Monocyte Nonspecific Esterase: Purification and Subunit Structure
By Joseph Youn

Monocyte nonspecific esterase has been purified from cultured cells of the acute myeloid leukemia cell line, ML-1. The purified enzyme shows the characteristic properties of the monocyte neutral serine carboxyI esterase, with high sensitivity to organophosphorus inhibitors and sodium fluoride inhibitor. The enzyme is a membrane protein which in the native state exists as a monomer of a mol wt of ~68,000 and a trimer of mol wt 205,000. These forms exhibit a complex pattern of dissociation and reassociation based on apparent noncovalent binding of subunits. The delipidated dissociated enzyme runs as a single protein chain of a mol wt of ~62,000 on sodium dodecyl sulfate (SDS) gel electrophoresis. The relation of the subunits to monocyte isoenzymes seen on isoelectric focusing (IEF) and polyacrylamide gel electrophoresis at pH 9.5 (pH 9.5 PAGE) of cell extracts is demonstrated. Availability of purified enzyme allows development of monoclonal antibodies and analysis of myeloid differentiation. In addition, the substrate specificity and function of the purified monocyte esterase are being examined.

MONOCYTE nonspecific esterase continues to be a key cytochemical marker in the classification of acute leukemias as recommended by the French-American-British (FAB) study group on acute leukemias. In addition, this enzyme marker is widely used in studies of myeloid and monocyte/macrophage differentiation. The high sensitivity of cytochemical monocyte esterase to organophosphorus inhibitors reveals that the enzyme belongs to the special group of carboxyl esterases that use a nucleophilic serine residue at the active site for hydrolysis. Electron microscopy suggests that the monocyte esterase is a membrane ectoenzyme, but the physiological role of this unique enzyme remains unknown. Although a wealth of studies has accumulated over the years on the cytochemistry of monocyte esterase, almost nothing has been reported on the purification and properties of the enzyme at the level of classical enzymology. Availability of purified enzyme is key to the production of monoclonal antibodies to monocyte esterase for improved subtyping of acute myeloid leukemias and for studies of monocyte differentiation. Furthermore, a description of the basic molecular and enzymologic properties of the enzyme is necessary to elucidate its molecular biology and its role in monocyte/macrophage physiology. This report describes the purification, subunit structure, and enzymology of the monocyte esterase from cultured cells of the acute myeloid leukemia cell line, ML-1.

MATERIALS AND METHODS

Source and Characteristics of Cultured ML-1 Cells

Acute myeloid leukemia (AML) cell line ML-15 was isolated from the peripheral blood of a 24-year-old man with AML by M.S. Lok and generously furnished to us by Dr. J. Minowada of Roswell Park Memorial Institute. Morphologically, ML-1 cells consist of a relatively monomorphic population of blasts, 15 to 25 μm in diameter, which on Wright’s stain show a moderate nuclear/cyttoplasmic ratio and agranular to finely granular basophilic cytoplasm. Cytologically, >90% of ML-1 cells are strongly positive for fluoride-sensitive nonspecific esterase, whereas 30% to 80% are positive for myeloperoxidase, Sudan Black B reactivity, and naphthol-ASD-chloroacetate esterase (J. Younno et al, manuscript in preparation). The ML-1 cell line therefore tests as myelomonocytic (AML-M4). Because ML-1 produces very high levels of monocyte esterase, it is an ideal source of enzyme for purification and molecular analysis.

Growth and Harvest of Cultured ML-1 Cells

Cultures of ML-1 were grown to plateau phase (20 to 25 x 10⁶ cells/mL) in sealed 2L roller bottles containing 500 mL of RPMI 1640 growth medium with 10% fetal calf serum (FCS) and 50 μg of gentamycin sulfate per milliliter. Pooled cultures of ~1.4 x 10¹⁰ cells in 1.6 L of growth medium were harvested, washed, and stored at −60 °C as previously described.

Preparation of Crude Extracts

Cell suspensions totaling 600 to 800 mL, containing ~1.6 x 10¹ⁱ cells in 20 mmol/L of 2[N-morpholino]ethane sulfonic acid (MES) buffer, pH 6.4, 20% glycerol, were thawed on ice. Triton X-100 (Sigma, St Louis) was added to a final concentration of 1%; the suspension was kept on ice for 30 to 60 minutes and stirred occasionally. The detergent-treated suspension was centrifuged at 16,000 g for 15 minutes, and the milky supernatant was taken as the crude extract. The pellet was resuspended in 50 mL of buffer, recentrifuged as above, and the wash was combined with the crude extract. The crude extract yielded about 1 to 1.5 x 10⁶ mU of ANB esterase and 3 to 4.5 x 10⁵ mU of ANA esterase.

Buffers

Buffer A consisted of 10 mmol/L of MES, pH 6.4, containing 20% glycerol. Buffer B consisted of buffer A with 33 mmol/L of NaCl; buffer C consisted of 1 mmol/L of KH₂PO₄, pH 6.8; buffer D consisted of 500 mmol/L of KH₂PO₄, pH 6.8; and AS consisted of saturated (NH₄)₂SO₄ containing 10 mmol/L of MES, pH 6.4.

Chromatography Materials

Diethylaminoethanol (DEAE)-cellulose (DH-52) was purchased from Whatman, Clifton, NJ; Sephadex G-200 from Pharmacia, Piscataway, NJ; and hydroxyapatite-agarose Ultrogel from LKB, Rockville, Md. Mol wt standards for gel-filtration chromatography (Sigma) included blue dextran, catalase, bovine serum albumin, and cytochrome C.

PAGE and IEF of Nonspecific Esterase

PAGE at pH 9.5 (pH 9.5 PAGE) and IEF were performed as previously described. Unless otherwise specified, all gels were run with a standard pH gradient from 3.5 to 9.0. ANA substrate was used to develop gels, since this stains monocyte esterases as well as...
ANB substrate does and reveals other nonspecific esterases that are not stained by ANB. SDS-PAGE was performed according to Laemmli. SDS was purchased from Bio-Rad, Richmond, Calif. Water-dialyzed specimens containing ~5 μg of protein were run. The specimens were made to 10% in methanol and extracted three times with an equal volume of heptane. The extracted aqueous layer was dried under an air stream, and the residue was dissolved in dissociating buffer. Mol wt standards for SDS-PAGE were purchased from Pharmacia.

**Enzyme Assays and Enzymological Studies**

Non-specific esterase activity was assayed by a kinetic ultraviolet (UV) method. Substrates included α-naphthyl acetate (ANA) and α-naphthyl butyrate (ANB). Inhibitor studies with sodium fluoride (NaF) and diisopropylfluorophosphate (DFP) were performed with substrate mixture made to the desired concentration of inhibitor from stock solutions of these (400 mmol/L of NaF in buffer A; 1 mol/L of DFP in isopropanol). Protein was determined according to Bradford as previously described.

**Enzyme Purification**

**Ion-exchange chromatography.** The detergent-treated crude extract in buffer A was applied to a DH-52 column equilibrated in the same buffer. The flow-through fraction was collected, and the bound fraction was eluted with a NaCl gradient in buffer A. Fractions collected were assayed for ANB esterase activity.

**Preparative gel-filtration chromatography.** The major esterase peak from the ion-exchange chromatography was pooled and dialyzed v AS solution. The resulting precipitate was collected by ultrafiltration through a glass fiber filter (Whatman GF/C). The collected protein was dissolved in 10 mL of buffer B and centrifuged at 16,000 g for ten minutes. The clarified supernatant and two washes each of 2 mL of buffer B were combined. Blue dextran marker was added to the enzyme solution to a final concentration of 1 mg/mL. The solution was subjected to gel-filtration chromatography through a 4.2 x 90 cm Sephadex G-200 column in buffer B, and 10-mL fractions were collected. Fractions volumes were measured to establish elution volume (V_e). The Blue dextran peak was determined spectrophotometrically at 625 μm to establish the void volume (V_v). Fractions were then analyzed for ANB esterase activity. Eluted enzyme was combined into three pools according to V_v, and the pools were dialyzed v AS solution. The G-200 column was calibrated by rechromatography with a mixture of mol wt standards: Blue dextran, mol wt 2 x 10^6, 1 mg/mL; catalase, effective mol wt 195,000, 1 mg/mL; bovine serum albumin, mol wt 68,000, 10 mg/mL; and cytochrome c, mol wt 12,500, 5 mg/mL. Blue dextran was measured as described above: catalase, albumin, and cytochrome C were measured by standard spectrophotometric methods.

**Hydroxypatite adsorption chromatography.** The major non-specific esterase peak from the G-200 gel-filtration chromatography, GII, average mol wt 205,000, was subjected to adsorption chromatography. The GII peak in buffer C was applied to a 5.0 x 4.0 cm hydroxypatite column equilibrated in the same buffer. The flow-through fraction was collected, and bound material was eluted with a linear KH_2PO_4 gradient. Fractions collected were assayed for ANB esterase activity. Enzyme peak fractions were pooled and dialyzed v AS solution. Rechromatography of hydroxypatite peaks was performed similarly. Column dimensions and gradient volumes were scaled down to maintain proportionality. Fractions of 1.25 mL were collected, assayed for ANB esterase and protein, pooled, and dialyzed v AS solution.

**Analytical Gel-Filtration Chromatography**

Conditions were as described above for preparative Sephadex G-200 chromatography except that 1.0-mL specimens containing 400 to 2 x 10^5 mU ANB esterase were run. Column dimensions were 1.6 x 90 cm. Fractions of 1.0 mL were collected and assayed for ANB esterase.

**Analytical Hydroxyapatite Adsorption Chromatography**

Conditions were as described above for preparative chromatography. Crude extract specimens of 1.0 mL containing 1,600 to 2,000 mU of ANB esterase were run on 1.5 x 6.0 cm columns, and fractions of 1.0 mL were collected.

**RESULTS**

Crude extract nonspecific esterase of cultured ML-1 cells (AML-M4) averaged about 1,800 mU/mL with ANB substrate and 500 mU/mL with ANA substrate. This activity equals that of the most active normal donor monocyte and acute monocytic leukemia (AML-M5) preparations we have seen.

**Analytical Chromatography**

When the crude extract was subjected to analytical gel-filtration chromatography on Sephadex G-200, an elution pattern characteristic of monocyte esterase was found. This pattern consisted of a major enzyme peak, GII, of a mol wt of ~205,000, and a subpeak, GIII, of a mol wt of ~68,000. A minor peak, GI, at the void volume, consisted of presumed aggregates of high mol wt (Fig 1). Recovery of enzyme was ~90% of applied activity. On pH 9.5 PAGE, the crude extract showed a zymogram similar to that of normal monocytes, consisting of: (a) an intense slower form, AB-2; and (b) a faster, less prominent form, AB-1. On pH 9.5 PAGE, the GII peak showed both forms at approximately equal intensity; the GIII peak enzyme showed predominantly the faster form, AB-1 (Fig 1, inset). These results suggest that the native enzyme, extracted from the cell membrane with nonionic detergent, consists of a moiety of a mol wt of ~68,000, AB-1, and a moiety of a mol wt of ~205,000, AB-2. Apparent enrichment of AB-1 in the GII peak suggests that this form is generated from AB-2.

On IEF, the crude extract showed the characteristic series of closely spaced monocyte esterase bands in the pl range 5.8 to 6.3: the more anionic forms were more prominent (Fig 2). On the same system, the GII peak showed a similar series of bands but with increased intensity of the less anionic forms; the GIII peak showed the less anionic forms predominantly. These results suggest that in unfractionated cell extracts AB-1 consists of two or more forms with a pl of 6.2 to 6.3, whereas AB-2 consists of multiple forms with slightly lower pl values.

The crude extract of ML-1 was also examined by analytical adsorption chromatography on hydroxypatite. Two peaks of esterase activity were obtained: a minor peak, HAI, eluting at about 100 mmol/L of phosphate; a major peak, HAII, eluting at about 200 mmol/L of phosphate (Fig 3). These peaks were also examined on pH 9.5 PAGE (Fig 3,
Fig 1. Analytical gel-filtration chromatography of ML-1 crude extract esterase. A 1-mL aliquot of crude extract containing 1,800 mU of ANB esterase in buffer B with 1 mg/mL Blue dextran was chromatographed on a 1.8 × 90 cm Sephadex G-200 column in buffer B; 1-mL fractions were collected, measured volumetrically, and assayed for ANB esterase. The column was next calibrated with a mixture of mol wt markers: Blue dextran (BD) (mol wt 2 × 10^6); catalase (CAT) (mol wt 195,000); bovine serum albumin (ALB) (mol wt 68,000); and cytochrome C (CYT) (mol wt 12,500). Arrows indicate midpoint of marker peaks. Elution profiles are expressed as ratio of peak elution volume, V_e, to BD elution volume, V_e, or void volume. The GI peak enzyme elutes near the void volume (V_e/V_e = 1.00), GII peak enzyme at mol wt ~205,000 (V_e/V_e = 1.30), and GIII peak enzyme at mol wt ~68,000 (V_e/V_e = 1.72). Inset: pH 9.5 polyacrylamide gel electrophoresis zymograms of monocyte esterase fractions from gel-filtration chromatography. ANA-Fast Blue BB stain. From left to right: unfractionated crude extract; peak GI; peak GII-leading edge, peak, trailing edge; peak GIII-leading edge, peak, trailing edge. Bold arrow indicates slower AB-2 form; fine arrow indicates faster AB-1 form. Enzyme from Sephadex peaks was applied to gel at a standardized volume of 60 μL for direct comparison. The amount of AB-1 appears increased in G200 fractions as compared with crude extract, and AB-1 is the major form in peak GIII. Also seen faintly in the crude extract are the fast migrating acetyl esterases A1 and A2, closely appositioned. These isoenzymes were found in all blood cells examined, i.e., genuinely nonspecific. Because the mol wts of A1 and A2 are similar to the mol wt of AB-1, these forms elute with the GII peak in gel-filtration chromatography of crude extract. Normal monocyte esterase gives a similar pattern of peaks on gel filtration chromatography (data not shown).

Preparative Chromatography

When the crude extract of ML-1 was subjected to ion-exchange chromatography on DEAE-cellulose, a major peak, DHI, was eluted at 50 mmol/L of NaCl (data not shown). This elution profile is similar to that of normal donor monocyte esterase. This peak contained 90% of recovered esterase and showed a typical monocyte zymogram on pH 9.5 PAGE and IEF (data not shown).

The concentrated enzyme from the DHI peak was next subjected to preparative gel filtration on Sephadex G-200, yielding an elution pattern resembling that given by crude extract of ML-1 and normal monocytes (data not shown).

Likewise, on pH 9.5 PAGE and IEF, the GI, GII, and GIII peak enzymes resembled those of the corresponding peaks obtained directly from the crude extract (data not shown).

The Sephadex G-200 major peak GII enzyme was next subjected to adsorption chromatography on hydroxyapatite-agarose. The enzyme was eluted in the characteristic two peaks: HAI and HAI1 (Fig 4). On SDS-PAGE, the delipidated, dissociated enzyme from each peak was found to be associated with a prominent single protein chain of a mol wt of ~62,000 (Fig 4, inset). The intensity of the protein band was proportional to the size of each HA peak. The HAI peak was nearly homogeneous with respect to this protein. These results clearly suggest that the fundamental subunit of monocyte esterase is a single protein chain of a mol wt of ~68,000 which, when delipidated, has a mol wt of ~62,000. The proportion of carbohydrate in this subunit remains to be determined.

The pooled enzyme peaks were rechromatographed on hydroxyapatite-agarose. The HAI peak consistently generated an HAI peak of about one-half its size in several runs.
Fig 2. (A) Isoelectric focusing (IEF) zymograms of monocyte esterase fractions from gel-filtration chromatography on Sephadex G-200 (see Fig 1). ANA-Fast Blue BB. From left to right: unfractionated crude extract; peak GI; peak GII-leading edge, peak, trailing edge; peak GI-peak, trailing edge. A characteristic closely spaced series of monocyte esterases is seen in PI region 5.8 to 6.3. Bold arrow indicates more anionic forms; fine arrow indicates less anionic forms. The amount of less anionic forms appears increased in fractionated material; these predominate in the GIII fractions. In this gel, only an expanded pH range of 5.0 to 8.0 was covered. The number of monocyte species is greater than that detected with standard gels covering pH range 3.5 to 8.0 (shown in right panel). (B) IEF zymograms of monocyte esterase fractions from hydroxyapatite (HA) adsorption chromatography (see Fig 3) pH 3.5 to 8.0. Conditions otherwise as described in left panel. From left to right: unfractionated crude extract; peak HAI-leading edge, peak, trailing edge; peak HAIL-leading edge, peak, trailing edge. Again there is an increase in activity of forms of monocyte esterase with highest pl values as compared with crude extract; these predominate in peak HAI.

Fig 3. Analytical adsorption chromatography of ML-1 crude extract esterase. A 1-ml aliquot of crude extract containing 1.600 mU of ANB esterase in buffer C was chromatographed on a 1.5 x 6.0 cm hydroxyapatite (HA) column, and enzyme was eluted with a linear gradient to 500 mmol/L of KPO4. Fractions of 1 ml were collected and assayed for ANB esterase. Inset: pH 9.5 polyacrylamide gel electrophoresis zymograms of monocyte esterase fractions from adsorption chromatography on HA. ANA-Fast Blue BB. From left to right: crude extract; HA peak I-leading edge, peak, trailing edge; HA peak II-leading edge, peak, trailing edge. Specimens were applied at standardized volume of 60 µL. Bold arrow indicates slower AB-2 form, fine arrow indicates the faster AB-1 form. Again the level of AB-1 activity appears increased in fractionated material as compared with crude extract, and AB-1 is the major form in peak HAI. The nonspecific acetyl esterases A1 and A2 are seen to elute just ahead of the HAi peak.
Fig 4. Hydroxyapatite-agarose adsorption chromatography of major esterase peak, GII, from gel-filtration chromatography. Experimental details are given in Materials and Methods section. Two peaks were eluted from the column: a minor peak, HAI, at \(-100\) mmol/L of KH\(_2\)PO\(_4\), and a major peak, HAIL, at \(-200\) mmol/L of KH\(_2\)PO\(_4\). Inset: sodium dodecyl sulfate polyacrylamide gel electrophoresis of successive chromatography fractions. A standard volume of 300 \(\mu\)L from each fraction was analyzed for direct comparison of intensity of proteins. A single major protein, mol wt 62,000, is associated with both esterase peaks (arrows). The larger peak, HAIL, is almost homogeneous with respect to this protein.

(Fig 5). To rule out contamination of the HAI fraction by the larger HAIL peak, cuts of the HAI peak not including the trailing shoulder were examined and similar results were obtained. Conversely, the HAIL peak consistently generated a minor HAI peak, even after the second rechromatography (Fig 6). Protein profiles closely matched enzyme peaks on rechromatography of the HAIL fraction. The HAIL peak showed the 62,000-mol wt subunit on SDS-PAGE in highly purified form (Fig 7). On pH 9.5 PAGE, the HAIL peak enzyme showed a major protein that closely matched the Rf.
Rechromatography of hydroxyapatite (HA) peak HAI on HA. Experimental details are given in Materials and Methods section. A minor HAI peak is consistently generated from HAIL. The Coomassie Blue protein peak at 495 μm matches the enzyme peaks closely.

of the AB-2 enzyme and a weaker series of two to three bands that matched the minor, AB-1 enzyme (Fig 8). It remains to be determined if the observed microheterogeneity in later stages of the purification represents hydrolytic processing of enzyme, enhancement of related minor forms, or greater resolving power of the system with purified material. The derivative minor HAI peak showed a similar but weaker pattern of bands. The rechromatographed HAI peak also generated similar esterase patterns on pH 9.5 PAGE; however, in this chromatography, protein stain showed residual contamination (HAI) or low levels of material (HAIL) (Fig 8).

On IEF, the HAI and HAIL peaks from the first chromatography showed the entire series of esterase bands with HAI enriched for the less anionic forms and HAIL enriched for the more anionic forms. This pattern approximated that of peaks obtained directly from chromatography of crude extract. When the HAI and HAIL peaks were individually rechromatographed on hydroxyapatite, no apparent further segregation of forms with higher and lower PI values was apparent in the derivative HAI and HAIL peaks obtained (Fig 9). The repurified HAIL peak on IEF showed a close match of protein and enzyme bands (Fig 9).

Approximately 1,000 μg of protein representing 10% of original ANB esterase activity was recovered in the final HAIL peak, representing a 350-fold purification based on activity protein (Table 1). The calculated final yield was 140,000 mU of ANA esterase and 2,600 μg of protein based on rechromatography of the entire HAIL fraction. Clearly, greater yields of highly purified HAIL enzyme are obtainable after the first and second adsorption chromatography. The purification data suggest that monocyte nonspecific esterase comprises ~0.25% of starting protein in crude extract (26 mg of enzyme protein calculated in 10,300 mg of total protein).

The HAIL peak enzyme was analyzed by gel-filtration chromatography on Sephadex G-200. Again, the characteristic monocyte elution pattern was seen, consisting of a major GII peak of a mol wt of ~205,000; a minor GIII peak of a mol wt of ~68,000; and a minor GI peak of high-mol-wt aggregates (Fig 10). On pH 9.5 PAGE, the repurified GII
Fig 8. pH 9.5 polyacrylamide gel electrophoresis zymograms of fractions from hydroxyapatite (HA) rechromatography of peaks HAI and HAIL. Top panel: ANA-Fast Blue BB. Bottom panel: Coomassie Blue protein stain. From left to right: HAI rechromatography peak HAI, peak HAIL; HAIL rechromatography peak HAI leading edge, peak, trailing edge; peak HAIL leading edge, peak, trailing edge, crude extract control. All column fractions were applied at standardized volume of 10 μL for enzyme and 60 μL for protein. Bold arrow indicates AB-2 form; fine arrow indicates AB-1. These specimens were dialyzed and concentrated v 0.1 mmol/L of 2[N-morpholino]ethane sulfonic acid (MES) buffer, pH 6.4, containing 20% glycerol. Predialysis specimens of the same fractions in KPO4 buffer showed predominance of the AB-1 forms in the HAI peaks. Here, the AB-2 form predominates in the same fractions. These results also indicate a marked tendency of AB-1 to generate AB-2 in these fractions, as seen on HA chromatography.

peak demonstrated the AB-2 form as a doublet, and the GIII peak demonstrated a mixture of AB-1 and AB-2 (Fig 10, inset). Possible reasons for the observed microheterogeneity are discussed above. On IEF, partial separation of more anionic (AB-2) forms in GII from the less anionic (AB-1) forms in GIII was again seen. The HAI peak showed a similar profile on G-200 rechromatography, except that the GIII peak was about twice the size of the GII peak (Fig 11). On pH 9.5 PAGE, this GII peak showed a mixture of AB-1 and AB-2, while GIII showed predominantly AB-1 (Fig 11, inset). IEF again demonstrated partial separation of less anionic (AB-1) forms in GIII from the more anionic (AB-2) forms in GII. These results suggest that hydroxyapatite adsorption chromatography separates monomers (HAI) and trimers (HAIL) but that an association–dissociation equilibrium characteristic of each fraction is reestablished. This equilibrium is shifted toward the trimer in the purified HAIL fraction and toward the monomer in the purified HAI fraction.

The GIII peak from preparative gel-filtration chromatography was of low specific activity due to heavy contamination by other proteins. On pH 9.5 PAGE and IEF, this peak showed the expected predominance of less anionic AB-1 forms. It also contained significant levels of the more anionic AB-2 forms.

Fig 9. Isoelectric focusing zymograms of fractions from hydroxyapatite (HA) rechromatography of peaks HAI and HAIL. Top panel: ANA-Fast Garnet GBC. Bottom panel: Coomassie Blue protein. Specimen makeup identical to that of Fig 11 except that crude extract control is on extreme left in top panel and is not included in bottom panel. All column fractions were applied at a standardized volume of 5 μL for enzyme and 25 μL for protein. Bold arrow indicates more anionic forms; fine arrow indicates less anionic forms. Because the enzyme bands in the third lane from the left were faint on the wet gel, the lane is replaced in the photograph by the same bands from the dried gel. Precise alignment of panels can be seen by horizontal ampholine bands in high pI range, which bind coupler dye nonspecifically and weakly.

Table 1. Purification of Monocyte Nonspecific Esterase

<table>
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<tr>
<th>Fraction</th>
<th>ANB Esterase Activity (mU/mL)</th>
<th>Protein (mg/mL)</th>
<th>Specific Activity (mU/mg)</th>
<th>Total Activity</th>
<th>Recovery (%)</th>
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<tr>
<td>Crude extract</td>
<td>830</td>
<td>1.690</td>
<td>12.4</td>
<td>136</td>
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<td>DH52 peak I</td>
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<td>1.4</td>
<td>1,960</td>
<td>1,100,000</td>
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<tr>
<td>G200 peak II</td>
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<td>4.100</td>
<td>0.53</td>
<td>7,740</td>
<td>530,000</td>
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<tr>
<td>HA-1 peak II</td>
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<td>2.500</td>
<td>0.075</td>
<td>33,000</td>
<td>400,000</td>
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<tr>
<td>HA-2 peak II</td>
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<td>ND</td>
<td>220,000</td>
<td>16</td>
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<td>HA-3 peak II</td>
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<td>5,000</td>
<td>0.104</td>
<td>48,000</td>
<td>50,000</td>
</tr>
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ANA, a-naphthyl acetate; ANB, a-naphthyl butyrate.

*An aliquot of HA-2 peak II containing 83,000 mU of ANB esterase was rechromatographed. Final yield is corrected accordingly. ANA esterase activity was 29% of ANB esterase activity in the crude extract. The ratio of ANA to ANB esterase decreased to 18% after DH52 chromatography and remained at 18% ± 1% through the final HAIL fractions.
Ve/Vo

Fig 10. Analytical gel-filtration chromatography of hydroxyapatite (HA) peak HAII on Sephadex G-200. Conditions were similar to those described in Fig 1. Approximately $2 \times 10^4$ mU of ANB esterase were applied. Inset: pH 9.5 polyacrylamide gel electrophoresis zymograms of Sephadex G-200 peaks. ANA-Fast Blue BB. From left to right: crude extract, peak Gil, peak Gill. Several enzymological parameters of the HAII peak enzyme were determined and found to be similar in all respects to those of normal monocyte enzyme. These parameters include the broad neutral pH optimum for ANB and ANA esterase from pH 6.5 to 8.5, high sensitivity to DFP inhibitor (50% inhibition of ANB esterase at $1 \times 10^{-3}$ mol/L, of ANA esterase at $2 \times 10^{-3}$ mol/L of DFP), and high sensitivity to NaF inhibitor (50% inhibition of ANB esterase and of ANA esterase at $1 \text{mmol/L of NaF}$).

DISCUSSION

Monocyte nonspecific esterase has been purified from cultured cells of AML-M4 cell line ML-1. The detergent-extracted membrane protein exists in the native state as a monomer of a mol wt of ~68,000 and a trimer of a mol wt of ~205,000 in a complex association–dissociation equilibrium. The trimer accounts for the bulk of extractable enzyme activity whereas the monomer is a relatively minor fraction. When dissociated and delipidated, the enzyme is seen to consist of a single major protein chain with a mol wt slightly lower than that of the native monomer—62,000. On pH 9.5 PAGE, the trimer migrates as the slower AB-2 form, and the monomer migrates as the faster AB-1 form. Analysis of crude extract shows that the trimer is associated with the more anionic forms, and the monomer is associated with the less anionic forms, as seen on IEF. A complex association–dissociation equilibrium and charge pattern of monomer and trimer is revealed with purified fractions. Further information is necessary to explain completely the various thermodynamic states of the enzyme in various environments and the details of the apparent noncovalent binding reaction of the subunits. A model incorporating the general features of monocyte nonspecific esterase subunit structure based on the data obtained from different systems is given in Fig 12.

We are now preparing polyclonal and monoclonal antibodies to monocyte nonspecific esterase for diagnostic and research purposes. The availability of such monoclonal antibodies will provide a new method of classification of acute

![Monomer and Trimer Structure](image)

**Fig 12. Model of monocyte nonspecific esterase subunit structure and noncovalent association–dissociation reaction-based chromatogram and zymogram data.**
leukemias and for subclassification of acute myeloid leukemias based on nonspecific esterase. In addition, antibodies to the monocyte esterase will be of use in studies of myeloid/monocyte differentiation. We have recently presented evidence for activation of a myeloid-associated zymogen of monocyte esterase during monocyte/macrophage differentiation of HL-60 induced by phorbol esters. An antibody probe for a zymogen epitope would furnish a new pan-myeloid marker and allow analysis of the mechanism of zymogen activation. An antibody probe for an epitope present on active enzyme only will allow more precise and facile subclassification of acute myeloid leukemias into monocytic and nonmonocytic types.

Leukocytes contain a large array of nonspecific esterases that are detected with ANA, ANB, or similar substrates. Although these esterases share the common property of activity with naphthol esters, their diverse natural substrates and physiological roles remain unknown. Monocyte esterase is a membrane enzyme with the properties of a neutral serum carboxyl esterase. Neutral serum proteases, such as those represented by several digestive enzymes and blood-clotting enzymes, are the best known examples of this group of esterases. Recently, a monocyte serine protease ectoenzyme with activity with serum amyloid A precursor was demonstrated. A lymphocyte serine protease ectoenzyme of possible significance in blast transformation has previously been described. Other types of carboxyl esterase may also operate by a similar reaction mechanism, among them lipoprotein lipase, phospholipases A1 and A2, and hormone-sensitive lipase. The availability of purified monocyte esterase will allow us to screen protein, lipid, and other substrates definitively in an attempt to define the natural substrate and the physiological role of this ectoenzyme. The monocyte esterase must subserve some unique and important function in inflammation, metabolic processing, or growth regulation. We are now in a position to study these interesting questions with the purified enzyme.

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Monocyte nonspecific esterase: purification and subunit structure

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