer, pH 7.0, to 0.1 M Na_3PO_4 (pH about 10.6). Fractions possessing an RZ of 0.80 and greater were pooled.

Trypsinization of 5.34 mg of MPO prepared above (possessing an RZ greater than 0.80) was performed by adding 0.25 mg of trypsin 1-300 (Nutritional Biochemical Co.) in 10 ml of 10 mM potassium phosphate buffer, pH 7.8, and incubating at 37° for 4 hours.

Two-directional disc electrophoresis was performed according to Felberg and Schultz, 1968, in 1.7 x 13 cm columns.

Amino acid analyses were performed, according to Spackman, et al., 1958, employing a Phoenix Amino Acid Analyzer with the accelerated column, on evacuated samples hydrolyzed in sealed vials at 105° with 6 N HCl for 24, 48, and 72 hours.

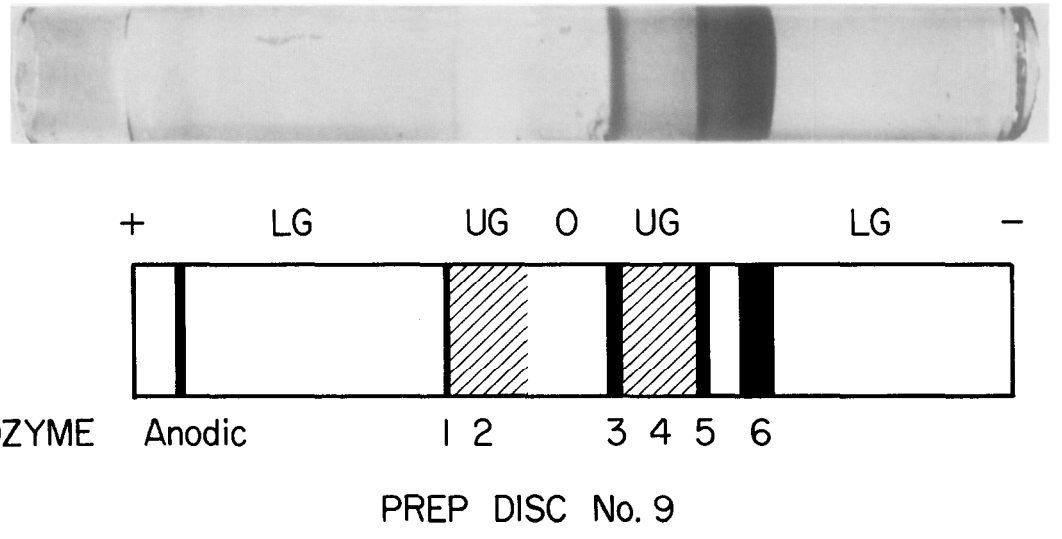

Figure 1. Preparative Disc #9: Two-directional disc electrophoresis of 9.12 mg of MPO prepared without trypsin (See METHODS AND MATERIALS) at 25 ma and 165 to 225 V for 5.2 hours at 5° in a 1.7 x 13 cm column. The sample was applied to the origin in 0.17 M K_2HPO_4 containing 38% sucrose at 6.67 mg/ml. A small green precipitate was observed at the origin at the completion of electrophoresis.

Absorption spectra were obtained on a Process & Instruments automatic recording spectrophotometer using Beckman DU optics. Reduction was accomplished by adding a few small crystals of sodium hydrosulfite to the sample.

RESULTS AND DISCUSSION

Figure 1 shows the zymogram obtained from two-directional disc electrophoresis of MPO prepared without the trypsin solubilization step and possessing an RZ greater than 0.80. Figure 2 is a zymogram of the same material after a four-hour digestion with trypsin. No change in peroxidase activity was observed during the digestion. The zymogram of the untrypsinized MPO was quite similar to that of MPO prepared by our earlier procedure (Schultz and Shmukler, 1964). Furthermore, trypsinization did not change the zymogram

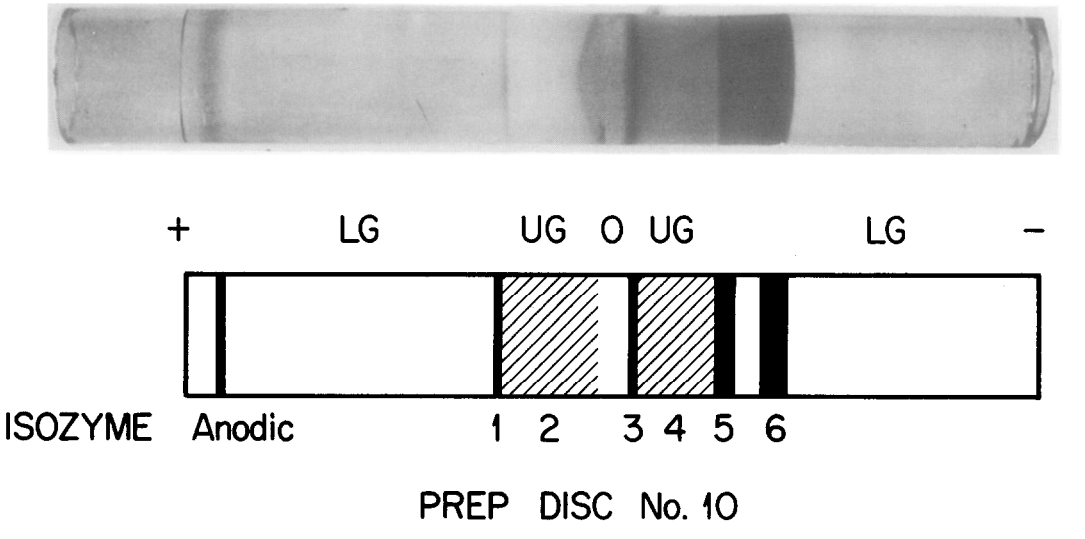

Figure 2. Preparative Disc #10: Two-directional disc electrophoresis of 5.34 mg of MPO prepared without trypsin after a 4 hour digestion with 0.25 mg of trypsin 1-300. Electrophoresis proceeded at 25 ma and 160 to 235 V for 5.6 hours at 5° in a 1.7 x 13 cm column. The sample was applied to the origin in 0.17 M K_2HPO_4 containing 38% sucrose at 6.67 mg/ml. A small green precipitate was observed at the origin at the completion of electrophoresis.

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of MPO prepared without trypsin (Figure 2).

Oxidized and reduced spectra (Figure 3) and amino acid analysis (Table 1) of MPO prepared without trypsin show that it is the same as the enzyme prepared with trypsin.

These results indicate that the heterogeneity observed in normal human leucocyte MPO is not due to partial digestion during the trypsin solubilization procedure of Schultz and Shmukler, 1964. It would appear that trypsin does not digest MPO, as evidenced by the similarities in the zymograms of MPO before and after trypsin treatment (Figures 1 and 2), and the retention of activity during the digestion. Studies to be reported elsewhere

(Felberg and Schultz, 1969) suggest that MPO exists as six dimeric molecules originating from three distinct monomeric subunits, thus providing an explanation for the observed heterogeneity.

TABLE I

AMINO ACID COMPOSITION OF MPO PREPARED WITHOUT TRYPSINIZATION[a]

Residue	24	48 (hours)	72	MPO Prepared with Trypsin[b]
Lys	10	11	11	11
His	3	4	3	4
Arg	39	39	42	35
Asp	50	54	55	51
Thr	22	22	20	23
Ser	22	21	18	21
Glu	42	40	42	37
Pro	20	22	18	31
Gly	26	26	25	26
Ala	26	30	27	26
Val	18	17	20	17
Met	11	11	11	12
Ileu	17	15	18	16
Leu	42	43	45	43
Tyr	10	9	6	10[c]
Phe	21	15	16	17
Total Residues[d]	379	379	377	380

[a]Samples hydrolyzed for the periods shown in 6 N HCl in sealed vials at 105°. Cysteine and Tryptophan were not specifically determined and those values were omitted.
[b]Schultz and Shmukler, 1964.
[c]Tyr determined spectrophotometrically by Schultz and Shmukler, 1964.
[d]Total residues normalized to the published values of Schultz and Shmukler, 1964.

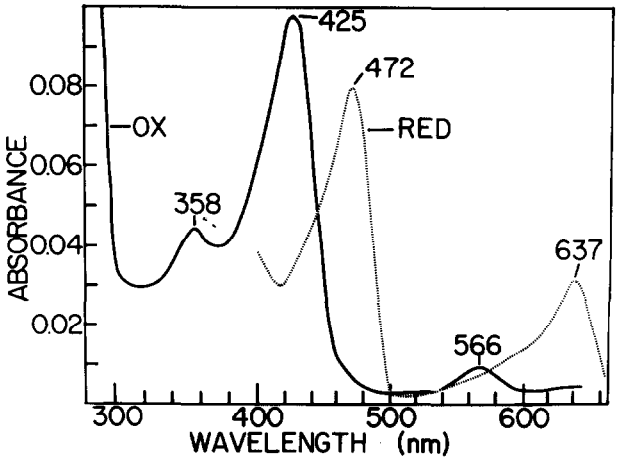

Figure 3. Oxidized (OX) and reduced (RED) spectra of MPO prepared without trypsin in 0.1 M potassium phosphate buffer, pH 7.0 (192 μg/ml). Treatment of this preparation with trypsin as described in METHODS AND MATERIALS, did not alter the oxidized spectrum.

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REFERENCES:

Agner, K., (1958) Acta Chem. Scand. 12:89
Felberg, N.T., (1969), Ph.D. Thesis, Hahnemann Medical College & Hospital Philadelphia, Pennsylvania
Felberg, N., and Schultz, J., (1968) Anal. Biochem. 23:241
Felberg, N.T., and Schultz, J., (1969) submitted to Biochemistry
Himmelhoch, S.R., Evans, W.H., Mage, M.G. and Peterson, E.A., (1969) Biochemistry 8:914
Schultz, J., Felberg, N., and John S., (1967) Biochem. Biophys. Res. Commun. 28:543
Schultz, J. and Shmukler, H.W., (1964), Biochem. 3:1234
Spackman, D.H., Stein, W.H., and Moore, S., (1958) Biochem. 30:1190
Zgliczynski, J.M., Stelmaszynska, T., Ostrowski, W., Naskalski, J., and Sznajd, J., (1968) European J. Biochem. 4:540