Partial Purification and Characterization of Lipase (EC 3.1.1.3) from *Propionibacterium acnes*

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(Received 10 September 1980; revised 7 November 1980)

Lipase from *Propionibacterium acnes* has been purified 4800-fold from crude culture supernatant. The purified enzyme preparation had no assayable protease, hyaluronate lyase or acid phosphatase activities. The molecular weight of the lipase was 46 770 as determined by gel filtration. Sodium dodecyl sulphate–polyacrylamide gel electrophoresis revealed a major protein component (mol. wt 41 190) together with two minor protein components (mol. wt 67 000 and 125 900). The lipase had a pH optimum of 6.8, was most stable in the pH range 5.0 to 6.0 and was completely inactivated after 30 min at 60 °C. The lipase hydrolysed trilaurin, triolein, trimyristin and tripalmitin at decreasing rates and did not exhibit phospholipase activity. Analysis of the reaction products from the hydrolysis of triolein by *P. acnes* lipase did not demonstrate an accumulation of 2-monoolein which suggested that the enzyme did not exhibit a positional specificity for the 1-position of the triacylglycerol.

Crude lipase preparations contained an aggregated high molecular weight form of the enzyme which was eluted with the void volume from Sephadex G-200. This aggregated form was dissociated to produce the lower molecular weight lipase species by subsequent dialysis and elution from Sephadex G-200 using buffer with a higher ionic strength.

INTRODUCTION

Propionibacterium acnes is the major bacterial inhabitant of the forehead and back of man (Marples & McGinley, 1974) and has been implicated in the disease acne vulgaris (Kligman, 1974). Lipases produced by *P. acnes* in vivo hydrolyse native sebum triacylglycerols to free fatty acids (Marples *et al.*, 1971). These acids may be effective in reducing the invasion of overt skin pathogens, but it has been suggested that they may, if over-produced, predispose the human carrier to acne (Marples *et al.*, 1971). Although the evidence for the role of free fatty acids as irritants is primarily circumstantial (Puhvel & Sakamoto, 1977) and there is evidence that free fatty acids are not involved in the acne process (Weeks *et al.*, 1977), it is still important to understand the nature and properties of *P. acnes* lipase. There is evidence that *P. acnes* lipase is produced in vivo (Marples *et al.*, 1971) and therefore the enzyme probably serves a function in the nutrition of the organism living on the skin.

The purification and properties of propionibacterial lipase have been described previously (Hassing, 1971; Fulton *et al.*, 1974; Pablo *et al.*, 1974). However, these investigations were carried out on the enzyme produced by strains of the *Corynebacterium acnes* group II species described by Voss (1970). This species is now recognized as *Propionibacterium granulosum* (Moore & Holdeman, 1974). *Propionibacterium granulosum* is isolated from the skin at a lower frequency than *P. acnes* (Marples & McGinley, 1974) and exhibits a greater lipolytic activity than *P. acnes* in vitro (Whiteside & Voss, 1973).

The study reported here was undertaken to describe the lipase produced by a typical strain

of *P. acnes*. There have been no previous studies reported in the literature of the purification and properties of the enzyme produced by this species.

METHODS

Reagents. Bovine serum albumin (fraction V), cytochrome *c* (horse heart, 95 to 100% pure), 1,2-diolein, 1,3-diolein, β -galactosidase (from *E. coli*; >98 % pure), glycerol, glycerol dehydrogenase, gum acacia, 1-monoolein (containing 10% 2-monoolein), oleic acid (98 % pure), ovalbumin (99%), L- α -phosphatidylcholine (from egg yolk), L- α -phosphatidylethanolamine (from ovine brain), silica gel G, sodium dodecyl sulphate, porcine pancreatic lipase (steapsin; specific activity 47 units mg⁻¹), triolein (95 % pure), trilaurin (90%), trimyristin (90%) and tripalmitin (90%) were obtained from Sigma. Acrylamide, *N,N'*-methylenebisacrylamide, 2-(*N*-morpholino)ethanesulphonic acid (MES), 3-(*N*-morpholino)propanesulphonic acid (MOPS), and *N,N*-bis(2-hydroxyethyl)glycine (bicine) were obtained from BDH. Yeast alcohol dehydrogenase [containing <0.1% (w/w) impurities] was obtained from Boehringer. Sephadex G-200 and G-100, CM-Sephadex C-50 and Blue Dextran 2000 were obtained from Pharmacia. All other chemicals were reagent grade.

Bacterial strain. A laboratory strain of *P. acnes* (type I of Marples & McGinley, 1974) isolated from a blackhead lesion on a patient in Leeds General Infirmary, and designated P-37, was used. Stock cultures were maintained in 40% (w/v) glycerol in 0.1 M-potassium phosphate buffered saline (pH 7.3) at -20 °C.

Enzyme production. Brain Heart Infusion broth (Difco) supplemented with 0.3% (w/v) glucose was dispensed in 400 ml portions into bottles and sterilized. A portion of an exponential-phase culture (0.5%, v/v) of *P. acnes* was added to each bottle and the cultures were incubated without stirring in an atmosphere of H₂/CO₂ (90:10, v/v) in cold catalyst anaerobic jars at 37 °C for 6 to 7 d. Culture supernatant containing the extracellular products was separated by centrifuging at 3000 g for 1 h. This supernatant was used as a source of lipase and designated fraction I enzyme.

Lipase assay. Lipase (EC 3.1.1.3) was assayed using triolein as substrate. The reaction mixture contained 0.5 ml triolein emulsion [10% (w/v) triolein emulsified in 5% (w/v) gum acacia using a Polytron homogenizer at maximum speed for 1 min], 0.5 ml of a suitable dilution of enzyme, and 2.5 ml 0.1 M-citrate/phosphate buffer (pH 6.5). A 1 ml portion of this reaction mixture was transferred to 5 ml Dole's reagent (propan-2-ol/heptane/1 M-H₂SO₄; 40:10:1, by vol.) before and after 1 h incubation at 37 °C. The amount of oleic acid released was determined by the method of Dole & Meinertz (1960) using oleic acid as the standard. One unit of lipase activity is defined as the amount of enzyme releasing 1 μ mol oleic acid min⁻¹.

Assays of other enzymes. Hyaluronate lyase (EC 4.2.2.1), acid phosphatase (EC 3.1.3.2) and protease were determined as described previously (Ingham *et al.*, 1979).

Enzyme purification. All procedures were carried out at 0 to 4 °C.

(i) **Ultrafiltration.** Culture supernatant was concentrated in an Amicon model TCF10 Diaflo cell fitted with a PM10 Diaflo membrane (mol. wt cut-off 10000). At later stages in the purification enzymically active fractions were concentrated and dialysed by ultrafiltration in a 50 ml Amicon model 52 cell fitted with a PM10 membrane.

(ii) **Equilibrium dialysis.** Equilibrium dialysis was carried out using Visking tubing (Gallenkamp). Enzyme solutions were dialysed against at least 10 times their volume of dialysis buffer (0.1 M-sodium phosphate buffer, pH 6, plus 0.5 M-NaCl) for 48 to 72 h.

(iii) **Sephadex G-100 chromatography.** Amicon-concentrated lipase (20 or 50 ml) was loaded on to a Sephadex G-100 column (4.2 $\times$ 90 cm) pre-equilibrated with 0.1 M-sodium phosphate buffer (pH 6). Fractions (9.5 ml) were collected at a flow rate of 20 ml h⁻¹. Fractions with a high lipase activity were combined, concentrated to 20 ml by ultrafiltration and either rechromatographed on Sephadex G-100 or loaded on to a CM-Sephadex C-50 column (2.4 $\times$ 30 cm) pre-equilibrated with 0.05 M-sodium phosphate buffer (pH 6). The column was eluted with 40 ml 0.05 M-sodium phosphate buffer (pH 6), followed by a 500 ml linear salt gradient (0 to 0.4 M-NaCl) in the same buffer. Fractions (4.5 ml) were collected at a flow rate of 20 ml h⁻¹.

Molecular weight determination. (i) **Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis.** This was carried out according to Weber *et al.* (1972) in 10% (w/v) gels. β -Galactosidase (mol. wt 130 000), bovine serum albumin (68 000), alcohol dehydrogenase (37 000) and cytochrome *c* (12 384) were used as reference proteins. Samples (60 μ g protein in 50 μ l) were prepared by heating to 100 °C for 2 min in 0.01 M-sodium phosphate buffer (pH 7) containing 1% (w/v) SDS and 1% (w/v) 2-mercaptoethanol. Samples were analysed in duplicate.

(ii) **Sephadex G-100 chromatography.** A 2.4 $\times$ 31 cm column equipped with a flow adaptor and pre-equilibrated with 0.1 M-sodium phosphate buffer (pH 6) was calibrated with Blue Dextran 2000, β -galactosidase, ovalbumin (mol. wt 43 000) and cytochrome *c*. A 2 ml sample of fraction V lipase (see Table 1) was loaded on to the column and fractions (3 ml) were collected at a flow rate of 10 ml h⁻¹.

Protein determination. The elution of protein from various columns was monitored by measuring absorbance at 280 nm. Protein determinations were made by the method of Lowry using bovine serum albumin as the standard.

Sephadex G-200 chromatography for the study of the molecular forms of crude lipase preparations. Amicon-concentrated samples of enzyme (5 ml) were loaded on to a Sephadex G-200 column (2.6 × 40 cm) pre-equilibrated with either 0.01 M- or 0.1 M-sodium phosphate buffer (pH 6). Fractions (4.5 ml) were collected at a flow rate of 10 ml h⁻¹ using 0.01 M- or 0.1 M-sodium phosphate buffer (pH 6) as eluant.

Glycerol determination. Free glycerol was determined according to Hagen & Hagen (1960).

Positional specificity in the hydrolysis of triolein. A purified preparation of *P. acnes* lipase (50 units; 15 ml) or pancreatic lipase (50 units; 15 ml) was incubated with 5 ml of an emulsion of 0.15 M-triolein and 5 ml Tris/HCl buffer (0.2 M; pH 7) at 37 °C. At zero time, and at 15 min intervals for 2 h, samples were taken from each reaction mixture and analysed for the reaction products. (i) 1 ml was transferred to 5 ml Dole's reagent and the amount of oleic acid released was determined as described above for the lipase assay. (ii) 0.5 ml was heated to 100 °C for 5 min to stop the reaction, then cooled, and the free glycerol present was determined. (iii) 1 ml was transferred to 1 ml Dole's reagent and the products were separated on silica gel G impregnated with boric acid. The thin-layer plates were prepared as described by Kates (1972). Standard lipids—1,2-diolein, 1,3-diolein, 1-monoolein, oleic acid and triolein—were prepared as 4% (w/v) solutions in chloroform. Samples and standard lipids (5 µl) were applied to the plates and the plates were run in one dimension in chloroform/acetone (96:4, v/v). The separated lipid spots were developed in iodine vapour.

RESULTS

Enzyme purification

Table 1 summarizes the purification of *P. acnes* P-37 lipase from 7.5 l of culture supernatant. Crude culture supernatant (concentrated to 250 ml) was dialysed by equilibrium dialysis against 0.1 M-sodium phosphate buffer (pH 6) plus 0.5 M-NaCl (this step was necessary to prevent aggregation of the enzyme—see below). Chromatography of 50 ml portions of this material in 0.1 M-sodium phosphate buffer on Sephadex G-100 resulted in a single peak of activity which eluted with the front of the major protein peak. Fractions with high enzyme activity from the chromatography of five such portions were combined and concentrated to 20 ml by ultrafiltration, and Sephadex G-100 chromatography was repeated twice until the peak of enzyme activity corresponded to the elution profile of the protein (fraction V; Table 1). Fraction V enzyme was concentrated to 20 ml by ultrafiltration and loaded on to a CM-Sephadex C-50 column. Protein impurities emerged without retardation and the lipase was eluted with 0.275 M-NaCl as a single peak of activity corresponding to a minor protein peak. Fractions with constant specific activity (per mg protein) were designated purified fraction VI lipase. This material was either used directly for the characterization studies or desalted by ultrafiltration and lyophilized.

Hyaluronate lyase, acid phosphatase and protease activities of the lipase fractions

Propionibacterium acnes P-37 produces extracellular hyaluronate lyase, acid phosphatase and protease (Ingham *et al.*, 1980). The activities of these enzymes were assayed in the various fractions during the purification of lipase. A low protease activity was present in the culture supernatant during the exponential phase of the growth of the micro-organism in

Table 1. *Purification of P. acnes* lipase

	Fraction	Total protein (mg)	Volume (ml)	Total activity*	Specific activity†	Purification factor	Yield (%)
I.	Crude culture supernatant	146 250	7500	87.3	0.0006	—	—
II.	Amicon ultrafiltration (mol. wt > 10000)	17 500	250	81.6	0.0047	7.8	93
III.	Sephadex G-100 (i)	1620	600	50.3	0.031	51.7	58
IV.	Sephadex G-100 (ii)	334	152	33	0.099	165	38
V.	Sephadex G-100 (iii)	119	150	24.5	0.206	343	28
VI.	CM-Sephadex C-50	2.87	117	8.3	2.89	4817	9.5

* µmol oleic acid released min⁻¹.

† µmol oleic acid released min⁻¹ (mg protein)⁻¹.

batch culture. Protease was not detectable in the culture supernatant during the stationary phase, when the cultures were harvested, in fraction II or in fraction III lipase. Hyaluronate lyase and acid phosphatase activities were separated by Sephadex G-100 chromatography and cation-exchange chromatography. Neither enzyme was detectable in fraction VI lipase.

*Extent of purification and molecular weight of *P. acnes* lipase*

The extent of purification of the lipase was analysed by SDS-polyacrylamide gel electrophoresis. Fraction VI lipase contained a major protein component (R_F 0.36, mol. wt 41 190) together with two minor protein components (R_F 0.25 and 0.16, mol. wt 67 000 and 125 900), indicating that the lipase preparation was heterogeneous. The molecular weight of the lipase was estimated to be 46 770 by Sephadex G-100 chromatography and therefore the major protein band from SDS-polyacrylamide gel analysis most probably represented the lipase. This was confirmed by SDS-polyacrylamide gel electrophoresis of fractions of low lipase specific activity eluted from CM-Sephadex C-50 ahead of the main peak. The minor band with a molecular weight of 67 000 obtained with the high specific activity lipase pooled fractions was greatly increased. This indicated that the component of molecular weight 67 000 was a contaminant protein which was eluted from the ion-exchange column at a slightly lower concentration of salt but which overlapped with the peak of lipase activity. It was unlikely that a protein of molecular weight 125 900 was present in the lipase preparation after repeated gel filtration during the purification procedure. It is possible that this component represented protein which was not fully reduced by the mercaptoethanol.

*Characterization of *P. acnes* lipase*

Molecular weight forms of crude lipase. During preliminary studies it was observed that when crude Amicon concentrates of lipase were chromatographed on Sephadex G-200 without prior dialysis two molecular weight forms of lipase activity were eluted from the column. The first peak of activity eluted with the void volume (mol. wt >200 000) and the second eluted with the front of the major protein peak (mol. wt 40 000 to 50 000 region). The effect of ionic strength on the molecular forms of the lipase was investigated by gel filtration.

Crude culture supernatant (500 ml) was concentrated to 10 ml by ultrafiltration and the concentrate was divided into two 5 ml portions. Figure 1(a) presents the elution profile of the lipase when one portion of the concentrate was dialysed against 0.01 M-sodium phosphate buffer (pH 6) by equilibrium dialysis and eluted from Sephadex G-200 using the same buffer. Of the total lipase activity recovered, 60% was eluted with the void volume and 40% was eluted as the lower molecular weight form. When the second portion of the concentrate was dialysed against 0.1 M-sodium phosphate buffer (pH 6) plus 0.5 M-NaCl and eluted from Sephadex G-200 with 0.1 M-sodium phosphate buffer, only the lower molecular weight form was observed (Fig. 1b). These results suggested that the lipase eluted in the void volume (Fig. 1a) was an aggregate of the lower molecular weight form of lipase which formed when the ionic strength of the dialysis and elution buffers was low.

To test this hypothesis, the void volume fractions (Fig. 1a) were combined, concentrated by ultrafiltration, dialysed against 0.1 M-sodium phosphate buffer (pH 6) plus 0.5 M-NaCl, and rechromatographed on Sephadex G-200 with 0.1 M-sodium phosphate buffer as eluant. The greater part of the lipase (70%) was now converted to the lower molecular weight form (Fig. 1c). Furthermore, it was possible to convert the low molecular weight lipase (Fig. 1b) to the aggregated form by precipitating the enzyme with $(\text{NH}_4)_2\text{SO}_4$ at 60% saturation, dialysing the precipitate against 0.01 M-sodium phosphate buffer (pH 6) and eluting from Sephadex G-200 with the same buffer (Fig. 1d). Thus, the molecular forms of the lipase could be interconverted by altering the salt concentration of the dialysis and elution buffers.

It was concluded that the lipase eluting with the void volume from Sephadex G-200 observed in preliminary experiments was an aggregate of the lower molecular weight form which could be dissociated in the presence of a higher concentration of salt.

Fig. 1. Molecular weight forms of crude *P. acnes* lipase. (a) Profile of elution of 5 ml of Amicon-concentrated culture supernatant after dialysis against 0.01 M-sodium phosphate buffer (pH 6) and elution from Sephadex G-200 with 0.01 M buffer. (b) Profile of elution of 5 ml of Amicon-concentrated culture supernatant after dialysis against 0.1 M-sodium phosphate buffer (pH 6) plus 0.5 M-NaCl and elution from Sephadex G-200 with 0.1 M buffer. (c) Profile of elution of the void volume lipase (from a) after dialysis against 0.1 M-sodium phosphate buffer (pH 6) plus 0.5 M-NaCl and elution from Sephadex G-200 with 0.1 M buffer. (d) Profile of elution of the low molecular weight lipase (from b) after precipitation with $(\text{NH}_4)_2\text{SO}_4$ at 60% saturation, dialysis against 0.01 M-sodium phosphate buffer (pH 6) and elution from Sephadex G-200 with 0.01 M buffer. ●, Lipase activity; ---, protein concentration (A_{280}).

Effect of pH on lipase activity. The effect of pH on lipase activity was determined by incubating fraction VI lipase (1 ml) at various pH values between 6.0 and 8.0 (at intervals of 0.4 pH unit) with 4 ml buffer (0.1 M-MES for pH 6.0 and 6.4; 0.1 M-MOPS for pH 6.8, 7.2 and 7.6; 0.1 M-bicine for pH 8) and 1 ml triolein emulsion. The initial reaction velocity at each pH tested was determined in duplicate at 37 °C. The pH optimum for activity was 6.8. The enzyme had high activity at pH 6.0 and 6.4 (73% and 77% of the maximum). Above pH 6.8 the activity was lower, with 39%, 26% and 19% of the maximum activity at pH 7.2, 7.6 and 8.0, respectively.

Effect of pH on lipase stability. The effect of pH on the stability of the lipase was determined at 37 °C and 4 °C. Fraction VI lipase (1 ml) was incubated in 0.2 M-citrate/phosphate buffer (2 ml) at various pH values between 4.0 and 8.0 (at intervals of 0.5 pH unit) for either 1 h at 37 °C or 7 d at 4 °C. The lipase activity in 1 ml portions of each enzyme solution at each pH was determined before and after incubation. Analyses were carried out in duplicate. After 1 h at 37 °C, the lipase retained more than 75% activity between pH 5.0 and 6.0; less than 50% activity was retained below pH 5.0 or above pH 6.0. The lipase exhibited maximum stability at pH 5.5, retaining 90% activity. After 7 d at 4 °C, the enzyme had lost more than 45% activity at all the pH values tested. It was most stable at pH 5.5 and 6.0, when 54% and 50% activity, respectively, was retained. Lyophilized fraction VI lipase retained 78% activity at pH 6 for at least 3 months at -20 °C.

Effect of temperature on lipase stability. The effect of temperature on the stability of the lipase was determined by incubating portions (1 ml) of fraction VI lipase at various temperatures between 4 °C and 70 °C for 30 min at pH 6. After incubation the enzyme solutions were immersed in ice prior to determining the residual activities at 37 °C. The lipase

Fig. 2. Thin-layer chromatography of the reaction products of *P. acnes* lipase and pancreatic lipase with triolein as substrate. For conditions see text: (a) authentic standards; (b) time zero control; (c) to (f) samples of the reaction mixture at 9%, 15%, 25% and 48% hydrolysis of triolein by *P. acnes* lipase; (g) and (h) samples of the reaction mixture at 17% and 50% hydrolysis of triolein by pancreatic lipase.

retained 100, 98, 95, 90, 60, 42, 10 and 0% activity at 4, 15, 37, 40, 45, 50, 55 and 60 °C, respectively.

Positional specificity in the hydrolysis of triolein. The reaction products formed from the hydrolysis of triolein by *P. acnes* and pancreatic lipase were compared. Pancreatic lipase exhibits a positional specificity towards the 1- and 3-positions of triacylglycerols and this is demonstrated by an accumulation of 2-monoacylglycerol during the reaction. Detectable amounts of free glycerol are formed only later in the reaction (Desnuelle, 1972).

The results of thin-layer chromatography of the samples from the hydrolysis of triolein by *P. acnes* and pancreatic lipase, representing various stages in the hydrolysis of 0.03 M-triolein, are shown in Fig. 2. The degree of hydrolysis was determined from the quantitative estimation of the oleic acid released. *Propionibacterium acnes* lipase produced only oleic acid and 1,2-diolein as major products. Pancreatic lipase showed oleic acid, 1,2-diolein and 2-monoolein as the major products. No appreciable amounts of 1,3-diolein or 1-monoolein accumulated in either reaction mixture. Determination of free glycerol in samples of the reaction mixtures showed that *P. acnes* lipase released approximately 1 µmol free glycerol for every 3 µmol oleic acid. Pancreatic lipase released free glycerol at detectable levels only after greater than 28% hydrolysis of the triolein. It was concluded that *P. acnes* lipase did not exhibit a positional specificity for the 1-position of the oleic acid of the triolein molecule as there was no accumulation of 2-monoolein in the reaction mixture and free glycerol was produced throughout the reaction.

Hydrolysis of various lipid substrates. The relative rates of hydrolysis of various lipids by *P. acnes* lipase were determined by incubating enzyme (1 ml) with 2.5 ml of a substrate buffer emulsion [0.15 M lipid emulsified with 0.1 M-citrate/phosphate buffer (pH 6.5) and gum acacia (10%, w/v)]. The free fatty acid content of the reaction mixtures was determined

before and after 1 h incubation at 37 °C. Assays were carried out in triplicate. *Propionibacterium acnes* lipase hydrolysed trilaurin (0.12 µmol free fatty acid ml⁻¹ min⁻¹), triolein (0.07 µmol ml⁻¹ min⁻¹), trimyristin (0.03 µmol ml⁻¹ min⁻¹) and tripalmitin (0.015 µmol ml⁻¹ min⁻¹). The lipase did not release detectable free fatty acid from phosphatidylcholine or phosphatidylethanolamine. It was not possible to determine the K_m of the lipase for the various substrates: the assay of lipase activity is dependent on the enzyme acting at a lipid/water interface, and the available substrate concentration is not dependent on the amount of substrate present, but on the surface area of emulsion particles. To determine the K_m values for various substrates would involve estimating lipase activity with varied substrate surface areas.

DISCUSSION

The molecular weight of *P. acnes* lipase was estimated to be 41 190 by SDS-polyacrylamide gel electrophoresis, and 46 770 by Sephadex G-100 gel filtration.

Attempts to purify the fraction VI lipase further by introducing an additional gel filtration step resulted in an almost complete loss of enzyme activity. The highly purified lipase lost 45% activity when kept at 4 °C for 7 d at pH 5.5. However, the enzyme remained relatively stable when lyophilized, which suggested that the inactivation was a result of the enzyme being in dilute solution. The rapid inactivation of lipases in purified preparations is not unusual and has been reported for both microbial (Lawrence *et al.*, 1967; Kimura *et al.*, 1972) and mammalian lipases (Greten & Walter, 1973). Further investigations would be necessary to determine whether the addition of stabilizing agents would enable further purification of the enzyme. Crude preparations of *P. acnes* lipase contained an aggregated molecular form of the enzyme. Aggregated molecular species are exhibited by lipases from a variety of sources, including human pancreatic lipase (Kimura *et al.*, 1972) and many microbial lipases (Tomizuka *et al.*, 1966; Lawrence *et al.*, 1967; Vadehra, 1974; Ishihara *et al.*, 1975). The nature of the enzyme reaction catalysed by lipases involves the enzymes acting at the interface of an insoluble lipid with water (Rietsch *et al.*, 1977) and this necessitates at least part of the enzyme molecule being hydrophobic in nature. It has been suggested that native high molecular weight forms of microbial extracellular lipases are aggregates of lipase molecules with other lipophilic substances (Breuil & Kushner, 1974; Fulton *et al.*, 1974; Henderson & Hodgkiss, 1973). It is possible that the aggregated form of *P. acnes* lipase was a combination of the enzyme with lipid or other lipophilic substances. It would be necessary to isolate the aggregated enzyme and determine which substances were present to demonstrate whether this was the explanation. The property of the aggregated form of *P. acnes* lipase of dissociation with an increase in salt concentration is similar to that observed for the lipase produced by *Mucor javanicus* (Ishihara *et al.*, 1975).

It is of interest to compare the results of this study with those of earlier studies of the purification and properties of *P. granulosum* lipase (Hassing, 1971; Fulton *et al.*, 1974; Pablo *et al.*, 1974). None of these workers succeeded in purifying the lipase produced by *P. granulosum* to homogeneity. There is difficulty in comparing the activities of the purified enzymes used in the present and the previous studies, because different substrates and assay procedures were used. The lipase produced by *P. granulosum* was reported to be totally excluded from Sephadex G-200 and G-100 during the purification studies and a lower molecular weight species was not demonstrated by gel filtration techniques. However, Fulton *et al.* (1974) and Pablo *et al.* (1974) reported that the native high molecular weight form of *P. granulosum* lipase dissociated when subjected to SDS denaturation to a low molecular weight form (mol. wt 54 000). This molecular weight was higher than our estimate of 41 190 for *P. acnes* lipase, made using the same technique, and might indicate that different enzymes are produced by the two species. In addition, *P. granulosum* lipase has an optimum pH for activity in the range of 7.6 to 8.0 (Hassing, 1971; Fulton *et al.*, 1974; Pablo *et al.*, 1974), whereas *P. acnes* lipase is most active at pH 6.8. Freinkel & Shen (1969) reported a pH

optimum around 7.0 for the lipases produced by four strains of *P. acnes* using crude enzyme preparations, which is in agreement with the results of the present study. The lower pH optimum of the *P. acnes* lipase is better fitted for the pH of the skin surface and blackheads (pH range 5.0 to 6.4; Noble, 1968; Holland *et al.*, 1978), than the pH optimum of the *P. granulosum* enzyme. The *P. acnes* lipase also exhibits maximal stability in the pH range of the skin.

Because of the nature of the lipid environment which is the natural habitat of *P. acnes*, it has been proposed that *P. acnes* might synthesize and secrete extracellular lipase in order to obtain free glycerol from triacylglycerols which could then be utilized as a carbon and energy source (Puhvel & Reisner, 1970; Rebello & Hawk, 1978). However, glycerol is a poor carbon and energy source, and the possibility should be considered that *P. acnes* utilizes free fatty acids released from sebum triacylglycerols either for catabolism or as precursors for the unusual fatty acids found in these bacteria. Oleic acid stimulates the growth of *P. acnes* in vitro (Ferguson & Cummins, 1978) and it has been suggested that oleic acid may substitute for biotin (Nieman, 1954), which is also growth stimulatory for *P. acnes* (Ferguson & Cummins, 1978; Holland *et al.*, 1979). It is conceivable that oleic acid, released from triolein in the pilosebaceous duct by the action of *P. acnes* lipase, might be important for the growth of *P. acnes* in vivo, especially if biotin were not available or were in limiting concentrations.

This work was generously supported by grants from the A. H. Robins Co., and the Leeds Area Health Authority (Teaching) Special Trustees.

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