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Application of the Phase Partition Method to a Hydrophobic Membrane Protein, Phospholipase A1 from *Escherichia coli*†

Per-Åke Albertsson‡

ABSTRACT: Phase partition has been applied to the purification of hydrophobic membrane proteins. Phase systems of up to four liquid phases contained various polymers and detergents in aqueous solution. The polymers dextran, Ficoll, poly(ethylene glycol), and poly(propylene glycol) produced phases with distinctive hydrophobic and hydrophilic characteristics. Partitions of the membrane-bound phospholipase A1 of *Escherichia coli* brought about a several-hundredfold purification of the enzyme and a separation from phospholipid and added detergents. The added polymers are also removed from the enzyme.

Isolation of pure membrane proteins is essential for a study of their properties and an understanding of their role in the intact membrane. The hydrophobic nature of membrane proteins presents special problems for conventional protein separation methods. Hydrophobic proteins form aggregates which can be broken only by drastic treatment with detergents, organic solvents, or extremes of pH. Membrane proteins also show a strong affinity for lipids.

We undertook to see whether phase partition could be used to separate hydrophobic proteins. With this technique, a substance may partition between two or several phases composed of different polymers dissolved in water. Previously, this technique has been applied only to hydrophilic substances such as the water-soluble proteins, nucleic acids, viruses, and cell organelles. The method should also be applicable to hydrophobic proteins, provided these can be solubilized and polymers with varying hydrophobicity are used as phase formers.

In order to make the phase partition technique generally applicable to hydrophobic proteins we sought first to apply it to phospholipase A1 of *Escherichia coli* (Scandella and Kornberg, 1971), an enzyme firmly bound to the outer membrane of *E. coli*. It is a stable enzyme with well-defined properties and is easily assayed.

We have found that polymer phase systems together with nonionic detergents were effective in the purification of phospholipase A1 several-hundredfold. It is hoped that the procedure will be applicable to other hydrophobic enzymes.

Experimental Procedures and Results

Materials and Methods. Dextran (10% moisture assumed) and Ficoll were obtained from Pharmacia Fine Chemicals,

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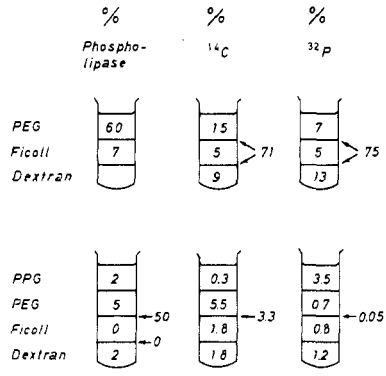

FIGURE 1: Distribution of phospholipase A1 activity and ^{14}C and ^{32}P counts of lysates from *E. coli* labeled with [^{14}C]leucine and [^{32}P]phosphate, respectively. The lysates were first partitioned in the three-phase system, upper row. The two upper phases (Ficoll and PEG phases) were then withdrawn and combined with a fresh dextran phase and a PPG phase to give the four-phase system (lower row). All figures are per cent of total input. For phase system composition see text and Figure 2.

Piskataway, N. J. Poly(ethylene glycol)[1] (PEG) 6000 and poly(propylene glycol) (PPG) 2025 Niax were from Union Carbide, New York, N. Y. Trimethylaminopoly(ethylene glycol) (TMA-PEG) and poly(ethylene glycol)sulfonate (S-PEG) were gifts from G. Johansson, Department of Biochemistry, University of Umeå, Umeå, Sweden. Triton X-100 was from Emulsion Engineering, Inc., Elk Grove Village, Ill., Ammonyx LO from Onyx Chemical Co., Jersey City, N. J., and sodium dodecyl sulfate from Matheson Coleman and Bell and recrystallized from ethanol.

The phospholipase assay was performed as described by Scandella and Kornberg (1971). The assay mixture contained 0.025 M Tris-HCl (pH 8.4), 0.05 M $CaCl_2$, Triton X-100 (0.5 mg/ml), and 5 nmol of [^{32}P]phosphatidylglycerol in 0.2 ml. The radioactive substrate was prepared from *Bacillus megaterium* as described by Bertsch *et al.* (1969). One unit of enzyme hydrolyzes 1 μmol of substrate per minute. When enzyme activity was measured in eluates from sodium dodecyl sulfate gels, the Triton X-100 was omitted from the assay mixture (Scandella and Kornberg, 1971).

Polyacrylamide gel electrophoresis was carried out as described by Scandella and Kornberg (1971).

Protein was determined by the method of Lowry *et al.* (1951). When polymers were present, the protein was first precipitated by trichloroacetic acid as described by Albertsson (1971).

For countercurrent distribution an automatic thin-layer apparatus was used (Albertsson, 1965, 1970). The apparatus is manufactured by Buchler Instruments, Fort Lee, N. J.

Escherichia coli particulate fraction was obtained as a wet paste and had been prepared by the Grain Processing Corp. of Muscatine, Iowa, as described by Scandella and Kornberg (1971). It consists of particulate matter obtained as sediment after homogenization of cells in a Manton-Gaulin press and centrifugation at 8000*g* for 30 min.

Solubilization Mixture. For solubilizing phospholipase from the particulate fraction, a combination of Triton X-100, high salt, and high pH was found to be effective. The following composition was used: 1% (w/v) Triton X-100, 0.01 M Tris-HCl, pH 9, 0.001 M EDTA, and 2 M NaSCN in water. (For other enzymes milder salts such as NaCl might be preferable. The salt concentration can also be varied.) Triton X-100 was chosen as an alternative to sodium dodecyl sulfate used by Scandella and Kornberg (1971) in their purification of phospholipase, since the nonionic detergent was considered to be milder and more generally applicable than sodium dodecyl sulfate.

Phase Systems. Phase diagrams and the general properties of aqueous polymer phase systems are described elsewhere (Albertsson, 1971). When two sufficiently different polymers are dissolved in water above certain concentrations, two immiscible aqueous phases are formed; one phase is rich in one of the polymers and the other phase is rich in the other. When three different polymers are used, three phases can be obtained; with four polymers, four phases, etc. Material can distribute between the liquid phases and the interfaces. In a four-phase system, for example, seven fractions are obtained: four liquid phases and three interface layers. Phases are numbered from the bottom up. The following phase systems were used (the figure in parentheses after each polymer gives the approximate per cent, overall, final concentration of the polymer): two-phase systems, dextran (11.1)–PEG (8.9); dextran (7)–PEG (4)–TMA-PEG (2); dextran (7)–PEG (4)–S-PEG (2); three-phase system, dextran (6.67)–Ficoll (8)–PEG (5.33); four-phase system, dextran (5)–Ficoll (6)–PEG (4)–PPG (25).

Partition of Escherichia coli Phospholipase A1 in Three- and Four-Phase Systems. E. coli particulate paste (0.56 g wet weight) was mixed with 5 ml of solubilization mixture (see above) and allowed to stand in a centrifuge tube for 1 hr at room temperature. To 4 ml of this mixture were then added 440 mg of Dextran 500, 480 mg of Ficoll, 320 mg of PEG 6000, and 760 mg of H_2O. The tube was shaken to dissolve the polymers and achieve equilibrium. The mixture was then centrifuged at 10,000*g* for 10 min at 20° to separate the phases. Phases 3 and 2 were clear; phase 1 was slightly turbid and highly viscous due to the presence of DNA. Two densely packed cakes were obtained at the two liquid–liquid interfaces. The lower one between phases 1 and 2 was yellowish, while the upper one between phases 2 and 3 was white. Small samples were taken and diluted ten times for enzyme assays. The results (Figure 1) show that 67% of the enzyme activity was found in the liquid phases 2 and 3.

Phases 3 and 2 were collected by pipet and transferred to another centrifuge tube containing a blank phase 1 (obtained by making a three-phase system as above but omitting the *E. coli* particulate fraction). In addition, 2 ml of PPG 2025 was added, and after vigorous shaking at room temperature the resulting phase system was centrifuged for 10 min at 10,000*g* and at 20° to separate the phases. Four clear phases were obtained. Thin, densely packed cakes were found at the interfaces between phases 1 and 2 and between phases 2 and 3. Samples were taken from all the liquid phases and the cakes were collected by placing the tip of a pipet or a syringe fitted with polyethylene tubing just above the interface and sucking up the interface cake. All samples were diluted ten times before assay.

The results (Figure 1) show that in the four-phase system most of the enzyme activity (70% of that in the total phase system and 50% of the original activity of the solubilizate) was found at the interface between phases 2 and 3.

Partition of ^{32}P- and ^{14}C-Labeled E. coli Lysate in Three- and Four-Phase Systems. E. coli B was grown in 450 ml of

[1] Abbreviations used are: PEG, poly(ethylene glycol); PPG, poly(propylene glycol); TMA-PEG, trimethylaminopoly(ethylene glycol); S-PEG, poly(ethylene glycol)sulfonate.

minimal medium containing 30 μCi of [^{32}P]P_i and harvested in late log phase. Packed cells (0.6 ml) were mixed with 4.5 ml of solubilization solution and allowed to stand for 1 hr. The lysate (4 ml) was then taken through a three-phase partition step followed by a four-phase partition step as described above. Samples were taken from different phases and the radioactivity was determined. Some samples were extracted with chloroform–methanol–water followed by partition in chloroform–methanol–water (Bertsch *et al.*, 1969) in order to determine the counts extracted into the chloroform layer.

The results (Figure 1) show that only 0.05% of the original ^{32}P counts of the lysate were found at the interface between phases 2 and 3 of the four-phase system (where 50% of the enzyme was found). Since 13% of the original counts of the lysate were chloroform-extractable, this means that at least 99.5% of the phospholipids were removed from the enzyme by these partition steps.

A similar experiment was carried out with *E. coli* grown in a medium containing [^{14}C]leucine. The result (Figure 1) shows the partition of ^{14}C between the different phases and can be taken as representative of the overall partition of *E. coli* proteins. Fifteen per cent of the counts are in phase 3 and, of these, only about 3% are at the interface 2–3 of the four-phase system.

Partition of Phospholipid and Triton X-100 in Three- and Four-Phase Systems. Partition of pure phosphatidylethanolamine and Triton X-100 was studied with the same three- and four-phase systems as described above. The ^{14}C-labeled phospholipid was prepared as described by Bertsch *et al.* (1969) and kindly supplied by M. Jazwinsky. Partition of Triton X-100 was determined by measuring the absorbance at 275 nm. In the three-phase system, over 90% of the phospholipid and the Triton X-100 was found in the PEG phase (Figure 2). Upon addition of PPG, the fourth phase, 99% of the phospholipid and 97% of the detergent were extracted into the PPG phase. Thus, the PPG phase can effectively remove phospholipids and detergents from the other aqueous polymer phases.

Partial Purification of Phospholipase A through a Three-Phase and a Four-Phase Step. The previous experiments demonstrate that most of the enzyme, and little protein, is found at the interface between the second and third phases of the four-phase system. Phospholipids and the detergent were extracted into the fourth phase. With these data, a partial purification procedure for phospholipase was devised. The Triton X-100 solubilized enzyme was partitioned in the three-phase system of dextran–Ficoll–PEG and separated from nucleic acids, cell debris, and a large part of other proteins. The enzyme was then transferred to the four-phase system which includes PPG 2025 in addition to the polymers of the three-phase system. The phospholipids and detergent were removed from the enzyme in this partition. The enzyme, no longer soluble, collected at the interface between phases 2 and 3.

E. coli particulate paste (5.4 g) was mixed with 50 ml of solubilization mixture and allowed to stand for 1 hr at about 25°. Solubilizate (40 ml) was added to 4.4 g of dextran 500, 4.8 g of Ficoll, 3.2 g of PEG 6000, and 7.6 ml of water. The mixture was shaken to dissolve the polymers and achieve equilibrium and then centrifuged for 10 min at 10,000*g* to separate the phases. Phases 2 and 3 were withdrawn (leaving the interfaces and phase 1 behind) and mixed with 20 ml of fresh phase 1 and 20 ml of PPG 2025. The resulting four-phase system was shaken vigorously at room temperature and then

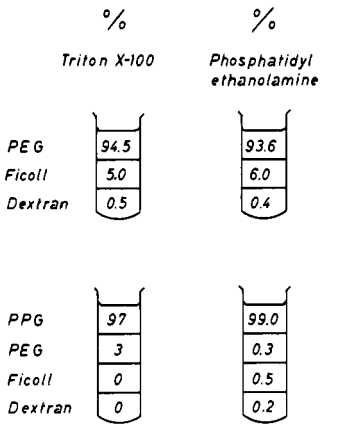

FIGURE 2: Distribution of Triton X-100 and phosphatidylethanolamine between the phases of a three-and a four-phase system. The three-phase system was made up by mixing 400 mg of Dextran 500, 480 mg of Ficoll, 320 mg of PEG 6000, 0.8 ml of H_2O, and 4 ml of 1% Triton X-100 in 2 M NaCNS. The four-phase system was obtained by adding 2 ml of PPG 2025 to the three-phase system.

centrifuged for 10 min at 10,000*g*. The cake at the interface between phases 2 and 3 was collected as described above. The interfacial material together with adjacent liquid phases was about 5 ml. It was transferred to a small centrifuge tube and centrifuged to pack the interface material to a dense cake. The clear liquid phases were withdrawn. The cake was dispersed in water to a volume of 3 ml and analyzed for enzyme activity and protein content (Table I).

Partition in Dextran–PEG Systems Containing Charged PEG. When charged PEG such as TMA-PEG or S-PEG is included in the dextran–PEG two-phase system, macromolecules partition mainly according to their net charge (Johansson, 1970). Figure 3 shows the partition of phospholipase in charged PEG systems as a function of pH. At and below pH 7.1 the enzyme behaves as a positively charged molecule, while at pH 7.9 and above it behaves as a negatively charged molecule. The detergent used in these experiments was Ammonyx LO, a lauryl dimethylamino oxide. Above pH 7.0 this detergent is nonionic, but below pH 7.0 it dissociates and becomes a cationic detergent.

Large-Scale Purification of Phospholipase from E. coli. A large scale purification procedure was devised, using first partition in dextran–PEG and dextran–PEG–PPG systems followed by sequential extraction with TMA–PEG. The results are shown in Table II. Frozen *E. coli* cell wall paste (200 g) was suspended in 1500 ml of a solubilization mixture containing 1% (w/v) Triton X-100, 2 M NaSCN, 1 mM EDTA, and 10 mM Tris; the pH was adjusted to 9 and the suspension was allowed to stand at room temperature for 1 hr. To 1600 ml of the suspension were added 222 g of dextran and 178 g of

TABLE I: Partial Purification of Phospholipase A1 from *Escherichia coli* Particulate Fraction (Envelope Fraction) through a Three- and Four-Phase Step.

	Enzyme (Units/ml)	Vol (ml)	Total Enzyme (Unit)	Total Protein (mg)	Purification
Solubilizate	0.007	40	0.28	360	1
Interface 2–3	0.067	3	0.20	11	23

TABLE II: Large Scale Purification of Phospholipase A1 from *E. coli* Particulate Fraction (200-g Envelope Fraction) by Phase Partition.[a]

	Vol (l.)	Enzyme (Units/l.)	Total Units	Total Protein (g)	Sp Act. (Units/g)	Purification	Yield (%)
Solubilizate	1.6	5	8.0	16.6	0.48	1	100
Fraction I	0.054	190	10.3	0.93	11.0	23	130
Fraction II	0.012	420	5.0	0.20	25	53	63
Fraction III	0.012	143	1.7	0.01	172	358	21

[a] For details see text.

PEG 6000. The polymers were dissolved by shaking and the resulting phase mixture was distributed into six centrifuge tubes and centrifuged at 8000*g* for 10 min in a Lourdes centrifuge. The clear top phases were decanted into six centrifuge tubes, about 160 ml per tube. To each of these were added 160 ml of poly(propylene glycol) (PPG 2025) and 90 ml of 20% dextran (w/v) in 2 M NaSCN. The resulting three-phase system was shaken vigorously and centrifuged at 8000*g* for 10 min at 20°. A thick interfacial layer was formed between phase 1, the dextran phase, and phase 2, the PEG phase. It was collected by drawing it up with a syringe fitted with 2-mm polyethylene tubing. The tip of the tubing was placed just above the interface. The collected interfacial material, together with some liquid of phases 1 and 2, drawn up with it, was distributed into four centrifuge tubes (40 ml capacity each) and centrifuged at 10,000*g* for 10 min. The clear upper and lower phases were drawn off and in each tube the interfacial material containing the enzyme was suspended in 25 ml of H_2O and centrifuged to wash away the polymers. The enzyme was insoluble and found in the pellets. These were combined, suspended in 50 ml of H_2O, and stored in the cold overnight. This material is designated fraction I. It was then centrifuged in the cold. The supernatant was discarded and the pellet was suspended in 20 ml of solubilization mixture and allowed to stand for 0.5 hr. The enzyme went into solution; insoluble material was removed by centrifugation at 10,000*g* for 10 min.

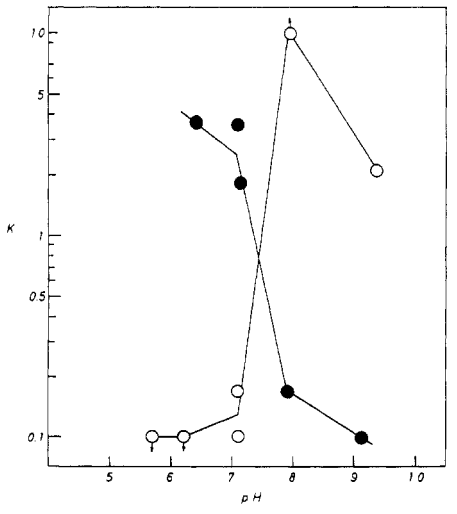

FIGURE 3: Cross partition of phospholipase A1 activity. Phospholipase was partitioned in phase systems containing either positively charged PEG (filled circles) or negatively charged PEG (open circles). Phase system composition: 7% (w/w) dextran 500, 4% (w/w) PEG 6000, 2% (w/w) TMA-PEG 6000 (open circles) or 2% (w/w) S-PEG (filled circles), 0.1% Ammonyx LO. The pH was adjusted by addition of Tris or H_2SO_4 and the pH was measured directly on the phase system.

The clarified solution was taken through a second cycle of two- and three-phase partitions in the same manner as above except that the scale was reduced because the enzyme was more concentrated. To 40 ml of the enzyme in the solubilization mixture was added 5.55 g of dextran and 4.45 g of PEG 6000. The polymers were dissolved by shaking and the resulting phase mixture was centrifuged at 10,000*g* for 10 min. The clear top phase, 22.6 ml, was drawn off and added to 22.6 ml of poly(propylene glycol) 2025 and 10 ml of 20% dextran 500 in 2 M NaSCN. The three-phase mixture was shaken vigorously and centrifuged at 20° at 10,000*g* for 10 min. A smooth layer of material was found at the interface between phases 1 and 2. It contained the enzyme and was collected as described above for the first cycle. After dilution with 20 ml of water it was centrifuged at 10,000*g* for 10 min. The pellet was resuspended and stored in the cold in 10 ml of H_2O. It is designated fraction II. This fraction gives 12–15 bands in sodium dodecyl sulfate gel electrophoresis.

Ammonyx–TMA-PEG Partition. One milliliter of fraction II was centrifuged at 10,000*g* for 10 min. The supernatant was discarded. Ammonyx (0.4 ml of a 1% solution) was added to the pellet. The pellet was suspended by shaking and allowed to stand at room temperature for 30 min, mixed with 1.2 ml of H_2O, and centrifuged at 10,000*g* for 10 min. The supernatant (1.6 ml), which contained most of the enzyme, was mixed with 0.4 ml of 20% (w/w) TMA-PEG 6000, 0.28 g of dextran 500, 0.16 g of PEG 6000, and 1.56 ml of H_2O. H_2SO_4 (2 μl, 0.125 M) was added to adjust the pH to 7.0. After shaking and phase separation by centrifugation, 10,000*g* for 10 min, the top phase was withdrawn and replaced by 2 ml of fresh top phase, for which a mixture of 6% (w/w) PEG 6000 and 2% (w/w) TMA-PEG 6000 in 0.1% Ammonyx was used. Tris (10 μl, 0.1 M), pH 9, was then added to raise the pH. After mixing and phase separation by centrifugation as above the top phase was withdrawn and a new top phase and 10 μl of 0.1 M Tris, pH 9, were added. In this way, the lower phase was sequentially extracted with top phase having pH values in the range between 7 and 9. The latter pH, obtained in the last extraction, was achieved by adding 25 μl of 0.1 M glycine buffer, pH 10.6.

The extractant top phases and the lower phase after the last extraction were assayed for phospholipase and protein. The results are shown in Figure 4. Most of the enzyme activity was extracted into the upper phase between pH 7.2 and 7.8.

The top phases of extractions with pH 7.2–7.6 were combined (10 ml). To recover the enzyme activity, dextran, PEG, and PPG were added to form a three-phase system in which the detergent Ammonyx LO was removed by a PPG phase and the enzyme was collected at an interface. Thus, 0.3 g of PEG 6000, 10 ml of 20% dextran in 2 M NaSCN, and 10 ml

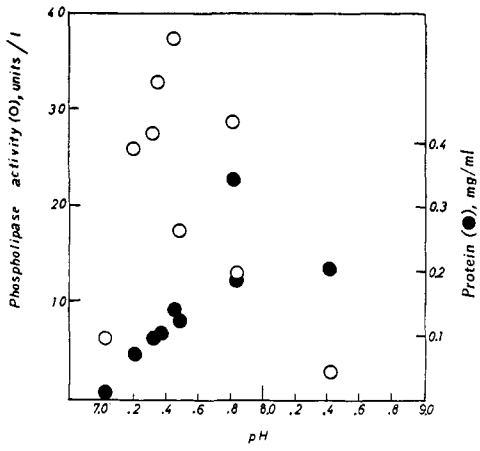

FIGURE 4: Extraction of phospholipase and protein into the upper phase of the dextran–TMA-PEG system. pH was increased before each new extraction. Successive fractions are denoted by the pH values on the abscissa. See text for details.

of PPG 2025 were added to the 10 ml of combined top phases. The mixture was shaken vigorously and centrifuged at 10,000*g* for 10 min. The material at the interface between phases 1 and 2 was collected (~0.5 ml), diluted with 1.5 ml of H_2O, and centrifuged. The pellet was suspended in 2 ml of H_2O, centrifuged, and resuspended in 1 ml of H_2O. This is designated fraction III.

Fraction III gives three, well-separated bands in sodium dodecyl sulfate gel electrophoresis with R_F values of 0.27, 0.75, and 0.99, respectively. The middle band contained the phospholipase activity. Since the enzyme retains activity in sodium dodecyl sulfate, it may be purified further by a preparative sodium dodecyl sulfate gel electrophoresis step as described by Scandella and Kornberg (1971). Elution of the enzyme from the middle band gave a preparation with a specific activity in the range 1.000–2.000 units/g of protein. The near-homogeneous preparation of Scandella and Kornberg had a specific activity of 2.000 units/g of protein.

Countercurrent Distribution. Fraction II (50 mg of protein) was solubilized in 1.2 ml of 1% Ammonyx LO for 1 hr, H_2O (3.5 ml) was added, and the mixture was centrifuged for 15 min at 15,000*g* to precipitate insoluble material. In the supernatant remained 90% of the enzyme activity and 40% of the protein. Supernatant (4.8 ml) was mixed with 0.09 g of glycine, 0.03 ml of 0.1 M EDTA, pH 8.0, 0.12 ml of 0.2 M Tris, 1.2 ml of 20% (w/w) TMA-PEG 6000, 0.84 g of dextran, 0.48 g of PEG 6000, and 4.68 ml of H_2O. After mixing and phase separation, the bottom and top phases were collected while the interphase was discarded. Cavities no. 0–6 in the countercurrent apparatus were each loaded with 0.7 ml of bottom and 0.7 ml of top phase. The remaining cavities no. 7–119 of the apparatus were loaded with 0.7 ml of each phase of a blank phase system having the composition described above but with no protein present. Transfers (120) were carried out at room temperature; the settling time was 6 min and the shaking time was 20 sec. When the countercurrent distribution was completed, the contents of the cavities were collected and assayed for phospholipase activity. The countercurrent distribution diagram, Figure 5, shows one main peak, the width of which is what could be expected for one component. The experiment therefore indicates that the enzyme activity is associated with one species of molecules which travel along the distribution train in a normal fashion. The specific enzyme activity of the peak was about 330 units/g of protein.

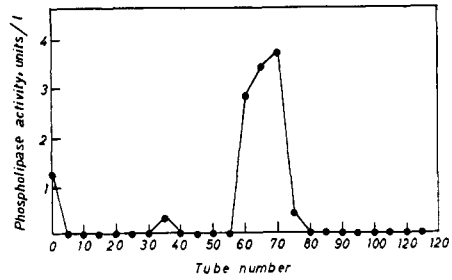

FIGURE 5: Countercurrent distribution of phospholipase A1.

Discussion

Little is known about the properties of membrane proteins and their interactions with lipids and detergents. Recent binding data (Helenius and Simons, 1972) show that delipidated membrane proteins bind large quantities of nonionic detergents such as Triton X-100, while soluble, hydrophilic proteins bind little or no detergent. Phase partition experiments on hydrophilic proteins in dextran–PEG systems with Triton X-100 show that this detergent has little or no effect on the partition of hydrophilic proteins, which also indicates that such proteins bind little or no detergent (Albertsson, unpublished experiments).

It is reasonable to suppose that when a protein binds a nonionic detergent, the hydrophobic part of the detergent molecule binds to hydrophobic parts of the protein. The hydrophobic regions of the protein will therefore be covered by detergent molecules and the hydrophilic part of the detergent molecules will be exposed to the aqueous surroundings, thereby rendering the protein soluble. Several nonionic detergents such as Triton, Tween, Brij, and Lubrol have a short PEG chain as the hydrophilic part. Proteins which are solubilized by these detergents would therefore expose a certain amount of "PEG surface" to the surroundings and thus favor the PEG-rich phase after partition in dextran–PEG or dextran–Ficoll–PEG phase systems (Figure 6). Phospholipase activity also favors the PEG phase of these systems when Triton X-100 is present. Partition in these phase systems would therefore seem to be a generally applicable procedure for enrichment of those proteins which are solubilized by Triton and similar nonionic detergents. By varying the type and concentration of detergent and the salt composition it should be possible to achieve selective distribution of membrane proteins between different phases.

Although all phases are mainly aqueous, it is justifiable to say that a PEG phase is slightly more hydrophobic in character than a Ficoll phase which, in turn, is more hydrophobic than a dextran phase. (Ficoll, for example, requires much higher concentrations of alcohol or acetone for precipitation than dextran does, and PEG is soluble in these solvents.) Proteins with different hydrophilic–hydrophobic surfaces, and therefore different degrees of detergent binding, would therefore be expected to partition differently between the polymer phases. This mechanism, schematically shown in Figure 6, is probably largely responsible for the purification obtained for phospholipase in the dextran–PEG or dextran–Ficoll–PEG systems.

Purification is also obtained by selective solubilization at each step at which the insoluble protein is treated with the detergents. For example, almost all enzyme activity but only part of the protein is solubilized when fraction I is treated with the solubilization mixture. That only a part of the protein is solubilized at this step, although it was earlier all soluble

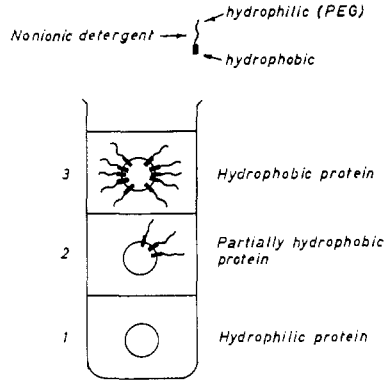

FIGURE 6: Proposed mechanisms for partition of proteins with different hydrophobic surfaces in a three-phase system of dextran–Ficoll–PEG. The lower phase (1) is the dextran phase; the middle phase (2) is the Ficoll phase, and the upper phase (3) is the PEG phase. The detergent molecules shown are of the ethylene oxide adduct type, such as, for example, Triton X-100, with a PEG chain as the hydrophile and octylphenyl as the hydrophobic part. It is proposed that hydrophobic proteins bind the hydrophobe of the detergent and the PEG part of the detergent would be exposed outward. Such coated proteins will have more affinity for the PEG-rich top phase (3) while hydrophilic proteins which do not bind detergent collect in the lower dextran-rich phase (1). Partially hydrophobic proteins will be enriched in the middle phase (2). Similar results would be obtained for other detergents containing a PEG part but different hydrophobic parts. Selectivity of phase partition would then depend on hydrophobic interaction between a protein and the hydrophobic parts of different detergent molecules.

when the *E. coli* particulate matter was treated, is probably due to exposure of new points of interaction between the proteins following the removal of the phospholipids during the formation of fraction I. Some of these new interactions may be too strong to permit solubilization by the detergent used in the initial solubilization.

Partition studies on soluble proteins, such as hemoglobin and glycolytic enzymes, in phase systems containing charged PEG, such as TMA-PEG or S-PEG, are described elsewhere (Johansson, 1970; Johansson *et al.*, 1973). The results show that if the ionic strength is kept low (~5 mM), a protein can be selectively transferred from one phase to the other by shifting the pH around the isoelectric point of the protein. The results in Figure 3 show that this technique can also be used for proteins solubilized by a detergent. Caution should be exercised, however, in drawing conclusions as to the isoelectric point of an enzyme from data like those in Figure 3. The transfer of enzyme activity from one phase to the other upon a shift in pH is probably the result of a change in the net charge of the protein–detergent micelles rather than of the enzyme alone.

The use of polyphase partition in combination with detergents for purification of hydrophobic membrane proteins offers several advantages. High selectivity can be achieved in each step. The method is flexible in that different types of detergents, salt concentrations, and pH values can be used. Thus, for the purification schemes described here, NaSCN can be replaced by NaCl as the latter may be milder for other, less stable, enzymes. Since nonionic detergents can be used and these have been shown to be fairly mild agents, the method should be generally applicable. Each step is simple and rapidly performed and can easily be scaled up. A particular advantage is that the method permits rapid removal of phospholipids and detergents. The polymers can then easily be removed by repeated washings and centrifugations, since the purified proteins are no longer soluble.

The interfaces between the different phases are as important as the bulk phases in the separation process. It has been shown for cells and organelles that the interface of the dextran–PEG system has a high capacity for adsorption of particles (Albertsson, 1971) and selective distribution between the interface and the bulk phases can be used for separation on a preparative scale. Also, partition of particles between different interfaces of a polyphase system can be used. This work shows that the same holds for particulate material from *E. coli* and that a solubilized membrane enzyme rendered insoluble after removal of the detergents can be selectively collected at a liquid–liquid interface. One may object that this could involve a risk of surface denaturation. It has been shown, however (Albertsson, 1971), that the interfacial tension between the phases is so low (0.0001–0.1 dyn/cm) that this risk is negligible.

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