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# Purification and Partial Characterization of a Bacterial Phospholipid: Cholesterol Acyltransferase*

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**A glycerophospholipid:cholesterol acyltransferase has been purified to near homogeneity from cell-free culture supernatants of *Aeromonas salmonicida*. The characteristics of the enzyme distinguish it from bacterial phospholipases; however, it shares several properties with the lecithin:cholesterol acyltransferase of mammalian plasma. Thus, the enzyme exhibits 2-positional specificity as an acyltransferase and it will act as a phospholipase $A_2$ in the absence of cholesterol. Furthermore, it has no divalent cation requirement and it is stimulated both by albumin and by human apolipoprotein A-I. Unlike the mammalian acyltransferase, however, the bacterial enzyme is not specific for phosphatidylcholine and in addition it can use human erythrocyte membranes as substrates. Similar to *Naja naja* phospholipase $A_2$, it acts asymmetrically on intact erythrocytes.**

Lecithin:cholesterol acyltransferase (EC 2.3.1.43) is an important component of the plasma lipoprotein lipid transport system. The enzyme catalyzes the esterification of high density lipoprotein cholesterol with fatty acids derived from the 2-position of phosphatidylcholine (1, 2). In spite of its importance in the maintenance of normal lipoprotein structures, little is known of the mechanism of acyl group transfer, although recent proposals suggest it may function via formation of an acyl-enzyme intermediate with the 2-acyl group of PC[1] (3). The enzyme's particular substrate requirements, the need for apolipoprotein A-I (4), and the lack of divalent cation dependence are some of its distinguishing features.

Bacteria in the family Vibrionaceae release a glycerophospholipid:cholesterol acyltransferase which is active against both liposome substrates and human erythrocyte membranes (5, 6). Reaction products with PC/cholesterol liposomes are cholesteryl ester, fatty acid, and GPC, indicating that crude preparations of the enzyme also contain lysophospholipase (5). Here we describe the purification of the acyltransferase and show that a single protein is responsible for acyltransferase, phospholipase, and lysophospholipase activities. In addition we show that a number of properties of this enzyme are similar to those of mammalian lecithin:cholesterol acyltransferase and quite different from the properties of other bacterial phospholipases thus far characterized.

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[1] The abbreviations used are: PC, phosphatidylcholine (lecithin); GPC, glycerophosphorylcholine; LPC, lysophosphatidylcholine; Apo-A-I, apolipoprotein A-I.

## EXPERIMENTAL PROCEDURES

*Materials*—Egg lecithin, lysolecithin, cholesterol, and bovine serum albumin (essentially fatty acid free) were obtained from Sigma. [4-$^{14}$C]Cholesterol (specific activity, 58 mCi/mmol), [1$\alpha$,2$\alpha$($n$)-$^3$H]cholesterol (40 mCi/mmol), and [1-$^{14}$C]oleic acid (57 mCi/mmol) were purchased from Amersham Corporation. 2-[1-$^{14}$C]Oleoyl-phosphatidylcholine was prepared according to the procedure of Pugh and Kates (7). Broken erythrocyte membranes were isolated as previously described (8). Human apolipoprotein A-I was a generous gift of Dr. J. Frohlich, Shaughnessy Hospital, Vancouver, Canada.

*Liposome Preparation*—Small unilamellar vesicles of lecithin or lecithin-cholesterol dispersions in 0.16 M KCl, 20 mM Tris-HCl, pH 7.4, were prepared by sonication followed by centrifugation at 110,000 × $g$ for 1 h to remove undispersed lipid (9). The actual concentrations of lecithin and cholesterol in the liposomes were determined immediately prior to use.

*Lipid Extraction and Isolation*—Lipids were extracted from liposome suspensions and from red cell membranes by the procedure of Folch *et al.* (10) and from intact erythrocytes by the method of Turner and Rouser (11). Neutral lipids were separated by thin layer chromatography in petroleum ether/ether/acetic acid (90:10:1), phospholipids by chromatography in $CHCl_3/CH_3OH/CH_3COOH/H_2O$ (25:15:4:2), or by two-dimensional thin layer chromatography (11). In general, plastic-backed plates (Polygram Sil G, Brinkmann) were used with radioactive lipids and glass-backed Silica Gel H (Merck) plates for subsequent chemical determinations. Spots were located by brief exposure to iodine vapor and either scraped from glass plates and eluted as described by Arvidson (12) or cut from plastic plates and counted in PCS (Amersham Corporation).

*Isolation of Enzyme-associated Membrane Fragments*—*Aeromonas salmonicida* ex-ATCC 14174 was obtained from the American Type Culture Collection, Rockville, MD. Cells were grown for 24 h as described previously and membrane fragments were isolated by the following modification of our earlier procedure (13). Ammonium sulfate was slowly added to cell-free culture supernatant to 85% of saturation at 0 °C. The resulting precipitate was isolated by centrifugation and resuspended in 20 mM Tris-HCl, pH 7.4. The suspension was centrifuged at 10,000 × $g$ for 10 min and the clear supernatant was centrifuged at 270,000 × $g$ for 3 h. The resulting pellet was washed by means of a second centrifugation using the same conditions. The final pellet was resuspended in a small volume of 20 mM Tris-HCl, pH 7.4.

*Enzyme Assays*—The standard acyltransferase assay system contained small unilamellar vesicles (0.25 μmol of cholesterol and approximately 0.25 μmol of lecithin) and 1.4% (w/v) bovine serum albumin (essentially fatty acid free) in a final volume of 0.25 ml of 0.16 M KCl, 20 mM Tris-HCl, pH 7.4. The reaction was stopped after 5 min at 37 °C by addition of 20 volumes of $CHCl_3/CH_3OH$ (2:1, v/v). Following extraction, samples of the lower phase were taken for lipid separation and measurement and samples of the upper phase for glycerophosphorylcholine determination. Phospholipase was assayed in a similar way except that cholesterol was omitted from the reaction mixture.

*Other Procedures*—Protein was quantitated either by the method of Markwell *et al.* (14) or by the procedure of Peterson (15). Phosphorus was measured as described by Bartlett (16) following sample

TABLE I

*Co-purification of acyltransferase and phospholipase*

Enzyme activities were determined using the standard assay conditions in the text. Values cited are from a purification started with 3.6 liters of culture supernatant.

| | Total protein | Phospholipase | | | Acyltransferase | | | |
|---|---|---|---|---|---|---|---|---|
| | | Total enzyme | Specific activity | Purification | Total enzyme | Specific activity | Purification | Recovery |
| | *mg* | *μmol min*$^{-1}$ | *μmol min*$^{-1}$ *mg*$^{-1}$ | *-fold* | *μmol min*$^{-1}$ | *μmol min*$^{-1}$ *mg*$^{-1}$ | *-fold* | % |
| Culture supernatant | 1404 | 1387 | 0.99 | 1 | 1186 | 0.85 | 1 | 100 |
| Membrane vesicles | 10.54 | 481 | 45.6 | 46 | 490 | 46.5 | 55 | 41.3 |
| Tween extract | 6.5 | 134 | 20.6 | 20.6 | 142 | 21.8 | 25.6 | 12 |
| G-100 peak | 4.44 | 356 | 80.7 | 81.1 | 338 | 76.1 | 90.0 | 28.5 |
| DEAE peak | 1.79 | 245 | 137 | 139 | 245 | 137 | 162.0 | 21.0 |

digestion with perchloric acid. Cholesterol and cholesteryl ester were determined following the procedure of Huang *et al.* (17). Sodium dodecyl sulfate-polyacrylamide electrophoresis was carried out in 12% gels according to Neville (18). Amino acid analysis was performed using a Beckman Model 118BL Autoanalyzer following sample digestion for 22 h with constantly boiling HCl.

## RESULTS

*Enzyme Isolation*—An outline of a representative purification is shown in Table I. Although the acyltransferase is a soluble protein, it is recovered bound to outer membrane fragments following their precipitation from cell-free culture supernatant with ammonium sulfate (13). Because there are a small number of proteins in the outer membrane (13), this results in a very large increase in specific activity (Table I). The acyltransferase was selectively removed from the fragments by extraction with 10 volumes of 0.1% (v/v) Tween 20 in 20 mM Tris-HCl, pH 7.4, for 45 min at room temperature. This was followed by centrifugation at 47,000 rpm for 3 h at 20 °C in a Beckman L5-65 ultracentrifuge using a Ti-50.2 rotor. The enzyme was concentrated from the supernatant, and detergent and lipid were removed by the addition of 2 volumes of acetone at −20 °C. The precipitate was isolated by centrifugation and washed with 1 volume of acetone followed by 1 volume of ether at −20 °C. The pellet was dried under reduced pressure and resuspended in a small volume of 1% (w/v) sodium deoxycholate, 0.2 M NaCl, 5 mM EDTA, 10 mM Tris-HCl, pH 8.1. The resulting solution was applied to a Sephadex G-100 column. Acyltransferase was recovered as a single peak (Fig. 1), effectively freed from contaminating lipopolysaccharide which was eluted earlier. Less than 0.02% of original membrane phosphorus was recovered in the active fractions. Peak fractions were pooled, diluted 1:1 with $H_2O$, and applied to a DEAE-Sepharose column which was eluted with a 300-ml linear gradient of 0.1-1.0 M NaCl (Fig. 2). The active fractions isolated between 0.5 and 0.55 M NaCl were either used directly or dialyzed and freeze-dried. The enzyme was stable at room temperature and was unaffected by repeated freezing and thawing.

The purified protein showed a single major staining band by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Fig. 3) accounting for more than 97% of total chromagenicity when stained gels were scanned photometrically at 590 nm. The front of the band was diffuse when samples were boiled for 3 min and much sharper in unboiled samples. By sodium dodecyl sulfate gel electrophoresis, the apparent molecular weight of the isolated enzyme was 23,600 ± 300 (mean of 5 determinations ± S.D.). The amino acid composition was not unusual (Table II) and the preparation contained no detectable phosphorus. Production of GPC was directly proportional to enzyme concentration up to at least 8 μg of protein ml$^{-1}$ (Fig. 4). Cholesteryl ester production was not linear, however, even at low enzyme levels.

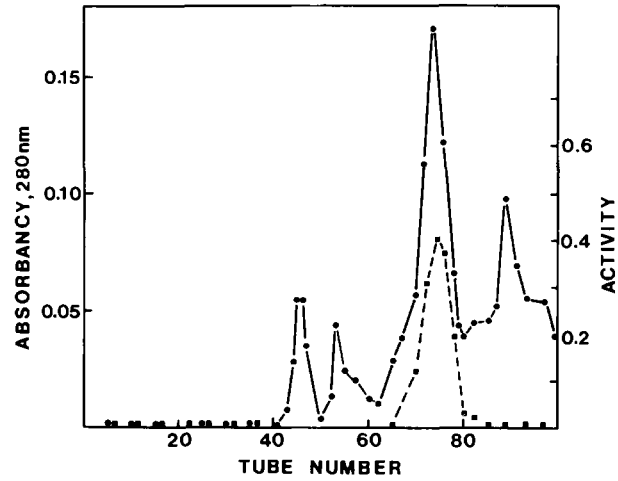


FIG. 1. **Sephadex G-100 gel filtration of the acetone-ether-extracted Tween 20 fraction.** Ten ml of the extract (6.5 mg of protein), solubilized as described in the text, were applied to a column (2.6 × 90 cm) equilibrated in 0.25% sodium deoxycholate, 0.2 M NaCl, 5 mM EDTA, 10 mM Tris-HCl, pH 8.1, and eluted with the same buffer at 9.0 ml/h. Fractions were 4.0 ml. ●, absorbancy; ■, enzyme activity in arbitrary units.

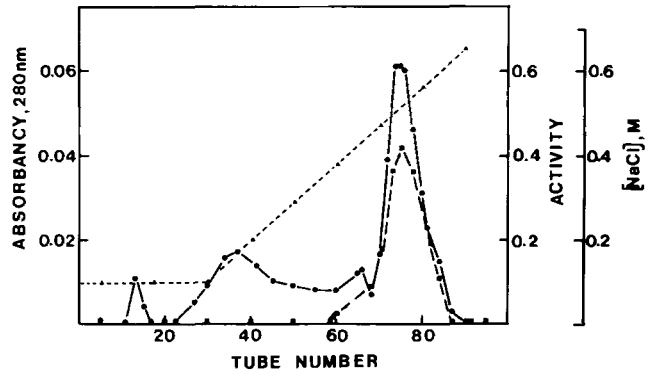


FIG. 2. **DEAE column chromatography of the Sephadex G-100 fraction.** Active fractions from the G-100 column were pooled, diluted 1:1 with $H_2O$, and applied to a column (1.6 × 20 cm) of DEAE-Sepharose CL-6B equilibrated in 20 mM Tris-HCl, pH 7.4, and eluted with a 300-ml linear gradient of 0.1-1.0 M NaCl in the same buffer. Flow rate was 15 ml/h and sample volume was 4.0 ml. ●, absorbancy; ■, enzyme activity; ▲, NaCl concentration.

*Enzyme Activities Associated with the Purified Protein*—Phospholipase and acyltransferase activities co-purified throughout the isolation procedure (Table I). In addition, the lysophospholipase, apparently responsible for GPC production, could be measured together with the phospholipase and acyltransferase in polyacrylamide gel fractions corresponding to the major protein band (Fig. 5).

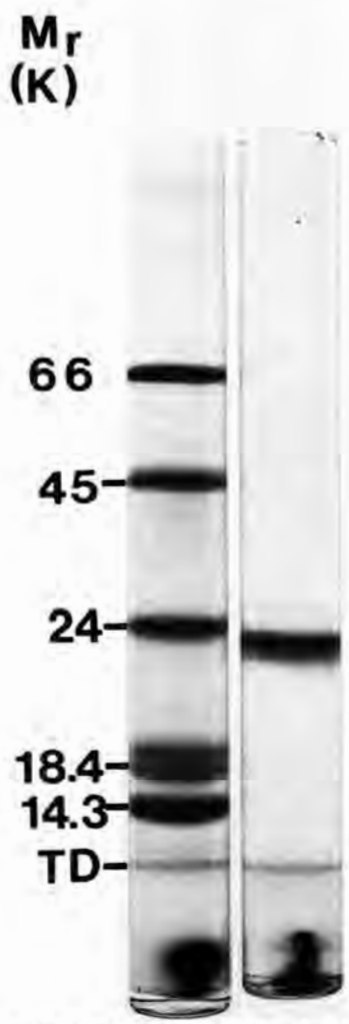


FIG. 3. **Sodium dodecyl sulfate-polyacrylamide electrophoresis of the purified acyltransferase.** Molecular weight markers were bovine albumin, egg albumin, trypsinogen, β-lactoglobulin, and lysozyme. *TD*, tracking dye. 15 μg of protein were applied.

TABLE II

*Amino acid composition of the purified acyltransferase*

| Amino acid | mol/$10^5$ g protein[a] |
|---|---|
| Lys | 32.2 |
| His | 16.8 |
| Arg | 19.6 |
| Asp | 87.2 |
| Thr | 34.4 |
| Ser | 75.2 |
| Glu | 78.9 |
| Pro | 43.4 |
| Gly | 114.6 |
| Ala | 95.7 |
| Val | 76.0 |
| Met | 12.7 |
| Ile | 22.1 |
| Leu | 65.6 |
| Tyr | 29.3 |
| Phe | 20.6 |

[a] Values are means of two independent determinations. Cys/2 and Trp were not measured.

The rate of production of cholesterol ester by acyl transfer in PC-cholesterol liposomes was very similar to production of fatty acid by hydrolysis in PC liposomes (Fig. 6). When 2-[1-$^{14}$C]oleoyl-PC was used in either liposome preparation, virtually all of the LPC formed was unlabeled, indicating acyl transfer and hydrolysis were at the 2-position of PC (Fig. 6). The rate of formation of GPC, due to lysophospholipase activity, was somewhat higher in PC liposomes.

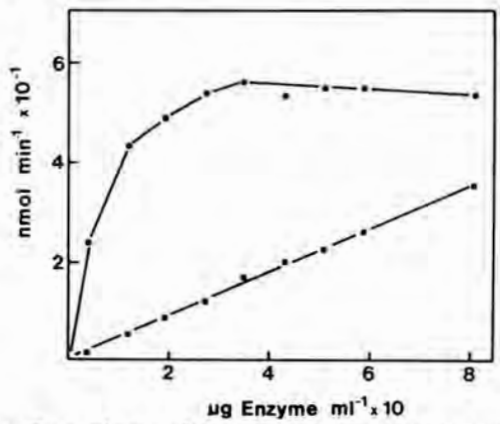


FIG. 4. **Dependence of reaction rate on enzyme concentration.** PC/cholesterol liposomes (1.0:1.0, mol/mol) containing [$^3$H] cholesterol were used in the basic assay system described in the text. ●, cholesteryl ester; ■, GPC.

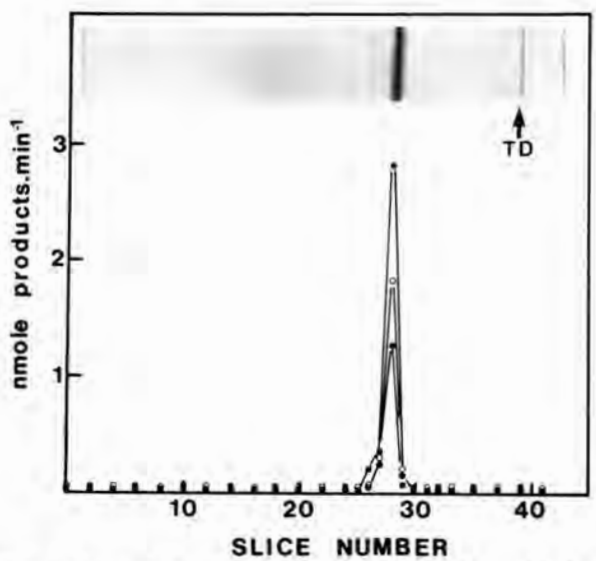


FIG. 5. **Enzymic activities associated with the purified protein.** Glycerophospholipid:cholesterol acyltransferase (approximately 2 μg of protein) was electrophoresed in a 12% SDS-polyacrylamide slab gel as described in the text except that the sample was not boiled. One portion of the gel was stained with Coomassie blue. The adjacent portion was sliced into 2-mm sections which were eluted with 30 mM Tris, pH 7.4. Samples were taken to measure fatty acid (●) release from [$^{14}$C]-PC liposomes and to measure cholesteryl ester (○) and GPC release (■) from PC-[$^{14}$C]cholesterol liposomes.

In a separate experiment, the preference of the enzyme for water or cholesterol was measured using PC-cholesterol liposomes. The results indicate that at early times (when little LPC and fatty acid have accumulated), most of the 2-position fatty acid of PC is transferred to cholesterol, but that at later times more of the fatty acid at this position is removed by hydrolysis (Table III). The results also show that all of the cholesteryl ester formed is derived by transfer from the 2-position of PC.

Because acyltransferase and phospholipase activities were 2-position specific and because no 2-acyl LPC accumulates, a sequential reaction mechanism was indicated (Fig. 7).

In agreement with this proposal, 1-acyl LPC would not act as a donor for cholesteryl ester formation. However, after a

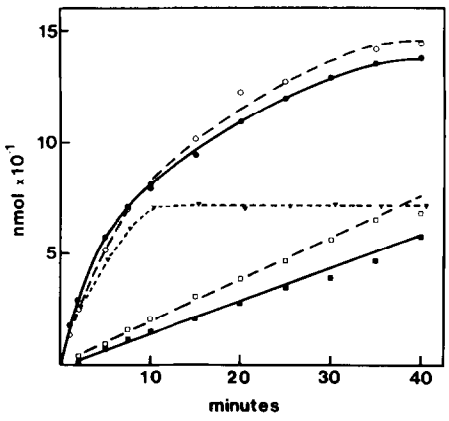


FIG. 6. **Comparison of PC and PC-cholesterol liposomes as enzyme substrates.** The reactions were carried out using the basic assay system with either PC or PC/cholesterol (1:1, mol/mol) liposomes containing 2-[1-$^{14}$C]oleoyl-PC or [$^{3}$H]cholesterol and 0.25 μg of enzyme. ●, [$^{3}$H]cholesteryl ester, and ■, GPC produced using PC-cholesterol liposomes; ○, $^{14}$C-labeled fatty acid, and □, GPC produced using PC liposomes; ▼, total LPC produced using PC liposomes. Virtually no [$^{14}$C]-LPC was produced under these conditions. Total LPC production was not measured using PC-cholesterol liposomes.

TABLE III

*Relative hydrolytic and acyltransferase activity in cholesterol-PC liposomes*

The reaction mixture contained 0.5 μmol of 2-[1-$^{14}$C]oleoyl-PC and of cholesterol in 0.5 ml as described in the text. Both the distribution of radioactivity in the three lipid fractions and the total amount of cholesteryl ester produced were measured after the indicated incubation times.

| Time | Total recovered label | | | Total CE formed | CE from 2-position[b] |
|---|---|---|---|---|---|
| | CE[a] | FA | LPC | | |
| *min* | | % | | *μmol* | % |
| 2.5 | 81.5 | 18.5 | 0 | 0.05 | 97 |
| 5.0 | 72.5 | 21.7 | 5.8 | 0.094 | 100 |
| 30 | 60.0 | 32.5 | 7.5 | 0.220 | 99 |

[a] CE, cholesteryl ester; FA, fatty acid.
[b] Calculated by comparing total CE formation with labeled CE.

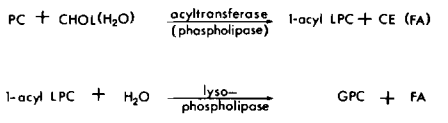


FIG. 7. **A proposed sequential reaction mechanism for the complete deacylation of PC by the bacterial enzyme.** *CHOL*, cholesterol; *CE*, cholesteryl ester; *FA*, fatty acid.

short delay, the rate of production of GPC was virtually the same using PC-cholesterol as it was using 1-acyl LPC-cholesterol liposomes (Fig. 8).

*Cofactor Requirements and pH Dependence*—Neither calcium nor magnesium was required for any of the enzyme activities associated with the protein. Addition of 5 mM $Ca^{2+}$, $Mg^{2+}$, or EDTA to the incubation mixture had no significant effect on reaction rates. Activity was independent of pH over the range 6.0–8.9 and was not influenced by sulfhydryl-reactive agents (0.75 mM dithiobis[2-nitrobenzoic acid], 0.1 mM $Ag^{+}$, 1 mM mercaptoethanol, 1 mM dithiothreitol).

*Influence of Liposome Composition*—Acyltransferase activity continued to increase with increasing proportions of cholesterol in the liposomes (Fig. 9) and was highest in liposomes containing equimolar PC and cholesterol. The addition of apolipoprotein A-I increased acyltransferase activity up to 3-fold (Fig. 10) in a manner similar to that reported for human

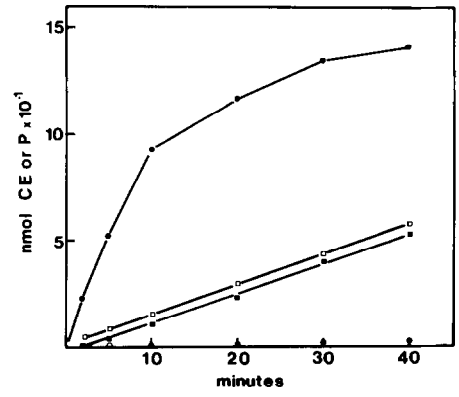


FIG. 8. **Comparison of PC-cholesterol and LPC-cholesterol liposomes as enzyme substrates.** The reactions were carried out using the basic assay system described in the text with PC/cholesterol (1.0:1.0) or 1-acyl LPC/cholesterol (1.0:1.0) liposomes containing [$^{3}$H] cholesterol. ●, cholesterol ester (*CE*), and ■, GPC production using PC-cholesterol liposomes; ○, CE, and □, GPC production using the LPC-cholesterol liposomes.

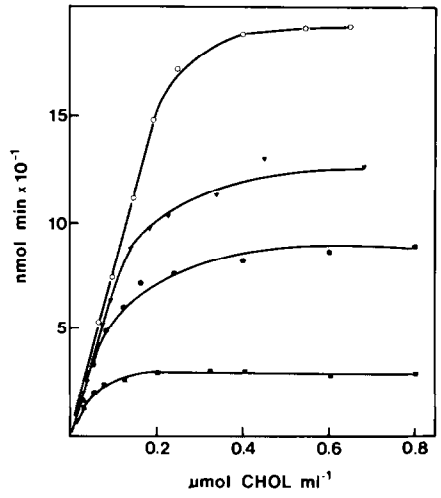


FIG. 9. **Effect of liposome concentration and composition on acyltransferase activity.** Four different liposome preparations, each containing [$^{3}$H]cholesterol (*CHOL*), were used in the basic assay system with 0.25 μg of enzyme. Cholesterol concentration was varied and cholesteryl ester production measured. ○, 1.0:1, PC/cholesterol; ▼, 1.6:1, PC/cholesterol; ●, 2.5:1, PC/cholesterol; ■, 4.6:1, PC/cholesterol.

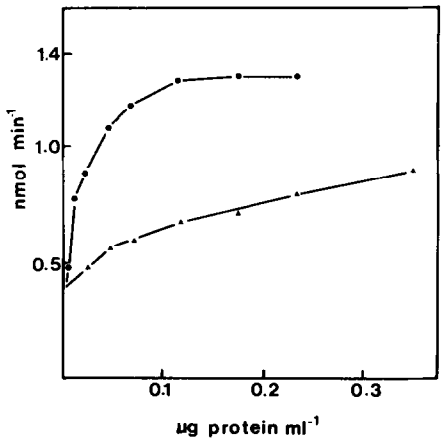


FIG. 10. **Acyltransferase activity as a function of medium albumin and apolipoprotein A-I.** The indicated concentrations of either apolipoprotein A-I or albumin were added to 1.1:1 PC/cholesterol liposomes (0.012 μmol of cholesterol) in 20 mM Tris-HCl, 0.16 M KCl in a total of 0.2 ml, pH 7.4.

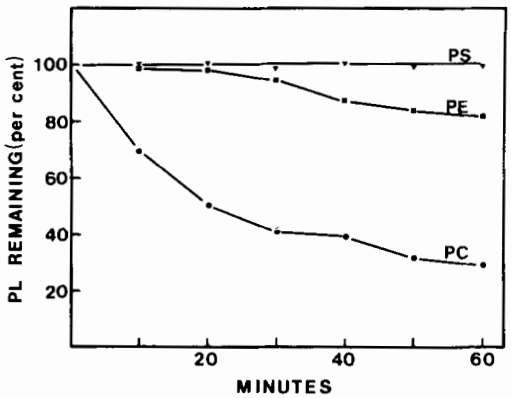


FIG. 11. **Enzymatic breakdown of intact erythrocyte phospholipids.** 50% suspensions of washed human erythrocytes in phosphate-buffered saline were incubated with the enzyme for the indicated times and extracted as described in the text. At 60 min, hemolysis was no higher in treated erythrocytes than in controls (less than 0.5% of total cells). More than 60% of the PC and phosphatidylserine and 70% of the phosphatidylethanolamine were broken down when similar quantities of hemolyzed cells were treated under the same conditions. *PL*, phospholipids.

lecithin:cholesterol acyltransferase (19). The addition of albumin also increased activity, but less dramatically and with no apparent optimum.

*Erythrocyte Membranes as Substrates*—We had shown earlier that all of the glycerophospholipids in the erythrocyte membrane would serve as substrates for the acyltransferase (5). When intact cells were used as substrates, 70% of the PC, 20% of the phosphatidylethanolamine, and none of the phosphatidylserine were degraded by the enzyme (Fig. 11), indicating that it does not penetrate the membrane bilayer, but that like *Naja naja* phospholipase $A_2$ (20), it acts only on one side of the intact membrane.

## DISCUSSION

The protein described in this communication is of unusual interest for several reasons. It exhibits three enzymic activities, it appears unique among bacterial phospholipases, and it shares a number of the characteristic features of mammalian lecithin:cholesterol acyltransferase. As well, the protein may be useful as a probe of cholesterol-phospholipid interactions in membranes.

Phospholipase, acyltransferase, and lysophospholipase activities were inseparable throughout the purification procedure (Table I and Fig. 5). In addition, selective binding of the acyltransferase to membrane vesicles in high salt resulted in parallel binding of the other two activities. Furthermore, acyltransferase mutants derived from *A. salmonicida* invariably also did not secrete the phospholipase or lysophospholipase (data not shown).

Unlike the 1-position specificity of nearly all bacterial phospholipases so far characterized, the enzyme exhibits 2-position specificity, both as an acyltransferase and as a phospholipase. The only microbial phospholipases capable of 2-position hydrolysis which have been isolated are the detergent-resistant $A_1$ of *Escherichia coli* which has been reported to hydrolyze either position (21) and the phospholipase B of *Penicillium notatum* (22). This latter enzyme, like the *A. salmonicida* protein, is a 2-position-specific phospholipase and a nonspecific lysophospholipase. In addition, it has no cation requirement; however, it is a glycoprotein with $M_r$ = 110,000 (23).

Virtually all of the phospholipases $A_2$ which have been purified from animal sources require divalent cations for activity, have $M_r < 20{,}000$ and do not act as lysophospholipases. It is generally agreed that a serine residue is not involved in the action of snake venom and other phospholipases $A_2$ (24) and that no detectable acyl-enzyme intermediate is found (25). These enzymes are therefore unable to carry out an acyl transfer. Pancreatic lipase is capable of using alcohols as acyl acceptors (26) and in addition, the phospholipase $A_1$ of rat liver, also called monoglyceride acyltransferase, will transacylate several alcohols including acylglycerol, but not including cholesterol (27). To our knowledge, the only phospholipase $A_2$ so far characterized which will also act as a cholesterol acyltransferase is mammalian lecithin:cholesterol acyltransferase. The protein described in this report has a number of other features in common with this enzyme. No divalent cation is required for activity and the enzyme is activated by both albumin and by Apo-A-I. For both enzymes, albumin presumably activates by complexing lysolecithin (28) and, in the case of the bacterial enzyme, fatty acid, whereas Apo-A-I appears to interact selectively with the surface of the liposome substrates (29). Clearly, the effect of Apo-A-I is somewhat different with lecithin:cholesterol acyltransferase which has an absolute requirement for this activator. However, it is interesting to note that similar Apo-A-I/cholesterol ratios were optimal for each enzyme. Both proteins act as 2-position-specific phospholipases in the absence of cholesterol and as 2-position-specific acyltransferases when cholesterol is included in the liposome substrates (30). The production of cholesteryl ester by lecithin:cholesterol acyltransferase has been reported to be maximal at cholesterol/lecithin ratios of 1:3 (31), 1:4 (28), 1:6 (32), or 1:7 (30) in the presence of Apo-A-I, whereas we observed highest bacterial acyltransferase activity with 1:1 liposomes. The bacterial enzyme assays were carried out without Apo-A-I, however, and it seems likely that this protein as well as nonionic detergents could profoundly affect the cholesterol/lecithin ratios which are optimal for the enzyme. Even in the absence of Apo-A-I, the purified acyltransferase had a much higher specific activity than lecithin:cholesterol acyltransferase (3, 29, 30). The bacterial enzyme is also a lysophospholipase whereas lecithin:cholesterol acyltransferase has been reported to carry out acyl transfer to lysolecithin by a mechanism requiring low density lipoprotein (3).

The action, if any, of lecithin:cholesterol acyltransferase on erythrocyte membranes has not been documented. The bacterial protein acts asymmetrically on human erythrocytes, thus providing data on the availability of phospholipids which is very similar to that obtained using several other procedures (33). Our preliminary evidence suggests that virtually all of the 2-position fatty acid in erythrocyte membrane phospholipids is transferred to cholesterol rather than released. The enzyme thus has been obvious potential applications as a probe of lipid interactions and asymmetry in plasma membranes.

## REFERENCES

1. Glomset, J. A., Parker, F., Tjaden, M., and Williams, R. H. (1962) *Biochim. Biophys. Acta* **58,** 398–406
2. Glomset, J. A. (1962) *Biochim. Biophys. Acta* **65,** 128–135
3. Subbaiah, P. V., Albers, J. J., Chen, C. H., and Bagdade, J. D. (1980) *J. Biol. Chem.* **255,** 9275–9280
4. Fielding, C. J., Shore, V. G., and Fielding, P. E. (1972) *Biochem. Biophys. Res. Commun.* **46,** 1493–1498
5. MacIntyre, S., and Buckley, J. T. (1978) *J. Bacteriol.* **135,** 402–407
6. MacIntyre, S., Trust, T. J., and Buckley, J. T. (1979) *J. Bacteriol.* **139,** 132–136
7. Pugh, E. L., and Kates, M. (1975) *Biochim. Biophys. Acta* **380,** 442–453
8. Buckley, J. T., and Hawthorne, J. N. (1972) *J. Biol. Chem.* **247,** 7218–7223

9. Barenholz, Y., Gibbes, D., Litman, B. J., Goll, J., Thompson, T. E., and Carlson, F. D. (1977) *Biochemistry* **16,** 2806–2810
10. Folch, J., Lees, M., and Sloane Stanley, G. H. (1957) *J. Biol. Chem.* **226,** 497–509
11. Turner, J. D., and Rouser, G. (1970) *Anal. Biochem.* **38,** 423–436
12. Arvidson, G. A. E. (1968) *Eur. J. Biochem.* **4,** 478–486
13. MacIntyre, S., Trust, T. L., and Buckley, J. T. (1980) *Can. J. Biochem.* **58,** 1018–1025
14. Markwell, M. A. K., Haas, S. M., Bieber, L. L., and Tolbert, N. E. (1978) *Anal. Biochem.* **87,** 706–210
15. Peterson, G. L. (1977) *Anal. Biochem.* **83,** 346–356
16. Bartlett, G. R. (1959) *J. Biol. Chem.* **234,** 466–468, 469–471
17. Huang, T. C., Chen, C. P., Wefler, V., and Raferty, A. (1961) *Anal. Chem.* **33,** 1405–1411
18. Neville, D. M. (1971) *J. Biol. Chem.* **246,** 5378–5334
19. Furukawa, Y., and Nishida, T. (1979) *J. Biol. Chem.* **254,** 7213–7219
20. Etemadi, A. H. (1980) *Biochim. Biophys. Acta* **604,** 423–475
21. Nishijima, M., Nakaike, S. Tamori, Y., and Nojima, S. (1977) *Eur. J. Biochem.* **73,** 115–124
22. Van den Bosch, H. (1980) *Biochim. Biophys. Acta* **604,** 191–246
23. Kawasaki, N., Sugatani, J., and Saito, K. (1975) *J. Biochem. (Tokyo)* **77,** 1233–1244
24. Heinrikson, R. L., Kreuger, E. T., and Kein, P. S. (1977) *J. Biol. Chem.* **252,** 4913–4921
25. Wells, M. A. (1971) *Biochim. Biophys. Acta* **248,** 80–86
26. Brockeroff, H., and Jensen, R. G. (1974) *Lipolytic Enzymes,* pp. 197–265, Academic Press, New York
27. Waite, M., and Sisson, P. (1974) *J. Biol. Chem.* **249,** 6401–6405
28. Fielding, C. J., Shore, V. G., and Fielding, P. F. (1972) *Biochim. Biophys. Acta* **270,** 513–518
29. Chung, J., Albano, D. A., Fless, G. M., and Scanu, A. M. (1979) *J. Biol. Chem.* **254,** 7456–7464
30. Aron, L., Jones, S., and Fielding, C. J. (1978) *J. Biol. Chem.* **253,** 7220–7226
31. Nakagawa, M., and Nichida, T. (1973) *Biochem. Biophys. Acta* **296,** 577–585
32. Nichols, A. V., and Gong, B. L. (1971) *Biochim. Biophys. Acta* **231,** 175–184
33. Op den Kamp, J. A. F. (1979) *Annu. Rev. Biochem.* **48,** 47–71