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Specificity and Induction of Undecyl Acetate Esterase from *Pseudomonas cepacia* Grown on 2-Tridecanone

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Undecyl acetate esterase from *Pseudomonas cepacia* grown on 2-tridecanone was strongly inhibited by organophosphates and other esterase inhibitors. Also, *p*-chloromercuribenzoate at 1×10^{-4} M showed a 70% inhibition of esterase activity. The enzyme hydrolyzed both aliphatic and aromatic acetate esters at substrate concentrations of 0.25 M. Under these conditions the highest reaction rate was toward undecyl acetate. No lipase or proteolytic activity was demonstrated. Undecyl acetate esterase was classified as a carboxylesterase (B-esterase). Cell-free activity studies on the production of undecyl acetate esterase grown on different carbon sources plus zymogram studies demonstrated that the enzyme was inducible when 2-tridecanone, 2-tridecanol, undecyl acetate and, to a lesser extent, 1-undecanol were growth substrates. Induction of undecyl acetate esterase during oxidation of 2-tridecanone supports the view that undecyl acetate is an intermediate in the degradation of the methyl ketone.

For convenience in discussing esterases, Whitaker (24) has divided carboxylic ester hydrolases (EC 3.1.1.1) into two groups, the nonspecific carboxylic ester hydrolases and the specific carboxylic ester hydrolases. The former group is made up of esterases and lipase whereas the latter group includes phospholipases, acetylcholinesterase, chlorophyllase, and pectinesterase. Nonspecific carboxylic ester hydrolases from tissue are further divided into carboxylesterase (B-esterase), arylesterase (A-esterase), and acetylesterase (C-esterase) based on substrate specificity and sensitivity to various activators and inhibitors. In the work reported here, characterization of undecyl acetate esterase from *Pseudomonas cepacia* was based on the scheme for classification of mammalian esterases.

Electrophoretic patterns of esterases from microorganisms have been used as possible aids in taxonomy (3, 10, 18). Comparative analyses of esterase patterns were obtained from extracts of different organisms grown on the same complex medium, so little insight was gained as to natural substrates or inducers for these esterases.

Goldstein and Goldstein (13) described an adaptive cholinesterase produced by a strain of *P. fluorescens* grown on acetylcholine as the sole carbon source. Recently, an inducible cholinesterase produced by *P. aeruginosa* was found to be sensitive to catabolite repression and to require cyclic adenosine 5'-monophosphate for its induction (12). An extracellular esterase has been isolated and characterized from *Aspergillus flavus*, induced by and capable of hydrolyzing the ester linkage of the depside, 2-protocatechuoyl-phloroglucinol carboxylic acid (4, 5).

This paper describes an esterase formed during the catabolism of the methyl ketone, 2-tridecanone. Electrophoretic mobility of this esterase compares favorably with an esterase induced for the catabolism of undecyl acetate, a proposed ester intermediate in 2-tridecanone degradation.

MATERIALS AND METHODS

Growth of the organism on 2-tridecanone, preparation of enzyme fractions, enzymatic assays, and polyacrylamide gel electrophoresis were described in the preceding paper (21).

Inhibitor studies. The radioactive assay for esterase activity was used for inhibitor studies (21). Various concentrations of each inhibitor were incubated with the esterase preparation at 30 C for 30 min before substrate addition. Polyacrylamide gel electrophoresis was used to assess esterase inhibition by several inhibitors. A partially purified esterase preparation was subjected to electrophoresis. After the run, gels were placed into 0.1 M phosphate buffer (pH 7.0) containing inhibitor at various concentrations. The control gel was incubated in buffer without inhibitor. Esterase staining was carried out except that the staining mixture contained the inhibitor at the same concentration as that used for incubation. Staining time was the same for all gels and the reaction was stopped by soaking the gel in 7% acetic acid. Gels were

scanned for stained esterase bands with a Gilford model 240 spectrophotometer equipped with a Linear Transport, model 2410, and a gel cuvette model 2412, at 470 nm with a slit plate of 0.05 by 2.36 cm. Scanning speed was 1.0 cm/min with the recorder chart speed at 1.0 inch/min.

Organophosphorus inhibitors used were diisopropyl fluorophosphate (DFP), diethyl-*p*-nitrophenyl phosphate (E 600), and bis-*p*-nitrophenyl phosphate (BNPP) (Sigma Chemical Co.), tri-*o*-cresyl phosphate (K and K Laboratories, Inc.), and tetraethyl pyrophosphate (TEPP) (Chemicals Procurement Laboratories, Inc.). Other inhibitors used were physostigmine sulfate (eserine) (K and K Laboratories, Inc.), sodium arsanilate (atoxyl), *p*-chloromercuribenzoate (PCMB), and sodium iodoacetate (Sigma Chemical Co.), sodium azide (Fisher Scientific Co.), phenylmethyl sulfonylfluoride (Calbiochem, San Diego, Calif.), and sodium fluoride (Mallinckrodt Chemical Works).

Tests for lipolytic and proteolytic activity. When undecyl acetate esterase was tested for its hydrolytic ability toward long-chain fatty acid esters, a titrimetric method was used (22). The reaction mixture contained a sonically emulsified suspension of 140 μmol of triolein, 10 μmol of $CaCl_2$, and 2% Triton X-155, plus enzyme preparation to a final volume of 3.0 ml. The pH was 7.8 to 8.0. When olive oil was used as substrate, 2.0 ml of the following sonic emulsion was used: 5.0 ml of olive oil, 6.0 ml of 0.075 M $CaCl_2$ solution, and 30 ml of 3% Triton X-155 solution. The reaction mixture was incubated in a 25-ml Erlenmeyer flask with shaking at 30 C in a Dubnoff metabolic shaking incubator for 1 h. The enzyme reaction was stopped by addition of 10 ml of isopropanol and 2 drops of 1% alcoholic phenolphthalein, followed by heating in a 70 C water bath for 5 min. The control was treated in the same manner except the enzyme preparation was replaced by distilled water. The amount of fatty acid liberated was determined by titration with a 0.010 N NaOH solution standardized by titration against potassium acid phthalate.

Unfractionated cell-free extract from *P. cepacia* and purified undecyl acetate esterase were tested for proteolytic activity employing Determatube TRY (*N*-benzoyl-L-arginine) and Determatube BTEE (*N*-benzoyl-L-tyrosine) (Worthington Biochemical Corp., Freehold, N.J.). The procedure followed was according to the instructions accompanying the Determatubes and the *Worthington Enzyme Manual* (26).

Esterase induction. *P. cepacia* was cultured in basal salts medium supplemented individually with the following carbon sources at a concentration of 0.3%: undecyl acetate, 2-tridecanone, 2-tridecanol, 1-undecanol, glycerol, Span 80 (sorbitan monooleate), acetate, lactate, and succinate. *P. cepacia* was also grown in nutrient broth and 1% yeast extract. Each culture was transferred three times in 100 ml of the medium containing the designated carbon source. A 100-ml amount of the third transfer was inoculated into a 2.8-liter Fernbach flask containing 1 liter of medium. Cells were harvested at log phase (determined by monitoring with a B and L Spectronic 20 spectrophotometer at 660 nm), and washed twice with 0.01 M potassium phosphate buffer. Cell-free extracts were prepared and checked for protein concentration and undecyl acetate esterase activity. A zymogram of esterases from the cell-free extracts was developed by analytical polyacrylamide gel electrophoresis followed by esterase staining.

Substrates. Substrates used were methyl acetate, ethyl acetate, *n*-butyl acetate, *t*-butyl acetate, *n*-pentyl acetate, *n*-hexyl acetate, *n*-heptyl acetate, *n*-dodecyl acetate, *n*-hexadecyl acetate, ethyl-*n*-propionate, ethyl-*n*-butyrate, ethyl-*n*-decanoate, ethyl-*n*-undecanoate, ethyl-*n*-laurate, *n*-butyl-*n*-propionate, *n*-butyl-*n*-butyrate, and potassium thioacetate (Eastman Organic Chemicals Co.), *n*-octyl acetate, *n*-nonyl acetate, *n*-decyl acetate, *n*-myristyl acetate, *n*-undecylenic acetate, phenyl acetate, and phenyl propionate (K and K Laboratories, Inc.), triacetin, tributyrin, triolein, *p*-nitrophenyl acetate, and α-naphthyl acetate (Sigma Chemical Co.), tripropionin (Chemicals Procurement Laboratories, College Point, N.Y.), and Span 80 (sorbitan monooleate) (Atlas Powder Co., Wilmington, Del.).

RESULTS

Inhibitors. Organophosphates and other esterase inhibitors were used to study their effect on undecyl acetate esterase. Esterase activity on undecyl acetate was strongly inhibited by all organophosphates tested (Table 1). Other esterase inhibitors were also inhibitory to undecyl acetate esterase but to a lesser extent. Sodium fluoride inhibited enzyme activity at high concentration. However, the fluoride ions were not the main factor in DFP inhibition since, at a concentration below 10^{-5} M, DFP was still exhibiting a good inhibitory effect, whereas sodium fluoride as the same concentration had no effect on the enzyme activity. Phenylmethyl sulfonyl fluoride (PMSF) was first used as an esterase inhibitor by Fahrney and Gold (11) as a relatively safe alternative for highly toxic DFP. PMSF inhibited undecyl acetate esterase more effectively than sodium fluoride but less so than DFP.

The effect of the following inhibitors was also determined by using polyacrylamide gel electrophoresis: DFP, E-600, BNPP, eserine, PCMB, and TEPP. Figure 1 demonstrates some representative scans showing that the esterase bands from a partially purified preparation were all inhibited to the same extent with the inhibitors tested.

Three of the organophosphates (TEPP, DFP, and BNPP) were further studied by plotting percentage of inhibition against $-\log [I]$ using unfractionated cell-free extract with undecyl acetate as substrate (Fig. 2). In all three cases, a biphasic curve was obtained. This same type of

TABLE 1. *Effect of esterase inhibitors on undecyl acetate esterase*[a]

Inhibitor	Concn (M)	Inhibition (%)
Diisopropyl fluorophosphate	2.4×10^{-3}	100
	2.4×10^{-5}	88
	2.4×10^{-7}	65
Tetraethyl pyrophosphate	3.0×10^{-5}	100
	3.0×10^{-7}	97
	3.0×10^{-9}	21
Bis-*p*-nitrophenyl phosphate	1.0×10^{-3}	100
	1.0×10^{-5}	98
	1.0×10^{-7}	54
Diethyl-*p*-nitrophenyl phosphate	1.5×10^{-4}	100
	1.5×10^{-6}	96
Tri-*o*-cresyl phosphate	5.0×10^{-5}	70
	5.0×10^{-7}	23
Phenylmethyl sulfonylfluoride	1×10^{-3}	100
	1×10^{-5}	14
	1×10^{-7}	0
Physostigmine (eserine)	1×10^{-3}	95
	1×10^{-5}	22
	1×10^{-7}	0
Sodium arsanilate (atoxyl)	1×10^{-2}	98
	1×10^{-4}	52
	1×10^{-6}	0
Sodium fluoride	1×10^{-1}	98
	1×10^{-3}	62
	1×10^{-5}	3

[a] Substrate concentration = 0.1 M undecyl acetate. Enzyme preparation was unfractionated cell-free extract. Inhibition (percent) was based on the enzymatic hydrolysis of the substrate under identical conditions but without inhibitor.

curve was also produced when PMSF, eserine, or atoxyl was used as inhibitor (Fig. 3).

Other enzyme inhibitors were used to test their effect on undecyl acetate esterase. Table 2 shows that, among the sulfhydryl-group inhibitors, 10^{-4} M PCMB demonstrated a 71% inhibition toward undecyl acetate esterase, but the others had no effect on the enzyme at the concentration tested. The chelating agent ethylenediaminetetraacetic acid did not inhibit nor activate the esterase.

Product inhibition was also tested by incubating 1-undecanol and acetate, singly or together, with the esterase before undecyl acetate was added to the reaction mixture. Table 3 shows that acetate had no effect on the esterase, but 1-undecanol inhibited esterase activity, especially at high concentration.

Substrate specificity. The activity of the esterase toward various acetate esters was determined. Undecyl acetate and all other esters were checked at a concentration of 0.25 M. Under these specified conditions and with activity for undecyl acetate set at 100%, a decrease in activity was shown as the alkyl side chain was varied in length (Table 4). Presence of a terminal double bond in the undecyl acetate substrate (ω-undecylenyl acetate) caused a slight reduction in enzyme activity. Relatively high enzyme activity was exhibited toward the aromatic acetate esters, *p*-nitrophenyl acetate and α-naphthyl acetate, with activity toward phenyl acetate being significantly lower. The above results demonstrate highest reactivity with undecyl acetate, but this may be misleading. Although the concentration of esters used (0.25 M) approached enzyme saturation in the case of undecyl acetate, this may not necessarily be the case with other ester substrates. For example, apparent K_m values for several of the other ester substrates were determined to be as follows: *n*-butyl acetate, 1.9×10^{-1} M; heptyl acetate, 5.6×10^{-2} M; nonyl acetate, 4.8×10^{-2} M; decyl acetate, 3.1×10^{-2} M; α-undecylenyl acetate, 3.3×10^{-2} M; dodecyl acetate, 9.1×10^{-2} M.

Hydrolytic activities of undecyl acetate esterase and hog pancreatic lipase were compared. Table 5 shows that the purified enzyme had no hydrolytic activity toward either triolein or the lipids in olive oil, but hydrolyzed undecyl acetate and *p*-nitrophenyl acetate. Unfractionated extract from cells grown on 2-tridecanone demonstrated slight lipase activity which was eliminated during enzyme purification. Lipase had negligible activity against undecyl acetate.

Proteolytic activity of undecyl acetate esterase was checked with the Worthington Determatubes TRY and BTEE. The purified enzyme showed no hydrolytic activity toward TRY used for testing protease activity of trypsin (8) and BTEE used for testing endopeptidase activity of chymotrypsin (16). When unfractionated extract from cells grown on 2-tridecanone was tested, no tryptic activity was detected, but a slight chymotryptic activity was present in the extract.

Induction. Cell-free studies on the production of undecyl acetate esterase by *P. cepacia* grown on different carbon sources demonstrated that the enzyme was inducible (Table 6). The low undecyl acetate hydrolytic activities in

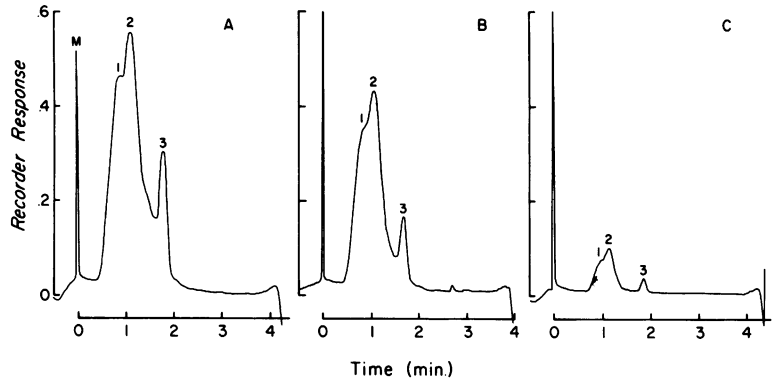

FIG. 1. *Scans of analytical polyacrylamide gels for inhibition of esterase bands. Bands were visualized with α-naphthyl acetate, Fast Blue RR salt, and a designated concentration of inhibitor. (A) No inhibitor; (B) 10^{-5} M esterine; (C) 10^{-6} M bis-p-nitrophenyl phosphate. Letter M indicates wire marking bromophenol blue front. Scanning was at an absorbance of 470 nm. Bands 1 and 2 correspond to the two isoenzymes purified as reported in the preceding paper (21). The partially purified esterase preparation used corresponds to fraction 7 (see Table 1 of reference 21).*

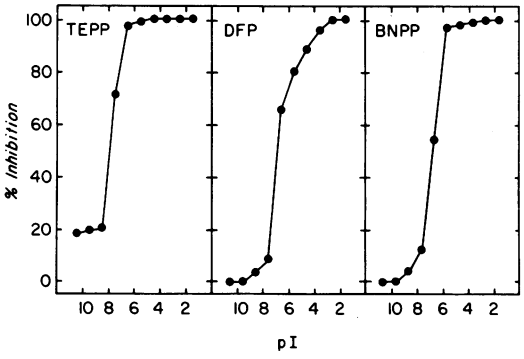

FIG. 2. *Organophosphate inhibition curves for the hydrolysis of undecyl acetate. Percent inhibition is plotted against the $-\log_{10}$ of the molar inhibitor concentration (pI). TEPP, tetraethyl pyrophosphate; DFP, diisopropyl fluorophosphate; BNPP, bis-p-nitrophenyl phosphate. Percent inhibition was based on the enzymatic hydrolysis of the substrate under identical conditions but without inhibitor. Unfractionated cell-free extract was incubated at 30 C for 30 min with varying concentration of inhibitor before addition of the substrate.*

cell-free extracts from cells grown on substrates other than undecyl acetate, 2-tridecanone, and 2-tridecanol may be from some other esterases. The secondary alcohol, 2-tridecanol, has been shown to be oxidized to the corresponding methyl ketone, so it was not surprising to have high undecyl acetate esterase activity in cells grown on this substrate.

Extracts from cells grown on various carbon sources were further analyzed by polyacrylamide gel electrophoresis, and a zymogram of esterases was developed (Fig. 4). Esterase bands were observed after incubating gels in a solution of α-naphthyl acetate and Fast Blue RR salt. Three esterase bands developed rapidly in gels of extracts from cells grown on undecyl acetate, 2-tridecanone, and 2-tridecanol. Among the

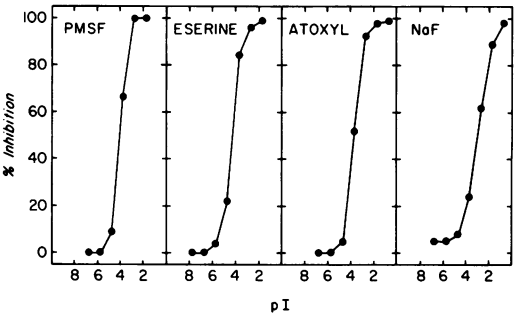

FIG. 3. *Inhibition curves for the hydrolysis of undecyl acetate. PMSF, phenylmethyl sulfonyl fluoride; esterine, physostigmine; atoxyl, sodium arsanilate; NaF, sodium fluoride. See legend of Fig. 2.*

TABLE 2. *Effect of other inhibitors on undecyl acetate esterase*[a]

Inhibitor	Concn (M)	Inhibition (%)
p-Chloromercuribenzoate	10^{-4}	71
Sodium iodoacetate	10^{-3}	0
Sodium azide	10^{-3}	0
Ethylenediaminetetraacetic acid	10^{-3}	0

[a] See footnote to Table 1.

TABLE 3. *Effect of end products on undecyl acetate esterase*[a]

Compounds	Concn (M)	Inhibition (%)
1-Undecanol	6.25×10^{-2}	69
	6.25×10^{-3}	17
	6.25×10^{-4}	10
Sodium acetate	6.25×10^{-2}	0
	6.25×10^{-3}	0
	6.25×10^{-4}	0
1-Undecanol plus sodium acetate	6.25×10^{-2} of each	67
	6.25×10^{-3} of each	22
	6.25×10^{-4} of each	14

[a] Substrate concentration = 6.25×10^{-2} M undecyl acetate. Esterase preparation corresponds to fraction 8 (see Table 1 of reference 21).

TABLE 4. *Hydrolysis of esters by undecyl acetate esterase*[a]

Substrate	Activity (%)
Methyl acetate	4.0
Ethyl acetate	11.9
n-Butyl acetate	16.9
i-Butyl acetate	15.2
t-Butyl acetate	12.0
n-Pentyl acetate	59.8
n-Hexyl acetate	63.8
n-Heptyl acetate	73.5
n-Octyl acetate	75.0
n-Nonyl acetate	81.4
n-Decyl acetate	87.6
n-Undecyl acetate	100.0
ω-Undecylenyl acetate	81.9
n-Dodecyl acetate	49.1
n-Tetradecyl acetate	10.8
n-Hexadecyl acetate	2.4
Triacetin	51.6
p-Nitrophenyl acetate	82.5
α-Naphthyl acetate	81.5
Phenyl acetate	42.2

[a] Substrate concentration = 0.25 M. Enzyme activities were assayed by the manometric method with the reference ester (undecyl acetate) set for 100%. Partially purified esterase preparation corresponds to fraction 7 (see Table 1 of reference 21).

three esterase bands, the two fast electromotive bands had mobilities corresponding to the purified esterase bands (21). These two bands also appeared after 30 min in the gel with extract from cells grown on 1-undecanol. According to the zymogram and the results presented in Table 6, it appears that 1-undecanol induced the esterase but at a lower level than that induced by undecyl acetate, 2-tridecanone, and 2-tridecanol. The third band appeared in gels of all cell-free extracts. However, this band developed faster in gels with extracts from undecyl acetate, 2-tridecanone, and 2-tridecanol. This band was recovered by preparative polyacrylamide gel electrophoresis and it was found to have a lower specific activity on undecyl acetate than the other two bands.

DISCUSSION

Enzyme inhibition tests revealed that organophosphate and non-organophosphate esterase

TABLE 5. *Comparison of hydrolytic activities of pseudomonad esterase and hog pancreatic lipase*

Enzyme	Substrate			
	Triolein	Olive oil	Undecyl acetate	*p*-Nitrophenyl acetate
Hog pancreatic lipase type VI	95.5[a]	84.0[a]	0.3[b]	0[b]
Unfractionated pseudomonad extract	2.9	1.9	2.1	1.7
Polyacrylamide gel electrophoretically purified pseudomonad fraction 8 (see Table 1 of reference 21)	0	0	175.3	149.0

[a] Microequivalents of substrate hydrolyzed per minute per milligram of protein.

[b] Micromoles of substrate hydrolyzed per minute per milligram of protein.

TABLE 6. *Undecyl acetate esterase activity in cell-free extracts of cells grown on various carbon sources*[a]

Growth substrate	Units/mg of protein
Undecyl acetate	3816.5
2-Tridecanone	3883.0
2-Tridecanol	3180.4
1-Undecanol	105.9
Span 80 (sorbitan monooleate)	33.4
Glycerol	16.5
Acetate	12.4
Succinate	12.4
Lactate	5.7
Yeast extract	13.8
Nutrient broth	10.1

[a] Unit = nanomoles of undecyl acetate hydrolyzed per minute. Substrate concentration = 0.1 M undecyl acetate. Protein concentration was approximately 0.05 mg/ml for cell-free extracts obtained from cells grown on undecyl acetate, 2-tridecanone, and 2-tridecanol, and was 2.5 mg/ml for the remainder.

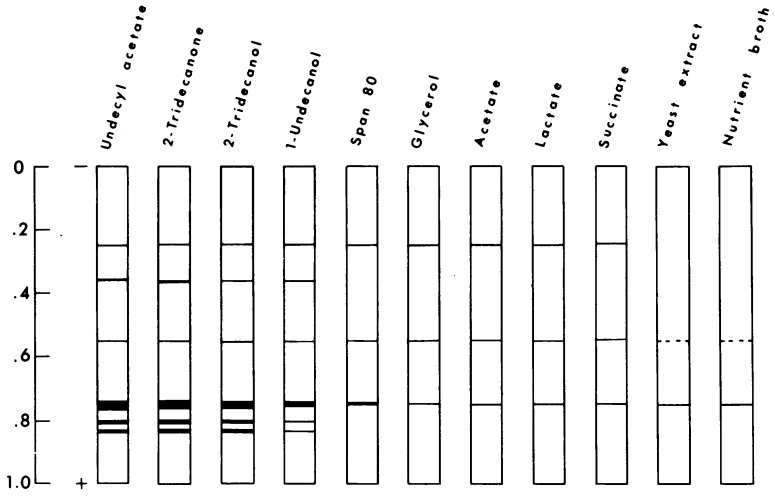

FIG. 4. *Zymograms of esterases from cell-free extracts of P. cepacia grown on various carbon sources. Esterase bands were developed after analytical polyacrylamide gel electrophoresis of the cell-free extracts. Gels with undecyl acetate, 2-tridecanone, and 2-tridecanol extracts developed with α-naphthyl-acetate and Fast Blue RR salt in 0.1 M phosphate buffer after 1 min. Other gels developed after 30 min.*

inhibitors strongly inhibited undecyl acetate esterase activity. Resistance of the esterase to inhibition by eserine at a concentration of 10^{-5} M indicates that this enzyme can be ruled out as a cholinesterase (15). The enzyme was inhibited by PCMB even though another sulfhydryl group inhibitor, iodoacetate, had no effect on the enzyme. This is similar to results obtained with a pancreatic lipase (23).

The biphasic character of the inhibition curves (Fig. 2 and 3) was similar to results obtained by Chow and Ecobichon (8) with inhibition of hepatic esterase by E-600 and DFP. These authors indicated that this type of curve was suggestive of the presence of two hepatic carboxylesterases, one being inhibited by low concentration of inhibitor and one by high concentration. Another possible explanation of the biphasic inhibition curve may be found in the proposal of Greenzaid and Jencks (14), who reported on the presence of two active sites in purified hog hepatic carboxylesterase, each site having different sensitivities to organophosphates. It seems likely that the biphasic nature of the inhibitor plots reported here was due to the presence of more than one organophosphate-sensitive carboxylesterase in the unfractionated cell-free extract. An indication of this is seen in the scans of polyacrylamide gels for esterase inhibition (Fig. 1) where the minor band and the two isoenzyme bands were inhibited by the organophosphate inhibitor, BNPP.

Classification of nonspecific carboxylic ester hydrolases (esterases) into three groups has been based on substrate specificity and sensitivity to various activators and inhibitors (organophosphates and a sulfhydryl reagents in particular). Arylesterases (EC 3.1.1.2), or A-esterases, rapidly hydrolyze aromatic esters and, in many cases, DFP (2). PCMB has also been shown to be inhibitory (15). Carboxylesterases (EC 3.1.1.1) or B-esterases are inhibited by organophosphates, carbamate, and organosulfur compounds (1, 2, 19), and these esterases have wide specificity hydrolyzing both aliphatic and aromatic esters. Acetylesterases (EC 3.1.1.6) or C-esterases do not hydrolyze organophosphate esters nor are they inhibited by them, and their greatest activity is on esters of acetic acid. However, these three groups of esterases have been shown to have overlapping substrate specificities (17, 19, 25). Also, it has been reported that the same esterase species may be differentially affected by organophosphorus compounds with respect to different substrates (7), and based on these observations, a subunit concept for nonspecific esterases was proposed (6). It was suggested that the different esterase species are integral parts of one enzyme built on a subunit structure that exhibits overlapping specificities through the sharing of common subunits.

Results obtained in the present investigation demonstrate that undecyl acetate esterase does not unequivocally fit into any one of the three categories mentioned above, for classification of esterases. This bacterial esterase demonstrated

overlapping substrate specificity, hydrolyzing both aliphatic and aromatic esters. Unlike A-esterases, the enzyme did not hydrolyze organophosphorus compounds but it was inhibited by them. However, it was similar to A-esterases in that it hydrolyzed aromatic esters and was inhibited by PCMB. The experimental esterase differs from C-esterases since it was inhibited by organophosphorus compounds at low dilutions. Although the bacterial esterases were similar to B-esterases with regard to inhibitor effects and ability to hydrolyze both aliphatic and aromatic esters, it differs in that it demonstrated a characteristic resembling lipase since it hydrolyzed insoluble substrates, i.e., undecyl acetate. However, triolein and triglycerides in olive oil were not hydrolyzed. Arbitrarily, undecyl acetate esterase may be considered as a B-esterase (carboxylesterase) since it possesses most of the characteristics of this group.

The esterase purified and studied from cells grown on the methyl ketone, 2-tridecanone, corresponded in electrophoretic mobility to the esterase induced when cells were grown on the ester, undecyl acetate (Fig. 4). The lower level of esterase induction by 1-undecanol may be explained by its structural similarity to undecyl acetate since 1-undecanol represents the alcohol moiety of the acetate ester. We interpret the induction of this esterase during methyl ketone oxidation as further evidence for an acetate ester intermediate in the catabolic pathway for 2-tridecanone. A close parallel is seen in the fungal mechanism of steroid oxidation. Progesterone, which can be regarded as a complex substituted methyl ketone, is converted to testosterone acetate by an oxygenase followed by an esterase-mediated split to give the steroid alcohol, testosterone, plus acetate (20).

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LITERATURE CITED

1. Aldridge, W. N. 1953. Serum esterases. 1. Two types of esterases (A and B) hydrolysing *p*-nitrophenylacetate, propionate, and butyrate, and a method for their determination. Biochem. J. **53:**110-117.
2. Aldridge, W. N., and E. Reiner. 1972. Enzyme inhibitors as substrates. Interaction of esterases with esters of organophosphorus and carbamic acids. North-Holland Publishing Co., Amsterdam.
3. Baptist, J. N., C. R. Shaw, and M. Mandel. 1971. Comparative zone electrophoresis of enzymes of *Pseudomonas solanacearum* and *Pseudomonas cepacia*. J. Bacteriol. **108:**799-803.
4. Child, J. J., T. Oka, F. J. Simpson, and H. G. Krishnamurty. 1971. Purification and properties of a phenol carboxylic acid acyl esterase from *Aspergillus flavus*. Can. J. Microbiol. **17:**1455-1463.
5. Child, J. J., D. W. S. Westlake, and F. J. Simpson. 1963. Degradation of rutin by *Aspergillus flavus*. Production, purification and characterization of an esterase. Can. J. Microbiol. **9:**653-664.
6. Choudhury, S. R. 1972. The nature of nonspecific esterases: a subunit concept. J. Histochem. Cytochem. **20:**507-517.
7. Choudhury, S. R., and A. M. Lundy. 1971. Differential inhibition of esterase isoenzymes by organophosphorus compounds. J. Histochem. Cytochem. **19:**390-392.
8. Chow, A. Y. K., and D. J. Ecobichon. 1973. Characterization of the esterases of guinea pig liver and kidney. Biochem. Pharmacol. **22:**689-701.
9. Desnuelle, P. 1960. Trypsin, p. 119-132. *In* P. D. Boyer, H. Lardy, and K. Myrback (ed.), The enzymes, vol. 4, 2nd ed. Academic Press Inc., New York.
10. El-Sharkawy, T. A., and D. Huisingh. 1971. Electrophoretic analysis of esterases and other soluble proteins from representatives of phytopathogenic bacterial genera. J. Gen. Microbiol. **68:**149-154.
11. Fahrney, D. E., and A. M. Gold. 1963. Sulfonyl fluorides as inhibitors of esterases. I. Rates of reaction with acetylcholinesterase, α-chymotrypsin and trypsin. J. Amer. Chem. Soc. **85:**997-1000.
12. Gilboa-Garber, N., V. Zakut, and L. Mizraki. 1973. Production of cholinesterase by *Pseudomonas aeruginosa*, its regulation by glucose and cyclic AMP and inhibition by antiserum. Biochim. Biophys. Acta **297:**120-124.
13. Goldstein, D. B., and A. Goldstein. 1953. An adaptive bacterial cholinesterase from a *Pseudomonas* species. J. Gen. Microbiol. **8:**8-17.
14. Greenzaid, P., and W. P. Jencks. 1971. Pig liver esterase. Reactions with alcohols, structure-reactivity correlations, and the acyl-enzyme intermediate. Biochemistry **10:**1210-1222.
15. Holmes, R. S., and D. J. Masters. 1969. The developmental multiplicity and isozyme status of cavian esterases. Biochim. Biophys. Acta **132:**379-399.
16. Hummel, B. C. W. 1959. A modified spectrophotometric determination of chymotrypsin, trypsin, and thrombin. Can. J. Biochem. Physiol. **37:**1393-1399.
17. Krisch, K. 1971. Carboxylic ester hydrolases, p. 43-69. *In* P. D. Boyer (ed.), The enzymes, vol. 5, 3rd ed. Academic Press Inc., New York.
18. Lund, G. 1965. A comparison by the use of gel electrophoresis of soluble protein components and esterase enzymes of some group D streptococci. J. Gen. Microbiol. **40:**413-419.
19. Oosterbaan, R. A., and H. S. Janz. 1965. Cholinesterases, esterases and lipases, p. 1-54. *In* M. Florkin and E. H. Stotz (ed.), Comprehensive biochemistry, vol. 16. Hydrolytic reactions; cobamide and biotin coenzymes. Elsevier Publishing Co., Amsterdam.
20. Rahim, M. A., and C. J. Sih. 1966. Mechanisms of steroid oxidation by microorganisms. XI. Enzymatic cleavage of the pregnane side chain. J. Biol. Chem. **241:**3615-3623.
21. Shum, A. C., and A. J. Markovetz. 1974. Purification and properties of undecyl acetate esterase from *Pseudomonas cepacia* grown on 2-tridecanone. J. Bacteriol. **118:**880-889.
22. Sierra, G. 1957. Studies on the bacterial esterases. I. Differentiation of a lipase and two ali-esterases during growth of *Pseudomonas aeruginosa* and some observations on growth and esterase inhibition. Antonie van

Leeuwenhoek J. Microbiol. Serol. **23**:241-265.
23. Verger, R., L. Sarda, and P. Desnuelle. 1971. On the sulfhydryl groups of porcine pancreatic lipase and their possible role in the activity of the enzyme. Biochim. Biophys. Acta **242**:580-592.
24. Whitaker, J. R. 1972. Principles of enzymology for the food sciences, p. 481-501. Marcel Dekker Inc., New York.
25. Wilkinson, J. H. 1970. Isoenzymes, 2nd ed. J. B. Lippincott Co., Philadelphia.
26. Worthington Enzyme Manual. 1972. Worthington Biochemical Corp., Freehold, New Jersey.