Human Monocyte Carboxylesterase

PURIFICATION AND KINETICS

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Human peripheral blood monocytes were isolated by density gradient centrifugation and purified by counterflow centrifugation elutriation. Membrane-localized carboxylesterase (CBE) was extracted with non-ionic detergent (Triton X-100) and purified by ion exchange (DEAE-cellulose), gel filtration (Sephacryl S-300), hydroxylapatite column, and high performance liquid chromatography. The purified enzyme migrated on 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis as a single protein band with a molecular weight of 60,000. Under non-denaturing conditions, monocyte CBE formed a trimer and eluted from a gel filtration column as a protein with an approximate molecular weight of 200,000. Electrophoretic pattern of the enzyme on polyacrylamide gels run at a neutral pH did not vary during enzyme purification. At least four major isoenzymes of human monocyte CBE were observed with isoelectric points between 7.5 and 7.8.

Pure human monocyte CBE hydrolyzed short chain α-naphthyl, o-nitrophenyl, and p-nitrophenyl esters. Amide esters and thioesters were not hydrolyzed by the enzyme. Short chain alcohols activated the enzyme and organophosphorus compounds, diphenyl carbonate, sodium fluoride, and phenylmethylsulfonyl fluoride inhibited the enzyme. EDTA and sulfhydryl agents had no effect on enzyme activity. The amino acid content of the enzyme was consistent with other CBEs. Inhibitors reacted either with the active or effect site of the enzyme. Purified enzyme now permits the characterization of CBE structure and regulation.

**Carboxylesterases (EC 3.1.1.1) are found in many cells and tissues (1) and have been extensively studied in liver preparations (2-15). Despite detailed biochemical studies, the actual physiological functions of these enzymes remain incompletely understood. The carboxylesterase present in the peripheral blood monocyte represents a unique and readily available source from which studies of this enzyme can be undertaken to elucidate its role both in physiological processes and xenobiotic metabolism.**

Monocyte carboxylesterase (CBE) represents a membrane-bound ectoenzyme of a peripheral blood cell whose primary biological functions are performed, to a large degree, by membrane-associated proteins (16, 17). Monocytes are migratory cells that differentiate into tissue histiocytes including Kupffer cells of the liver. The carboxylesterase, as a monocyte ectoenzyme, is ideally situated to interact with the extracellular environment as shown by the inhibition of its activity by organophosphorus compounds and by the use of this property as a biomarker of organophosphorus exposure (18). Further, the absence of human monocyte carboxylesterase, an enzyme scavenger used for the detoxification of toxic organophosphorus esters and perhaps other toxic organic esters, has led to speculation that such human hosts are at increased risk for cancer (19, 20). Variation in the expression of carboxylesterase isoenzymes also functions as a marker for monocyte maturation arrest in leukemias (21, 22). Finally, monocyte/macrophage cell lines represent easily obtainable sources of this enzyme for the investigation of carboxylesterase regulation if such enzymes can be shown to be similar to the native human enzyme.

To clarify the biochemical properties of human monocyte carboxylesterase and its role in xenobiotic metabolism, we have purified to homogeneity peripheral blood monocyte carboxylesterase. Our studies show that the purified human monocyte carboxylesterase has a preference for certain ester substrates, is inhibited by organophosphorus agents, is activated by alcohols, and has an amino acid content comparable to other enzymes in the esterase family.

**EXPERIMENTAL PROCEDURES**

**Chemicals and Radioisotopes**—Substrates and modifiers of carboxylesterase were all obtained from Sigma with the following exceptions. The aminofluorene carcinogens, 2-acetylaminofluorene and N-hydroxy-2-acetylamino-5-fluorenone, were kindly provided by the National Cancer Institute repository for carcinogens. Triphenyl phosphate was purchased from Aldrich. Pure diphenyl carbonate and tetraphenylresorcinol diphasphate were kindly provided by General Electric Plastics. The solvents used for high performance liquid chromatography were all high performance liquid chromatography grade solvents. Radiolabeled diisopropyl fluorophosphate was obtained from Du Pont-New England Nuclear, and radiolabeled diphenyl carbonate

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‡ The abbreviations used are: CBE, carboxylesterase; DFP, diisopropyl fluorophosphate; ANP, α-naphthyl butyrate; ANA, α-naphthyl acetate; TPF, triphenyl phosphate; DPC, diphenyl carbonate; SDS-PAGE, sodium dodecyl sulfate-polyacrylamide gel electrophoresis; TEMED, N,N,N′,N′-tetramethylethylenediamine.
Polyacrylamide gel electrophoresis was performed at a neutral pH by gradient centrifugation through Ficoll-Hypaque (Pharmacia LKB Biotechnology, Inc.) and counterflow centrifugation elutriation using a Beckman JE-6B elutriator as previously described (23). The monocyte preparation was more than 95% pure as judged by Diff-Quik staining (American Scientific Products, McGaw Park, IL) for morphological analysis and esterase activity using standard methods.

Extraction of Human Monocyte CBE—Human monocyte membrane CBE was extracted from cells by the nonionic detergent, Triton X-100 (Sigma). Briefly, 4 × 10⁶ cells were suspended in 100 ml of 20% glycerol (Sigma), 10 mM Tris (pH 7.5), and 1% Triton X-100. The monolayer membrane was kept on ice for 60 min with occasional shaking. The suspension was then centrifuged at 27,000 × g for 30 min. Unless otherwise specified, all protein manipulations were carried out at 4 °C. The enzyme was then purified from the detergent-extracted monocyte membrane by the following methods.

Ion-exchange Column Chromatography—The detergent-extracted enzyme (crude extract) was applied to an ion-exchange column (DE52, Whatman) and eluted with a linear gradient of NaCl (0–0.5 M) in 10 mM Tris-HCl containing 20% glycerol. Column fractions were then assayed for protein and esterase activity, and those column fractions corresponding to the esterase peak were pooled. The pooled fractions were desalted by shaking with 30% ethanol and then dialyzed against 0.5 M Tris-HCl, 0.1% sodium polyethylene glycol (Carboxax, PEG 8000, Fisher). The concentrated sample was then dialyzed against 50 mM Tris (pH 7.5) containing 10% glycerol and applied to a gel filtration column.

Preparation of Substrate Solutions—The following methods for determining rate constants are described in detail by Johnson (37). The effect of assay pH on human monocyte carboxylesterase activity was determined by adding 0.4 mg of pure enzyme to 1 ml of reaction mixture containing 50 mM buffer and 0.2 mM AN. Sodium chloride was used as the buffer for pH values between 4 and 6, whereas Tris-HCl was used for pH values between 7 and 12. The reaction mixture was incubated for 10 min at room temperature, and enzyme activity was determined as described previously.

Inhibition of human monocyte carboxylesterase was measured by the following method. After preincubating enzyme and buffer for 5 min at 37 °C, inhibitor solution or solvent alone was added, and the mixture incubated for fixed time periods at 37 °C in the absence of substrate. At the termination of the incubation, substrate was added and residual monocyte carboxylesterase activity was determined (30). Determination of the percentage of inhibition was calculated by dividing the enzyme activity in samples preincubated with inhibitor by the activity of samples preincubated with solvent only. Linear regression was used to calculate the slopes of the lines derived from the percentage enzyme activity remaining as a function of time. The reciprocal of the slopes of each of the lines was plotted as a function of the reciprocal of the inhibitor concentration used to derive each linear regression. From the resultant straight line \(1/k_{\text{inactiv}} \) the intercept of the line with the abscissa and \(1/k_{\text{inactiv}} \) was obtained. The value of \(k_{\text{inactiv}} \) (the dissociation constant for the inhibitor-receptor complex) and \(k_{\text{max}} \) (the rate of enzyme inactivation by inhibitor) were calculated from these graphs. These methods for determining rate constants are described in detail by Johnson (37).
The dissociation constants for enzyme-inhibitor complexes (K_i) and the determination of competitive and noncompetitive inhibition were obtained by the simple graphical method described by Dixon (38). Straight lines were obtained when the reciprocal values for enzyme velocity in the presence of varying inhibitor and constant substrate concentrations were plotted as a function of different inhibitor concentrations. The intercept of straight lines determined with two different substrate concentrations gave the value for -K_i. With competitive inhibitors in the plots described for two different substrate concentrations, the lines cross giving a point above the abscissa for -K_i, whereas with noncompetitive inhibitors the lines meet at a point on the abscissa which gives an intercept of -K_i.

RESULTS

Purification Human monocytes were purified by counter-flow centrifugation elutriation (23), and their membrane esterases solubilized by a nonionic detergent (Triton X-100). The solubilized membranes were then applied to a column of DEAE-cellulose (DE52) and eluted with a linear gradient of sodium chloride (Fig. 1). This column resolved the solubilized monocyte membranes into multiple protein peaks, but the majority (more than 95%) of the enzyme activity eluted as a single peak to the left of the first protein peak. Less than 5% of the enzyme did not bind to the column and eluted with the detergent (data not shown). The enzyme peak from the ion-exchange column was concentrated and then applied to a gel filtration column (Sephacryl S-300) which had been calibrated with standard molecular weight proteins (Fig. 2). The majority (95%) of the enzyme eluted with β-amylase (Mr 200,000) and only 5% of the enzyme eluted with bovine serum albumin (Mr 66,000). Thus, the majority of the enzyme was eluted in its high molecular weight form. Higher molecular weight forms of the enzyme were not eluted from this column. Electrophoretic gel patterns of the enzyme from this column separation showed that the enzyme was only 50% pure (Fig. 4A, lane 4), and additional steps were required for the purification of the enzyme. Enzyme peak I from the gel filtration column was applied to a column of hydroxylapatite and eluted with a linear gradient of potassium phosphate (Fig. 3). The enzyme eluted as a single peak from this column with a salt concentration of 0.2 M potassium phosphate.

The enzyme peak from the hydroxylapatite column was concentrated and analyzed along with samples from previous steps by SDS-PAGE (Fig. 4A) and polycrylamide gel electrophoresis at a neutral pH to determine whether the protein bands exhibited esterase activity (Fig. 4B). The results of these experiments show that the enzyme was successfully purified to homogeneity by three chromatographic steps (Fig. 4A). The pure enzyme (Fig. 4A, lane 5) had a Mr of ~60,000 as determined from SDS-PAGE. Further, the enzyme migrated as a single band of high molecular weight, possibly a trimer, in a polyacrylamide gel run at a neutral pH. The migration distance of samples from the different purification steps remained constant (Fig. 4B). In the early steps of the purification, there were larger aggregates of the enzyme than the trimeric form (Fig. 4B, lanes 1 and 2).
In order to determine whether the single protein band on SDS-PAGE (Fig. 4A, lane 5) consisted of a single or multiple isoforms, the purified enzyme was analyzed on isoelectric focusing gels. The enzyme was shown to consist of at least four major isozymes (Fig. 4C). Gel slices from the isoelectric focusing gel were eluted with double-distilled water to measure the pH, and the isoelectric points determined for these four isozymes. The pI values for these isoenzymes were between pH 7.5 and 7.8.

Approximately 800 µg of pure enzyme was recovered from 4 X 10^9 human monocytes. This represented 0.22% of the total protein of the detergent-extracted monocyte homogenate and a 146-fold purification based on the specific activity of the enzyme as measured with α-naphthyl butyrate as substrate (Table I).

Amino Acid Composition—The results of the amino acid analysis of human monocyte carboxylesterase expressed as residues (%) per 60,000 g of enzyme are shown in Table II. As can be seen in that table, the amino acid composition of human monocyte carboxylesterase is similar to published data on the amino acid composition of other human and nonhuman esterases.

Enzyme Stability—The addition of 10% glycerol to the enzyme during purification is used as a stabilizing factor. The enzyme stored in glycerol at -85 °C is stable for months.

Table I

| Sample                  | Total protein | Volume | Total activity | Specific activity | Yield | Purification |
|-------------------------|--------------|--------|----------------|------------------|-------|--------------|
| Crude extract           | 350          | 100    | 3 x 10^4       | 85.7             | 100   | 1            |
| DE52 peak               | 15           | 10     | 2 x 10^4       | 1,333            | 66    | 15           |
| Sephacryl peak 1       | 2            | 47     | 1.5 x 10^4     | 7,609            | 50    | 88           |
| Pure enzyme (hydroxylapatite pool) | 0.8   | 9.2   | 1 x 10^4       | 12,507           | 33    | 146          |

* Protein determination was done by the method of Lowry (29).

** Measured with α-naphthyl butyrate as substrate.
not hydrolyzed by the enzyme. For the para-nitrophenyl esters, activity is determined with acetate, propionate, butyrate, valerate, caproate, and caprylate esters. No activity was observed with higher carbon chain substrates in this class. Enzyme activity was slightly higher with the o-nitrophenyl and α-naphthyl esters than with p-nitrophenyl esters. The highest activity ($V_{\text{max}}$) was seen with butyrate esters. Since liver carboxylesterases also hydrolyze thioesters and amide esters, the classical substrates (acetanilide, acetyl-coenzyme A, and butyl-coenzyme A) for these enzyme reactions were evaluated as substrates for the purified monocyte esterase (32–34). Purified enzyme showed no activity with these esters as substrates. Further, the carcinogens, 2-acetylaminofluorene and N'-hydroxy-2-acetylaminofluorene, were not hydrolyzed by monocyte esterase.

**Effects of Alcohols**—The effects of several nucleophilic small molecules on the esterase catalyzed hydrolysis of α-naphthyl butyrate are summarized in Table IV. Short chain alcohols like methanol and ethanol activated the enzyme under the conditions of this assay, whereas more complex alcohols (ethylene glycol monoethyl ether and ethylene glycol) had little effect on enzyme activity except at high concentrations (1 M). n-Butanol increased monocyte carboxylesterase activity at low concentrations (0.1 M), but at high concentrations (1 M) it inhibited enzyme activity. Dimethylsulfoxide and phenol at high concentrations also inhibited the hydrolysis of α-naphthyl butyrate. The former compound only inhibited the enzyme at high concentrations (1 M), whereas phenol inhibited the enzyme at one-tenth that concentration. Neither of these latter agents increased α-naphthyl butyrate hydrolysis.

**Inhibitors**—Monocyte esterase activity was inhibited by a variety of organophosphorus compounds as well as sodium fluoride in high concentration ($10^{-2}$ M). Sulphhydril inhibitors (p-chloromercuribenzoic acid and N-ethylmaleimide) and a metal chelator (EDTA) caused no change in enzyme activity over a range of concentrations. Further, eserine (a cholinesterase inhibitor) had no effect on enzyme activity. On preincubation for 10 min at 37°C, the rank order of potency of the organophosphorus compounds as measured by dose-inhibition curves was disisopropylfluorophosphate (DFP) > diethyl phosphorothioate (paraoxon) > tetraphenylresorcinol diphosphate > triphenyl phosphate (TPP) > phenylmethylsulfonyl fluoride > diphenyl carbonate > sodium fluoride (data not shown).

In experiments where electrophoresis was used to examine the type of binding between radioactively labeled inhibitor and pure enzyme (Fig. 6A), radioactivity migrating with the purified esterase when 3H-labeled DFP was incubated with the enzyme and the resultant enzyme-inhibitor mixture elec-

![FIG. 5. Enzymatic activity of human monocyte carboxylesterase with different substrate.](https://example.com/figure5.png)
with esterase, respectively. In B, human monocyte carboxylesterase was labeled with either \(^{3}H\)DFP, \(^{14}C\)TPP, or \(^{14}C\)DPC. Human monocyte carboxylesterase (4 \(\mu\)g) was labeled with \(1 \times 10^{6}\) cpm of either \(^{3}H\)DFP, \(^{14}C\)TPP, or \(^{14}C\)DPC, and incubated at room temperature for 30 min. The samples were then electrophoresed through SDS-PAGE slabs (12% acrylamide). The gel was fixed, dried, and examined by autoradiography. The exposure time was 5 days. Lanes 1, 2, and 3 of A showed the binding activity of \(^{3}H\)DFP, \(^{14}C\)TPP, and \(^{14}C\)DPC with human monocyte carboxylesterase, respectively. In B, human monocyte carboxylesterase was labeled with \(^{3}H\)DFP by methods identical to those used in A, lane 1, except that the enzyme was preincubated for 30 min with either no unlabeled inhibitor (lane 1), or \(10^{-2}\) M TPP (lane 2), or 0.1 M DPC (lane 3). The enzyme was subsequently labeled with \(^{3}H\)DFP. The samples were then electrophoresed through SDS-PAGE (12%), and the gel was examined by autoradiography. The exposure time was 9 days.

Electrophoresed under denaturing conditions. With the other two radiolabeled inhibitors (TPP and DPC), little or no radioactivity was observed to comigrate with the enzyme when these enzyme-inhibitor mixtures were subjected to electrophoresis and autoradiography. Such data suggested that DFP formed a covalent bond with the monocyte esterase, whereas DPC and TPP did not form stable covalent bonds. Such mechanisms were further supported by experiments in which the enzyme-inhibitor mixture was subjected to electrophoresis in a nondenaturing gel. Under these conditions, enzyme activity was recovered after electrophoresis of the DPC/enzyme and TPP/enzyme mixtures, whereas no enzyme activity was observed with a DFP/enzyme mixture when it was subjected to electrophoresis under the same conditions (data not shown).

To elucidate further the interactions between these organophosphorus agents and monocyte carboxylesterase, competitive binding experiments were done (Fig. 6B). In these experiments, pure monocyte carboxylesterase was preincubated for 30 min with either no unlabeled inhibitor or TPP or DPC, these mixtures were subsequently incubated with identical quantities of radiolabeled DFP. The latter mixtures were then subjected to electrophoresis as described previously. The results of these experiments show that the carboxylesterase, preincubated with TPP and then treated with radiolabeled DFP, incorporated significantly less radioactivity (Fig. 6B, lane 2) than the carboxylesterase incubated with radiolabeled DFP alone (Fig. 6B, lane 1) (no DPC preincubation). These data suggest that DFP and TPP compete with the same site on the enzyme. An identical experiment using DPC in place of TPP showed quite different results. In the case of DPC, there was no significant difference in the quantity of \(^{3}H\)DFP incorporated into enzyme (Fig. 6B, lane 3) as compared to the amount of radiolabel incorporated into the control (Fig. 6B, lane 1) (no DPC preincubation). These data suggest that TPP and DFP compete for an identical site on monocyte carboxylesterase, whereas DPC interacts with a site other than the active site of the enzyme.

Using two different substrate concentrations of \(\alpha\)-naphthyl butyrate (0.1 and 0.2 mM), a Dixon plot (38) was constructed to determine the mode of action of the apparent reversible inhibitors, DPC, TPP, and tetraphenylresorcinol diphosphate. The latter inhibitor, like its analog, TPP, did not bind covalently to the enzyme (data not shown). By Dixon plot, TPP and tetraphenylerosorcinol diphosphate, were observed to be competitive inhibitors of carboxylesterase, whereas DPC was determined to be a noncompetitive inhibitor. These observations confirm the electrophoretic data characterizing enzyme-inhibitor interactions between TPP and DFP binding site but showing no interaction between DPC and that binding site. The inhibitor constants \((K_i)\) for each reversible inhibitor are shown in Table V. To calculate the affinity of the inhibitors for monocyte carboxylesterase as well as their inactivation constant \((K_{\text{activation}})\), we have assumed the following reactions.

\[
 E + 1 \xrightarrow[K_{\text{association}}]{(\text{tight binding})} E1 \xrightarrow[K_{\text{activation}}]{(\text{loose binding})} E1' 
\]

By this kinetic scheme, \(K_{\text{association}}\) is equal to the reciprocal of \(K_d\). The data derived from these assumptions are also shown in Table V. The \(K_{\text{association}}\) values provide a rank order of affinity of the various inhibitors for carboxylesterase which are consistent with the inhibitor constants \((K_i)\).

**DISCUSSION**

In this report, we have described a relatively simple method for purifying to homogeneity the membrane-associated human monocyte carboxylesterase from normal, resting peripheral blood monocytes using only three different column chromatographic systems for purification and high performance liquid chromatography to remove nonprotein contaminants. Under non-denaturing conditions, the majority of the enzyme (>95%) present in detergent-extracted human monocyte homogenates had a molecular weight of approximately 200,000 (Figs. 2 and 4B, lanes 1-4), and the pure enzyme protein migrated as a single band on SDS-PAGE as observed with silver staining (Fig. 4A, lane 5). By the latter electrophoretic method, the molecular weight was determined to be 60,000. Treatment with SDS presumably resulted in the dissociation of the trimeric form of the enzyme to a monomeric species. Although our data were not analyzed by a Ferguson plot to derive a Stokes radius for the enzyme, we believe existing data are sufficient to permit the reasonable inference that human monocyte carboxylesterase exists in monomeric and trimeric forms. Others have shown that covalent cross-linking of pig and ox liver carboxylesterase subunits with dimethyl suberimidate, and subsequent polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate gives three bands with molecular weights of 60,000, 120,000, and 180,000 (40). Identical data have been derived for human liver carboxylesterase (3). Further, electron microscopic examination of negatively stained pig liver esterase has shown enzyme particles to be clover-leaf shaped trimers (41). In a review of carboxylesterase molecular weights and subunit structures, these same enzymes have been examined from nine different species including the human by a variety of methods and found to have a trimeric structure (42). Such findings are also compatible with those observed by Yourn (43) who purified an esterase from an acute myeloid leukemia cell line (ML-1). In that report, a monomer of 68 kDa and a trimer with a molecular mass of 205 kDa were described and observed by PAGE run at pH 9.5. In contrast to that report, human monocyte carboxylesterase was observed as a single band in

**FIG. 6.** SDS-PAGE autoradiograph of human monocyte carboxylesterase labeled with either \(^{3}H\)DFP, \(^{14}C\)TPP, or \(^{14}C\)DPC. Human monocyte carboxylesterase (4 fig) was labeled with 1 \(\times 10^{6}\) cpm of either \(^{3}H\)DFP, \(^{14}C\)TPP, or \(^{14}C\)DPC, and incubated at room temperature for 30 min. The samples were then electrophoresed through SDS-PAGE slabs (12% acrylamide). The gel was fixed, dried, and examined by autoradiography. The exposure time was 5 days. Lanes 1, 2, and 3 of A showed the binding activity of \(^{3}H\)DFP, \(^{14}C\)TPP, and \(^{14}C\)DPC with human monocyte carboxylesterase, respectively. In B, human monocyte carboxylesterase was labeled with \(^{3}H\)DFP by methods identical to those used in A, lane 1, except that the enzyme was preincubated for 30 min with either no unlabeled inhibitor (lane 1), or \(10^{-2}\) M TPP (lane 2), or 0.1 M DPC (lane 3). The enzyme was subsequently labeled with \(^{3}H\)DFP. The samples were then electrophoresed through SDS-PAGE (12%), and the gel was examined by autoradiography. The exposure time was 9 days.
its presumed trimeric form on PAGE run at pH 7. These subunit and undissociated enzyme molecular weights are similar to those observed for human liver carboxylesterase, human intestinal esterase, human brain esterase, and esterases purified from nonhuman species (3, 6, 15, 44-48). An esterase from the human brain with a preference for butyl ester substrates is an exception to these findings since it has been found to have a molecular mass of 340 kDa (46). Whether this enzyme represents an aggregate of lower molecular weight enzymes or a different enzyme cannot be determined from the data. The similarity of the amino acid content of esterases lends additional support to the possibility that the primary structure of these enzymes share common elements between species (Table II). Sequence data from human monocyte carboxylesterase and other esterases would provide a more quantitative estimation of the proposed homogeneity of this enzyme from species to species.

Isoelectric focusing gel electrophoresis of the purified human monocyte carboxylesterase showed at least four major isoenzymes with pIs between 7.5 and 7.8 (Fig. 4C). Multiple esterase isoenzymes have also been identified in a number of human tissues, and investigations by Coates and his colleagues (49) have reported that at least nine structural gene loci control the expression of human esterase isoenzymes. The recent cloning of carboxylesterase from rat liver provides further evidence that a family of carboxylesterase genes exist (15). Like the monocyte CBE isolated by us, rat liver microsomal CBE is a high mannose-type glycoprotein (50, 51).

Despite the reported capacity of carboxylesterases to hydrolyze both xenobiotic and endogenous substrates, the true biological function of this family of enzymes remains poorly understood. Human monocyte CBE did not hydrolyze long chain saturated or unsaturated fatty esters (C12-C18) in aqueous solution. Further, the use of lipid micelles containing a variety of pure phospholipids as well as mixtures of phospholipids along with these long chain fatty acid esters as substrates for CBE did not result in ester hydrolysis. Other investigators (52) have reported that monocyte CBE metabolized N-acetylated arylamines to their carcinogenic metabolites using an indirect assay. In our hands, purified human monocyte CBE did not deacetylate the potent acetylated aminofluorene carcinogens using a direct method to measure hydrolytic products.

Another xenobiotic, triphenyl phosphate, has been reported to be hydrolyzed to phenol and monophenyl phosphates by monocyte CBE (53). These works used a whole cell preparation and radiolabeled TPP as an enzyme source and substrate, respectively. Our results demonstrated TPP to be a relatively potent CBE inhibitor, but we did not observe the generation of any hydrolytic products (phenol and phenyl phosphates) during its incubation with purified monocyte CBE. Since TPP in aqueous solution hydrolyzes slowly to phenol and phenyl phosphates, the hydrolysis observed by others (63) may represent the chemical hydrolysis of TPP or the activity of another organophosphorus-hydrolyzing enzyme (64). We have now examined crude and partially purified hepatic preparations of porcine, rabbit, rat, and human CBE for their capacity to hydrolyze TPP under a variety of experimental conditions and find no evidence of enzymatic hydrolysis of TPP using several different assays to measure the products of hydrolysis, phenol, and phenylphosphates. Neither amidase nor thioesterase activity was demonstrated using classical substrates for these reactions and purified monocyte CBE. In summary, purified monocyte CBE was unable to hydrolyze xenobiotics proposed as substrates for this enzyme and was incapable of metabolizing long chain fatty acid esters which would have characterized a physiological role for this enzyme.

Human monocyte CBE did hydrolyze α-naphthyl esters, p-nitrophenyl esters, o-nitrophenyl esters of different carbon chain lengths (Fig. 5), but the role of these short chain esters in physiological processes remains elusive. Relatively low concentrations of alcohols (ethanol or ethylene glycol monooethyl ether) caused a significant increase in the total rate of α-naphthyl butyrate hydrolysis by pure monocyte CBE. These findings are consistent with the existence of an acyl-enzyme intermediate as proposed by other workers (55), who showed that substrate activation occurred via effector sites on the enzyme which were different from catalytic sites. Effector site occupancy may induce a conformational change in the enzyme permitting a more rapid ingress and egress of substrates to the catalytic site. Recent investigations of pig liver esterases have determined that modulators of its catalytic reaction after inhibition kinetics (56), and the results indicated that an aromatic or hydrophobic structure and a carbonyl group were required for optimal interaction with the effector site. Since the human monocyte CBE inhibitor, diphenyl carbonate, has these properties, our data are consistent with the possibility that DPC interacts with an effector site on the enzyme. On the basis of the inhibitor profile of human monocyte CBE, the enzyme is likely to be a serine esterase without metal or sulfhydryl requirements essential for its activity. Its inhibitor profiles are similar to human hepatic and intestinal esterases which are also inhibited by DFP, paraaxon, and phenylmethylsulfonyl fluoride, but not by EDTA (6, 44). Our data also suggest that there is more than one enzyme site with which inhibitors interact since diphenyl carbonate is a noncompetitive inhibitor whereas the organophosphorus compounds are competitive inhibitors. We have postulated on the basis of its structure that DPC may interact with an effector site on the enzyme, and studies are underway to test this hypothesis.

We have attempted to sequence the purified human monocyte carboxylesterase directly from the N terminus but the data suggested that the N terminus was blocked. Nonetheless, the purification of this monocyte enzyme to homogeneity provides enzyme protein for sequencing by other methods and the subsequent synthesis of oligonucleotide probes for cloning the monocyte carboxylesterase. Such information will permit a better understanding of the molecular basis of both the catalytic and effector sites of this enzyme, the structural variations of the human mutant enzymes (20, 57), and the biological functions of this enzyme.

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| Compound                        | $K_a$ | $K_d$ | $K_{association}$ | $K_{dissociation}$ | $t_{1/2}$ | Type of inhibition |
|--------------------------------|-------|-------|-------------------|---------------------|----------|-------------------|
| Diphenyl carbonate              | 3.7 x 10^{-3} | 1.7 x 10^{-6} | 6.0 x 10^{-9}     | 0.011               | 63       | Noncompetitive    |
| Triphenyl phosphate             | 8.0 x 10^{-9} | 3.2 x 10^{-8} | 3.1 x 10^{-7}     | 0.033               | 21.06    | Competitive       |
| Tetraphenylresorcin diphosphate | 3.0 x 10^{-10}| 3.3 x 10^{-10} | 1.9 x 10^{-6}     | 0.014               | 49.64    | Competitive       |

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