Disaggregation of Adenylate Cyclase during Polyacrylamide-Gel Electrophoresis in Mixtures of Ionic and Non-Ionic Detergents

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1. Adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] solubilized from the rat liver plasma membrane with 1% Lubrol PX and partially purified by gel filtration in buffer containing 0.01% Lubrol PX was physically characterized by polyacrylamide-gel electrophoresis. 2. The molecular radius determined for the partially purified enzyme was 4.9 nm, compared with the value of 3.9 nm obtained for the enzyme before gel filtration. 3. This difference, representing an approximate doubling of the molecular volume of the enzyme, implied that aggregation with itself or other proteins had occurred during partial purification. 4. Aggregation was not reversed by electrophoresis in the presence of high Lubrol concentrations. 5. Substitution of deoxycholate or *N*-dodecylsarcosinate for Lubrol PX either for solubilization or during electrophoresis led to poorer resolution of membrane proteins at concentrations giving greater than 70% loss of enzyme activity. 6. Partially purified adenylate cyclase was electrophoresed in the presence of mixed micelles of Lubrol PX and deoxycholate or Lubrol PX and *N*-dodecylsarcosinate. Different mixtures were examined simultaneously in a suitable apparatus. 7. Electrophoresis in the presence of 0.1% Lubrol plus 0.03% deoxycholate decreased the molecular radius of the cyclase to 4.0 nm, with greater than 90% recovery of enzymic activity. The net charge of the enzyme was also increased, indicating ionic detergent binding. 8. With 0.1% Lubrol plus 0.03% *N*-dodecylsarcosinate the molecular radius was 4.3 nm, recovery approx. 50% and net charge similar to that seen in Lubrol plus deoxycholate. 9. The resolution of cyclase from bulk protein, on an analytical scale, was improved in the presence of detergent mixtures, as compared with resolution in Lubrol alone. 10. The results demonstrate the usefulness of polyacrylamide-gel electrophoresis to detect and overcome aggregation problems with membrane proteins and suggest that detergent mixtures in specific ratios may be useful in the purification of adenylate cyclase and other intrinsic membrane proteins.

The separation of intrinsic membrane proteins with maintenance of biological function for the purpose of purification or physical characterization depends on the disruption of some, but not all, of their mutual interactions. Excessive disruption may lead to a loss of quaternary structure and hence of activity. Insufficient disruption permits interactions between different membrane proteins, thereby obscuring the unique size and charge properties of the protein of interest and confounding attempts at purification. Dissociation of membranes is usually

achieved by dispersion with detergents (for extensive reviews see Helenius & Simons, 1975; Tanford & Reynolds, 1976). Strongly ionic detergents (most commonly dodecyl sulphate) generally yield individual detergent-complexed polypeptides, but these are usually denatured and inactive. Non-ionic detergents and bile salts often preserve functions, but are less effective solubilizers. Certain protein–protein interactions may not be disrupted even though the protein–detergent complexes are not sedimentable (Kagawa, 1972; Wasserman, 1974).

In a recent paper (Welton *et al.*, 1978) we described the partial purification of adenylate cyclase [ATP pyrophosphate-lyase (cyclizing), EC 4.6.1.1] solubilized with Lubrol PX from the rat liver plasma membrane. We postulated that our failure (and possibly that of other workers: Swislocki & Tierney, 1973; Johnson *et al.*, 1976; Swislocki *et al.*, 1977) to

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Abbreviations used: K_R , retardation coefficient; Y_0 , relative free electrophoretic mobility; p[NH]ppG, guanylyl-5'-yl imidodiphosphate.

purify the enzyme beyond a fairly crude stage was due to aggregation of the enzyme with other proteins. A gel-electrophoretic fractionation was therefore devised (Newby *et al.*, 1978a) to characterize physically the active enzyme in Lubrol dispersion. The present study is an attempt to characterize the partially purified enzyme and to seek improved conditions of dispersion for the active enzyme.

Experimental

The source of materials used here was the same as given previously (Newby *et al.*, 1978a). Lubrol PX was a gift from ICI organics, Blackley, Manchester M9 3DA, U.K. Sodium cholate, sodium deoxycholate and sodium *N*-dodecylsarcosinate were from Sigma Chemical Co., St. Louis, MO 63178, U.S.A.

Membranes and soluble fractions

The preparation and storage of rat liver plasma membranes, treatment of membranes with glucagon and guanylyl-5'-yl imidodiphosphate, solubilization of membranes at 10mg of protein/ml with 1% (w/v) Lubrol PX and chromatography of the soluble fraction on Ultrogel AcA 22 (LKB Instruments, S-161 25 Bromma, Sweden) in buffer containing 0.01% Lubrol have all been described in detail (Welton *et al.*, 1977, 1978). The partially purified enzyme was concentrated at 4°C to approx. 1mg of protein/ml by ultrafiltration at 1000g for 30min through a Centriflo cone (Amicon, Lexington, MA 02173, U.S.A.).

Membranes were also solubilized with ionic detergents after pretreatment with glucagon and guanylyl-5'-yl imidodiphosphate as described by Welton *et al.* (1978). Membranes were resuspended at 4°C to approx. 10mg of protein/ml in buffer containing 10mM-Tes (2-[[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]amino]ethanesulphonic acid), pH7.5, 1mM-dithiothreitol, 1mM-EDTA and 25% (w/v) sucrose. Ionic detergents were added to a final concentration of 1% for cholate and 0.3% for *N*-dodecylsarcosinate. The mixture was vortex-mixed for 30s and centrifuged at 49000g for 30min at 4°C, and the supernatant was aspirated and used.

Electrophoresis

The apparatus and procedures for electrophoresis have been described previously (Chrambach *et al.*, 1976; Newby *et al.*, 1978a). For experiments involving mixtures of ionic and non-ionic detergents the upper buffer reservoir of a Hoefer Scientific Instruments (San Francisco, CA 94107, U.S.A.) GT-5 (model 155; Bio-Rad, Richmond, CA 94804,

U.S.A.) electrophoresis apparatus was modified. Nine open cylinders of 3cm internal diameter were glued to the floor of the chamber. Holes were then bored to accommodate conventional 8mm rubber grommets. The voltage was applied by a circular platinum electrode passing through each chamber. By this arrangement the same voltage was applied across each gel. A complete description of the construction and uses of this apparatus and a similar design allowing variation of lower buffer is given by Newby *et al.* (1978b).

Samples (20–50 μ l, containing approx. 20 μ g of protein for partially purified enzyme and 100 μ g for initially solubilized material) were loaded on gels by overlaying in the buffers containing 25% sucrose used for solubilization and gel filtration. The amount of cyclase activity loaded was in the range 20–200pmol of cyclic AMP produced/10min. Electrophoresis was conducted at 0°C for 3h at 2.0mA/gel.

The compositions of the gels and multiphasic buffer system used were exactly as given in Newby *et al.* (1978a). As detailed there, to maximize the electrophoretic mobility of the cyclase the buffer used in both stacking-gel and resolving-gel polymerization mixture was 55mM-imidazole/HCl, pH6.0. This gave an operative pH of 7.5 in both gels. Unstacking of the enzyme was achieved simply by increasing the restrictiveness of the gel. The moving boundary was marked with the red component of RBY dye (product 51250; Gelman; Ann Arbor, MI 48106, U.S.A.).

Assays

Adenylate cyclase activity of membranes, soluble fractions and that recovered from gel slices was measured at 30°C as described previously (Newby *et al.*, 1978a) and gels were stained for protein by the method of Fairbanks *et al.* (1971). Membrane protein concentration was determined as described by Welton *et al.* (1978). Densitometry of stained gels was conducted at 690nm with a Gilford Instruments (Oberlin, OH 44074, U.S.A.) gel scanner at a linear transport rate of 0.5cm/min. Detergents were detected in gels by fixation for 1h or more in 12.5% (w/v) trichloroacetic acid (approx. 60ml/gel). Lubrol PX, deoxycholate and *N*-dodecylsarcosinate all formed white precipitates, which could be detected by eye or photographed against a dark field.

Data analysis

The determination of R_F for adenylate cyclase and for standard proteins, construction of Ferguson (1964) plots by computerized linear regression analysis and derivation of molecular radius from a standard curve of $(K_R)^{\frac{1}{2}}$ against geometric mean radius for standards have all been described (Rodbard & Chrambach, 1974; Chrambach *et al.*, 1976).

These references also detail the statistical methods employed. R_F was defined as the ratio of the migration distance in the resolving gel of the component of

interest to that of the moving boundary marker. The same standard proteins were used as previously (Fig. 7 of Newby *et al.*, 1978a).

Fig. 1. Electrophoretic profiles for adenylate cyclase, by slicing and enzyme assay and protein, by staining and densitometry. Partially purified adenylate cyclase was electrophoresed with 5.5% total monomers in the resolving gel and (a) 0.1% (w/v) Lubrol PX in the stacking and resolving gels; (b) 0.03% deoxycholate in the upper buffer and gels; (c) 0.01% *N*-dodecylsarcosinate in the upper buffer and gels; (d) 0.1% Lubrol PX in the upper buffer and gels plus 0.03% deoxycholate in the upper buffer; (e) 0.1% Lubrol PX in the upper buffer and gels plus 0.03% *N*-dodecylsarcosinate in the upper buffer. —, A_{690} ; ●, adenylate cyclase activity.

Results

Electrophoresis in the presence of Lubrol PX of adenylate cyclase before and after partial purification

Partial purification of adenylate cyclase solubilized with 1% Lubrol PX by gel filtration in buffer containing 0.01% Lubrol led to an approx. 5-fold increase in specific activity as previously reported (Welton *et al.*, 1978). Most of the detergent used for solubilization was separated from the enzyme by this procedure (Welton *et al.*, 1978). The partially purified material was examined by gel electrophoresis with different concentrations of Lubrol in stacking and resolving gels. A single peak of enzymic activity (e.g. Fig. 1a) was seen at all Lubrol concentrations. The polydispersity of the enzyme as judged by band width at half-height of the enzyme activity against slice-number profile (Newby *et al.*, 1978a) was minimized at Lubrol concentrations of 0.01% or above (Table 1).

Ferguson-plot analysis (Ferguson, 1964) was conducted to determine the size and charge parameters of the enzyme. Studies were conducted with 0.1% Lubrol in stacking and resolving gels. This concentration was chosen because it yielded the 'smallest and least polydisperse size' for the active enzyme before partial purification (Newby *et al.*,

1978a). Electrophoresis was conducted at six gel concentrations each, simultaneously, in the same apparatus for adenylate cyclase before and after partial purification. Linear plots of $\log R_F$ against gel concentrations were obtained (Fig. 2). The slope of this plot (K_R) is a measure of molecular size, whereas the R_F at zero gel concentration is related to molecular net charge (Ferguson, 1964; Rodbard & Chrambach, 1974). The values of K_R were converted into the less precise but more readily understandable parameter geometric mean radius by use of a standard curve constructed with ten standard proteins (Rodbard & Chrambach, 1974).

The results in Table 2 show that adenylate cyclase was significantly larger ($P < 0.01$ by Student's *t* test, with 20 degrees of freedom), as judged by the K_R value after partial purification. Net charge was also increased consistent with a larger species of similar average surface charge density. The values of geometric mean radius (Table 2) allow us to calculate that the molecular volume and hence presumably molecular weight (assuming the same partial specific volume) were approximately doubled during partial purification. It was concluded that the enzyme had aggregated either with itself or other proteins. Even when electrophoresis was conducted with 1% Lubrol (results not shown) the size of the enzyme was not decreased.

Table 1. R_F , band width and recovery of adenylate cyclase activity after polyacrylamide-gel electrophoresis. Electrophoresis of partially purified adenylate cyclase was conducted with Lubrol in stacking and resolving gels. Resolving gels contained 5.5% (w/v) total gel monomers. Ionic detergents were added together with Lubrol at the concentration also present in the gels into the upper buffer. Enzyme activity was determined as described after slicing gels into 2mm slices. The front was marked by the red component of RBY dye mixture (Gelman no. 51250). R_F and band width at half height were calculated from hand-drawn plots of activity against slice number. Recovery was estimated by summing the activity recovered from each gel slice and relating the sum to the activity in a sample of partially purified enzyme assayed in an identical manner. Abbreviation: N.D., not determined.

Lubrol concn. in upper buffer and gels (% w/v)	Concn. of ionic detergent in upper buffer (% w/v)	Parameters of adenylate cyclase derived from profiles of activity against slice number		
		R_F	Band width	Recovery (%)
0.00	—	0.36	0.18	113
0.01	—	0.42	0.08	129
0.10	—	0.39	0.08	118
1.00	—	0.36	0.06	83
<i>N</i> -Dodecylsarcosinate				
0.10	0.00	0.36	0.10	118
0.10	0.01	0.54	0.08	96
0.10	0.03	0.70	0.08	62
0.10	0.10	0.60	N.D.	1.8
Deoxycholate				
0.10	0.00	0.35	0.08	110
0.10	0.01	0.59	0.10	85
0.10	0.03	0.67	0.08	100
0.10	0.10	0.67	N.D.	54

Fig. 2. Ferguson plots obtained for adenylate cyclase. Ferguson (1964) plots were obtained with 0.1% (w/v) Lubrol in stacking and resolving gels for adenylate cyclase before ($\blacktriangle$) and after ($\circ$) partial purification in 0.01% Lubrol. Ferguson plots for partially purified enzyme when the upper buffer was supplemented with 0.1% Lubrol and 0.03% deoxycholate ($\triangle$) or 0.1% Lubrol and 0.03% *N*-dodecylsarcosinate ($\square$) are also shown. Each point represents the mean for two or three observations at the same gel concentration.

Electrophoresis of partially purified adenylate cyclase in the presence of deoxycholate or *N*-dodecylsarcosinate

In view of the failure of 0.1% or even 1% Lubrol to reverse the aggregation of adenylate cyclase that occurred during chromatography in 0.01% Lubrol, the influence of harsher ionic detergents was investigated. The final concentration of deoxycholate (0.03%) or *N*-dodecylsarcosinate (0.01%) that inactivated the enzyme by 20% when added to the partially purified enzyme for 3 h at 0°C was determined. Increasing the concentrations of either detergent led eventually to complete inactivation. The above stated concentrations were then added to the upper buffer during electrophoresis of the partially purified enzyme. Since the detergents were small highly charged molecules, they rapidly overtook the enzyme during electrophoresis (see below), and the enzyme was exposed to them at the concentrations stated. Moreover, Lubrol formed mixed micelles with the ionic detergents and was therefore removed from the enzyme (see below). Hence this procedure resulted in the transfer of the enzyme from a Lubrol to an ionic detergent environment. The profiles of activity against slice number obtained are shown in Figs. 1(b) and 1(c). Both cyclase and bulk proteins (by staining and densitometry) exhibited lower R_F

values in either deoxycholate or *N*-dodecylsarcosinate. Recoveries of enzyme activity were 14–29% with deoxycholate and 18–25% with *N*-dodecylsarcosinate (two experiments each). It was concluded that further aggregation of cyclase and bulk proteins had occurred at the low detergent concentrations necessary to preserve even this modest enzyme activity.

Electrophoresis of adenylate cyclase solubilized with cholate or *N*-dodecylsarcosinate

An alternative approach was to solubilize membranes directly with ionic detergents. When membranes were pretreated with glucagon and p[NH]ppG (Welton *et al.*, 1978) a persistently activated state of adenylate cyclase was produced. The enzyme could then be solubilized by 1% cholate with approx. 40% recovery of stimulated enzyme activity. Electrophoresis of the cholate-solubilized material with 0.1% cholate in the upper buffer led to 22% recovery of loaded activity (8% of membrane activity). The activity was confined to the top of the stacking gel, indicating gross aggregation.

N-Dodecylsarcosinate (0.3%) also solubilized the preactivated adenylate cyclase. Approx. 40% of stimulated enzyme activity was recovered in the supernatant. After electrophoresis with 0.01% *N*-dodecylsarcosinate in the upper buffer no enzymatic activity was recovered.

Electrophoresis of the adenylate cyclase in mixtures of Lubrol PX and deoxycholate or Lubrol PX and *N*-dodecylsarcosinate

Since ionic detergents alone had failed to disaggregate the cyclase at concentrations that did not inactivate the enzyme, the efficacy of mixtures of Lubrol and ionic detergents was investigated. It was argued that the range of detergent concentrations over which such effect might be seen would be small and it was therefore necessary to vary the ionic detergent concentration in a systematic manner. This was facilitated by a simple modification of the upper buffer reservoir of our polyacrylamide-gel-electrophoresis apparatus, which allowed up to nine gels to be electrophoresed simultaneously with different upper buffer constituents.

Partially purified adenylate cyclase was subjected to electrophoresis with mixtures of Lubrol PX and deoxycholate or Lubrol PX and *N*-dodecylsarcosinate in the upper buffer. Subsequent experiments showed that the same results were obtained when ionic detergent was included in the sample and gels as well. Samples were electrophoresed simultaneously with 0.1% Lubrol alone and 0.1% Lubrol plus 0.01, 0.03 or 0.1% of ionic detergent in upper buffer. The results of these experiments are shown in Fig. 1 and Table 1. Inclusion of either deoxycholate or *N*-dodecyl-

sarcosinate with Lubrol in the upper buffer led to an increase in R_F of the cyclase: 0.03% ionic detergent appeared to cause complete transition to the higher R_F (Table 1). Increasing ionic detergent concentrations further led to greater loss of enzyme activity. The recovery of enzyme with optimal deoxycholate was close to 100%, whereas with *N*-dodecylsarcosinate it was approx. 50%.

An increase in R_F of the cyclase at a single gel concentration could be explained either by an increase in molecular net charge or by a decrease in size. Ferguson-plot analysis was therefore conducted with mixtures of detergents in the upper buffer. Fig. 2 shows the pooled results from two experiments with each detergent mixture. Values of K_R and Y_0 computed from these data are given in Table 2. In the presence of either 0.1% Lubrol plus 0.03% deoxycholate or 0.1% Lubrol plus 0.03% *N*-dodecylsarcosinate the enzyme was significantly smaller than in Lubrol alone. Despite the decrease in size the electrophoretic mobility was in each case increased, indicating a large increase in charge density on the protein. This presumably reflected binding of negatively charged detergent molecules. The values of K_R for the partially pure enzyme in the presence of mixed detergents was indistinguishable from that of the enzyme before partial purification (Table 2). It was therefore concluded that the enzyme had been disaggregated to its 'minimum active size' observed before purification.

Electrophoresis of detergents and detergent mixtures alone

To explain the behaviour of adenylate cyclase with different gel and upper buffer constituents, it was necessary to determine the electrophoretic behaviour of the detergents themselves in the buffer system used. As seen in Fig. 3 (gel *a*), Lubrol alone did not migrate, as expected for an uncharged detergent. Small samples (0.5 ml) of deoxycholate and

N-dodecylsarcosinate travelled as a concentrated stack at the dye front (Fig. 3, gels *b* and *c*). When added

Fig. 3. Electrophoresis of detergents and detergent mixtures. Resolving gels (1.0 ml) with 5% (w/v) gel concentration were polymerized. Detergents were added to resolving-gel polymerization mixture and 0.5 ml sample gels polymerized on top of the resolving gels. After filling the upper reservoir, tracking dye (RBY, Gelman No. 51250) was layered over the gels in upper buffer containing 25% sucrose. Electrophoresis was conducted as for adenylate cyclase, until the red component of the dye was approx. 0.5 cm from the end (indicated by an arrow). Gels were then removed and fixed overnight in 60 ml of 12.5% (w/v) trichloroacetic acid. Gels were then transferred to test tubes containing 12.5% trichloroacetic acid and photographed against a dark background. Sample gels contained: (a) 0.1% Lubrol PX; (b) 0.1% deoxycholate; (c) 0.1% *N*-dodecylsarcosinate; (d) 0.1% Lubrol+0.03% deoxycholate; (e) 0.1% Lubrol+0.03% *N*-dodecylsarcosinate; (f) 0.1% Lubrol+0.1% deoxycholate; (g) 0.1% Lubrol+0.1% *N*-dodecylsarcosinate.

Table 2. Size and charge parameters of adenylate cyclase

Electrophoresis was conducted as described in the legend to Fig. 2. Values of K_R and Y_0 and their errors were derived from computerized analysis of the Ferguson (1964) plots shown (Fig. 2). Geometric mean radius was obtained by linear interpolation from a standard curve relating $K_R^{\frac{1}{2}}$ to geometric mean radius for ten standard proteins as described previously (Rodbard & Chrambach, 1974; Newby *et al.*, 1978a). Standard deviations for K_R were 0.007 and for Y_0 0.2; 95% confidence limits for molecular radius were 0.7 nm.

Cyclase preparation	Detergent present in upper buffer	$-K_R$	Y_0	Geometric mean radius (nm)
Solubilized in 1% Lubrol PX	—	0.106	1.5	3.9
Solubilized in 1% Lubrol PX and partially purified in 0.01% Lubrol	—	0.147	2.0	4.9
	0.1% Lubrol PX+0.03% deoxycholate	0.108	2.6	4.0
	0.01% Lubrol PX+0.03% <i>N</i> -dodecylsarcosinate	0.118	2.8	4.3

to the upper buffer the large volume (500ml) compared with the gel (2ml) implies that a stack of detergent followed by unstacked detergent at approximately the concentration in the starting upper buffer would migrate into the gel (Ornstein, 1964). When mixtures of Lubrol and ionic detergents were electrophoresed Lubrol migrated into the gels, indicating the formation of mixed micelles (Fig. 3, gels *d-g*). With a high proportion of ionic detergent these behaved similarly to ionic detergent alone (Fig. 3, gels *f* and *g*). With lower proportions of ionic detergent some mixed micelles of lower ionic mobility were formed, which failed to stack (Fig. 3, gels *d* and *e*). Thus under all conditions where mixtures of Lubrol and ionic detergents were present in the upper buffer, electrophoresis proceeded in the presence of mixed micelles. However, if ionic detergent alone was present in the upper buffer the small amount of Lubrol in the sample would be swept into the stack as mixed micelles and hence removed from the cyclase.

Discussion

There is as yet no general strategy for the purification of intrinsic membrane-bound proteins. Such attempts as have been successful have relied on particularly favourable circumstances, for instance choice of an extremely rich source (MacLennan, 1970; Jørgensen *et al.*, 1971; Oesterhelt & Stoekenius, 1971; Meunier *et al.*, 1974; Marchesi *et al.*, 1976), the availability of high-affinity ligands (Cuatrecasas, 1972; Meunier *et al.*, 1974) or the exceptional stability of the protein (Widnell, 1974; Marchesi *et al.*, 1976). Many proteins of biological interest possess none of these properties. In particular, adenylate cyclase is, according to estimates based on the catalytic-centre activity of the bacterial enzyme (Takai *et al.*, 1974), only a minor component (less than 0.1 %) of any plasma membrane in which it occurs. It is readily inactivated by membrane-disruptive agents, and although hormones activate the membrane-bound enzyme at low concentrations this property is lost on solubilization (Swislocki & Tierney, 1973; Neer, 1974; Pfeuffer & Helmreich, 1975; Queener *et al.*, 1975; Roy *et al.*, 1975; Johnson *et al.*, 1976; Catt & Dufau, 1977).

Simply solubilizing membranes and applying traditional methods for fractionation of soluble proteins has led to some successes (Evans & Gurd, 1973; Hokin, 1974; Slavik *et al.*, 1977). However, the choice of the nature and concentration of detergents used has generally been based solely on the criterion of maintaining enzymic activity. A barrier has frequently been reached beyond which further purification was difficult, but could be overcome by a change of detergent (Imai & Sato, 1974; Hoeven & Coon, 1974; Arinc & Philpot, 1976), by use of chaotropic ions (Davis & Hatefi, 1971), by use of a

detergent mixture (Widnell, 1974) or by removal of hydrophobic material during dodecyl-Sepharose chromatography (Homcy *et al.*, 1978). One of the particular problems encountered in the purification of membrane proteins is their tendency to aggregate with one another (Kagawa, 1972; Wasserman, 1974; Homcy *et al.*, 1978). This is a particular danger when detergent concentration is lowered, a universally applied procedure necessitated by the instability of proteins at high detergent concentrations. We therefore suggest that a better criterion for determining conditions of solubilization is those giving rise to the 'smallest and least polydisperse active enzyme'.

Partial purification of adenylate cyclase by gel filtration in 0.01 % Lubrol PX led to an increase from this minimum active size correlated with an incapacity to purify the enzyme further (Welton *et al.*, 1978). The objection could be raised that this increase reflects altered detergent or lipid binding. We consider that this is unlikely for four reasons. First, the increase in size occurs under conditions of lowered detergent concentrations and removal of phospholipid as detergent lipid micelles, both of which would be expected to decrease detergent and lipid binding. Secondly the increase in size was accompanied by an increase in net charge. This would not be expected if binding of uncharged detergent or neutral phospholipid was responsible. Thirdly, after disaggregation the enzyme reverted to a size indistinguishable from that of the initially solubilized material, despite the different detergent and lipid environment. Fourthly, the size of the disaggregated enzyme was very similar with deoxycholate and *N*-dodecylsarcosinate in addition to Lubrol, despite the large differences in micellar size of the pure detergents (Helenius & Simons, 1975). These arguments support the contention that alterations in the protein content of the protein-lipid micelles were responsible for the changes in size observed and imply that the partially purified enzyme was an aggregated form. In view of the fact that several other chromatographic procedures led to preparations of the enzyme of similar specific activity and intractability to further purification (Swislocki & Tierney, 1973; Johnson *et al.*, 1976; Swislocki *et al.*, 1977; Welton *et al.*, 1978), aggregation may not be peculiar to the gel-filtration procedure used.

The failure of deoxycholate and *N*-dodecylsarcosinate alone to disaggregate the enzyme is at first surprising. However, the concentrations used were relatively low. Higher concentrations may have eventually disaggregated the enzyme, but only in a totally inactive form. The ability to titrate the enzyme with non-ionic and ionic detergents, especially in direct-comparison experiments, allowed us to determine precisely the optimal conditions for disaggregation. The narrow range over which a disaggregated but active species was produced

justifies such a systematic approach. The ability of mixtures of detergents to induce disaggregation may be related to the observation that ionic detergents increased the net charge on the protein-lipid micelles. Conceivably, electrostatic repulsion between detergent-complexed membrane proteins may overcome their tendency to aggregate through hydrophobic interactions. The combined effect of the non-ionic and ionic detergents is to achieve solubilization of the proteins at a sufficiently low concentration of the ionic detergents to avoid denaturation. It will be interesting to determine whether detergent mixtures can facilitate the removal of hydrophobic material from adenylate cyclase in a similar manner to that reported by Homcy *et al.* (1978) using hydrophobic chromatography.

Conducting electrophoresis in the presence of detergent mixtures led to increased mobilities for other proteins as well as adenylate cyclase (Figs. 1*d* and 1*e*). Many of the proteins were so accelerated as to remain in the stack at 5.5% gel concentration. Adenylate cyclase became one of the slowest-moving proteins. The observed changes in relative mobilities of cyclase and bulk protein should be exploitable in a preparative-scale polyacrylamide-gel electrophoresis.

Wasserman (1974) has pointed out the value of gel electrophoresis in detecting gross aggregation of membrane proteins. Since many of the problems of designing polyacrylamide-gel-electrophoretic fractionation systems for membrane proteins with retention of biological function have been overcome (Newby *et al.*, 1978*a*), this analysis can now be refined to detect relatively small changes of aggregation state. Such fractionation systems are also essential for determining ultimate purity (Chrambach *et al.*, 1976; Tanford & Reynolds, 1976). Given this, we consider that purification could be monitored at each stage with little extra investment of effort. Investigators could then be immediately alerted to aggregation problems and much frustration would be avoided. Polyacrylamide-gel-electrophoresis has also proved a rapid, economical and versatile tool for designing conditions to reverse aggregation.

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