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INTERACTION OF A SOLUBILIZED MEMBRANE ATPase WITH LIPID BILAYER MEMBRANES

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SUMMARY

A membrane ATPase derived from *Streptococcus fecalis* has been purified, and found to interact with phospholipid bilayers causing a 10^2–10^4-fold increase in d.c. conductance. The membrane conductance increases with the concentration of the soluble ATPase, but shows a saturation effect at high concentration. There is no ion selectivity conferred on the membrane by the ATPase interaction, however, a significant increase in the water permeability has been detected. At extremely low concentrations of the ATPase (around 10^{-10} M) discrete conductance fluctuations are seen. These conductance fluctuations are made up of integral multiples of a unit "channel", approximately 10^{-10} Ω^{-1} in magnitude. Both the incidence and duration of these conducting channels increase markedly as the voltage bias on the membrane is raised. The results indicate the field-dependent formation of aqueous-filled pores in the bilayer–ATPase system. The purified ATPase has been shown to be lipid-free and non-particulate. It is, therefore, considered that information relevant to the nature of lipid–protein interactions at the membrane level can be obtained by studying the bilayer–ATPase system.

INTRODUCTION

In an earlier publication it was reported that a large increase in lipid bilayer electrical conductance occurred as a result of interaction with a solubilized membrane protein[1]. The membrane protein studied was the ATPase extracted from *Streptococcus fecalis* membrane ghosts[2]. The magnitude of increase in conductance was dependent on the presence of Mg^{2+} and upon the Na^+ and K^+ concentrations in the range of 10^{-2}–10^{-1} M. An additional 10-fold increase in conductance was observed when ATP was added to the interactant system. The characteristics of this interaction led to the suggestion that the bilayer–ATPase interactant complex might be similar in structure and properties to the membrane–ATPase complex in the intact organism.

The solubilized ATPase has since been highly purified and the interaction between this material and phospholipid bilayers has been studied in considerable detail. Binding studies using aqueous dispersions of phosphatidylcholine have demonstrated the existence of a bilayer–ATPase complex (unpublished observations).

In the present paper, we describe the further study of the electrical changes which take place as a result of the bilayer–ATPase interaction. The membrane system chosen for this investigation, namely the planar bilayer formed from synthetic diphytanoyl–phosphatidylcholine in decane, has been characterized and its properties reported earlier[3]. The reproducibly high specific resistance and excellent chemical and mechanical stability of these membranes provide an ideal system in which to study the conductance change accompanying lipid–protein interactions. In the initial report of this interaction, it was demonstrated that the direct current conductance of the interactant membrane is an intrinsic property of the bilayer region[1]. The bilayer–ATPase system is therefore relatively well defined and an attractive model for the study of lipid–protein interactions at the membrane level.

MATERIALS AND METHODS

The ATPase preparations employed in this study were first separated on DEAE-cellulose and then subjected to molecular sieve chromatography on an Agarose A 1.5 m column (Bio-Rad, Richmond, Calif.), following the procedure of Schnebli and Abrams[4]. A typical absorbance tracing of the Agarose column effluent is shown in Fig. 1. This separation is qualitatively similar to that reported

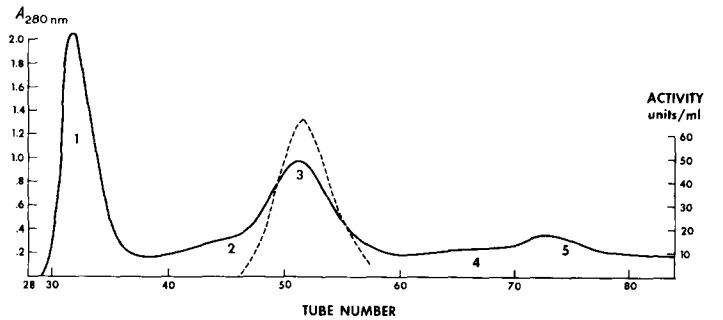

Fig. 1. Elution of ATPase from an Agarose A 1.5 m column in Tris–$MgCl_2$ buffer. The enzyme activity is represented by the dotted line.

by Schnebli and Abrams[4]. Fractions under Peak 3 between Tubes 51 and 54 had a constant specific activity of ATPase corresponding to 65 units per absorbance unit at 280 nm; where one unit of enzyme activity is defined as that amount of enzyme which catalyzes the release of 1 μmole of inorganic phosphate from ATP per min at a Mg–ATP concentration of 5 mM at 38 °C. Enzyme assays were carried out according to the method of Abrams[2]. All other fractions under Peaks 1, 2, 4 and 5 (Fig. 1) contained no measurable ATPase activity. The enzyme was stored at 4 °C in Tris–$MgCl_2$ buffer[4], which consisted of 0.01 M $MgCl_2$ and 0.02 M Tris titrated to pH 7.2 with HCl at 22 °C. The ultraviolet spectrum of the enzyme in this buffer was identical to that reported for the purified protein[4].

Bilayer membranes were formed by the brush technique from chromatographically pure synthetic 1,2-diphytanoyl-3-*sn*-phosphatidylcholine in *n*-decane at a concentration of 6 mg/ml. The bilayers were formed on a 1.3 mm diameter aperture in the teflon cell described earlier[1]. The membranes were observed with reflected

light through a low power telemicroscope and their areas estimated with the aid of a calibrated reticule in the microscope eyepiece. The electrical system employed in this study was similar to apparatus used to characterize diphytanoyl-phosphatidylcholine bilayers[3]. A Keithly Model 610 electrometer was used to measure transmembrane potential differences. In most cases, reversible Ag–AgCl electrodes were used to monitor membrane conductance. The aqueous phase generally consisted of 0.1 M NaCl in Tris–$MgCl_2$ buffer. Experiments were conducted at 22 ± 0.5 °C. Measurement of the net volume flux through the bilayer in the presence of an osmotic pressure gradient was carried out using a cell described by Huang and Thompson[5]. Solutions in both types of cells were stirred magnetically.

RESULTS

The time course of the rise in transmembrane current at constant voltage after the addition of the soluble ATPase to the aqueous solution on one side of the bilayer is depicted in Fig. 2. The initial rapid increase in conductance was usually followed by a slow rise until a constant value was attained. In some cases, it was impossible to measure the maximum conductance owing to the decreased mechanical and electrical stability of the membrane in the presence of the ATPase. In addition to the large increase in the membrane conductance, a concomitant decrease in the membrane specific capacitance from 0.40 to 0.36 $\mu F \cdot cm^{-2}$ was observed.

The time lag between addition of ATPase and increase of bilayer conductance

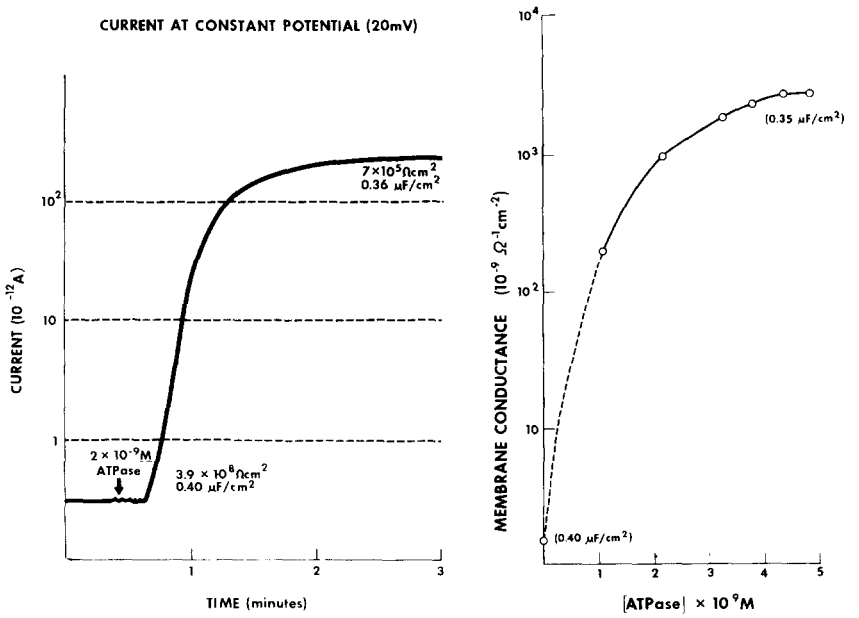

Fig. 2. Increase in membrane current under 20 mV voltage clamp after the addition of ATPase to the outer chamber. Aqueous phase: 10^{-2} M KCl, 10^{-2} M NaCl in Tris–$MgCl_2$ buffer.

Fig. 3. Effect of ATPase concentration on the membrane conductance. The dotted line indicates that it was not possible to measure stable conductance values below 10^{-9} M ATPase.

appeared to be determined largely by the diffusion time of the protein towards the membrane. This could be minimized by slowly injecting the ATPase through a fine hypodermic needle directly at the bilayer membrane. With this method of addition however, an open circuit potential, of several mV magnitude lasting a few minutes, frequently developed unless the composition of the enzyme buffer solution was similar to that surrounding the bilayer. The volume of ATPase solution added in a typical experiment was less than 10 μl compared with 30 ml for the solution surrounding the bilayer. Consequently, no significant perturbation in the equilibrium ionic composition of the buffer resulted from the addition of the ATPase solution.

The dependence of the bilayer conductance on the concentration of soluble ATPase is illustrated in Fig. 3. The data were obtained on a single bilayer membrane with successive additions of the ATPase to the outer chamber. Protein was added to the bulk solution with constant stirring, and bilayer conductance recorded when it reached a stable value. Each point, therefore, represents the equilibrium conductance value. Although the magnitude of the conductance change varied between bilayers for a given ATPase concentration, the saturation effect was reproducible.

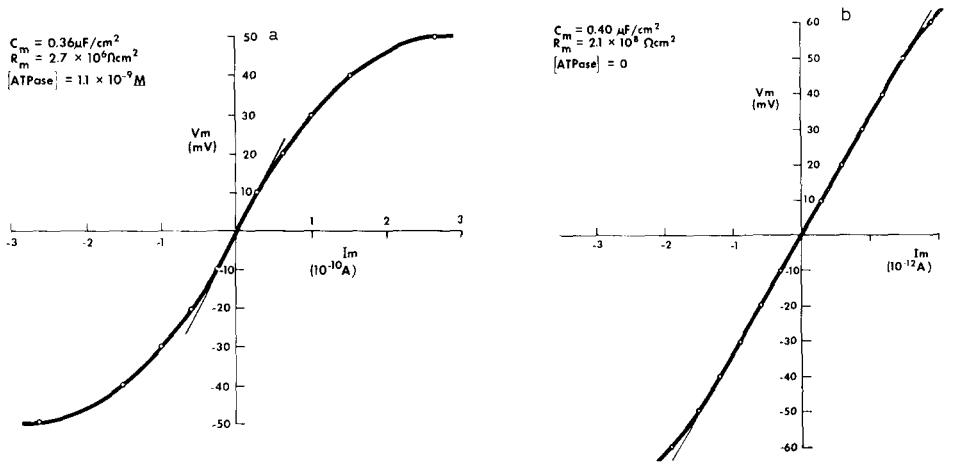

Fig. 4. Current–voltage characteristic of a diphytanoyl–phosphatidylcholine membrane. (a) in the presence of ATPase; (b) prior to the addition of the enzyme. Bilayer area was $7.1 \cdot 10^{-3}$ cm^2 in both cases. Aqueous phase: 10^{-2} M KCl, 10^{-2} M NaCl in Tris–$MgCl_2$ buffer.

The voltage–current curve for the bilayer–ATPase system is illustrated in Fig. 4a. It may be directly compared with the curve in Fig. 4b for the unmodified diphytanoyl–phosphatidylcholine bilayer obtained prior to the addition of ATPase. In this experiment, membrane resistance at zero applied voltage dropped from $2.1 \cdot 10^8$ to $2.7 \cdot 10^6\ \Omega \cdot$cm^2 on the addition of ATPase while the span of ohmic behavior, centered about zero voltage, decreased from almost 100 mV to about 20 mV. It can also be seen that in the ATPase-treated bilayer there was a marked continuous decrease in resistance at voltages greater than ± 50 mV. Dielectric breakdown of the interactant membrane frequently occurred around 60 mV or slightly higher. In contrast, the dielectric breakdown voltage for untreated diphytanoyl–phosphatidylcholine membranes has been found to be about 200 mV[3].

The time course of increase in transmembrane current at constant voltage in a typical experiment is presented in Fig. 5. ATPase was added to the bulk solution with constant stirring. Following a short time lag, intermittent conductance changes were observed. These conductance increments increased both in magnitude and

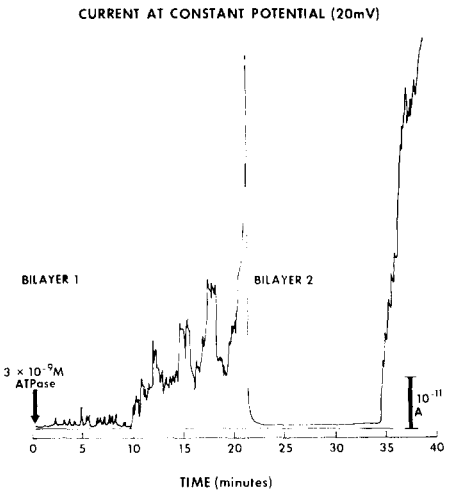

Fig. 5. Recorder tracing of current under 20 mV voltage clamp following membrane interaction with ATPase. The first bilayer was formed prior to the addition of the ATPase, allowed to interact for 20 min, then broken deliberately. Membrane 2 was formed immediately from fresh lipid in the presence of the soluble ATPase. Both traces show the current fluctuations referred to in the text.

frequency until the characteristic rapid rise in conductance occurred. In this experiment, the first bilayer was broken deliberately, and another membrane was formed immediately from fresh lipid using the brush technique. Unlike the rapid formation of pure diphytanoyl–phosphatidylcholine bilayers, the drainage of the membranes formed in the presence of the ATPase was always very slow. In the experiment presented in Fig. 5, approximately 15 min elapsed under 20 mV voltage clamp before the first observable optically black region appeared in the membrane, whereupon a rapid increase in the membrane conductance occurred. The slow formation of the bilayer region is probably the result of adsorption of the ATPase at the lipid–water interfaces. The observation that no increase in membrane conductance occurred until bilayer regions appeared in the thick film strongly suggests that the membrane–ATPase interaction leading to increased conductance is a bilayer phenomenon. It may be seen in Fig. 5 that the increase in membrane conductance following addition of ATPase to the system was not a continuous process but rather proceeded by the propagation of discrete conductance increments[6]. This phenomenon is better illustrated in Fig. 6, which is a current record of a bilayer–ATPase interactant system under 40 mV voltage clamp. At this extremely low concentration ($2 \cdot 10^{-10}$ M) of ATPase in the aqueous solution, the membrane conductance fluctuated between discrete levels. The start of the trace shows the switching transient following the application of the 40 mV bias. The initial level of the current was higher than that expected for the unmodified diphytanoyl–phosphatidylcholine bilayer. Superimposed

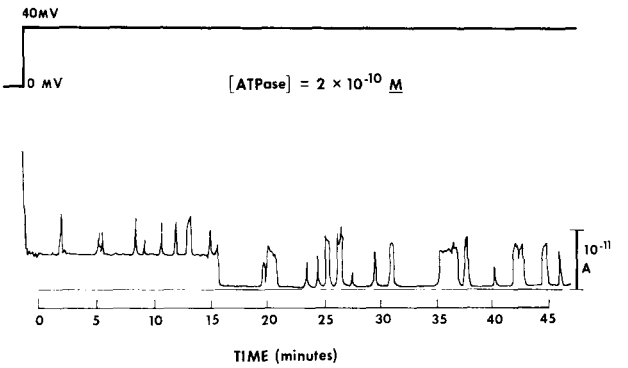

Fig. 6. Current record showing discrete current fluctuations for a bilayer–ATPase interactant system under 40 mV voltage clamp. Further description in the text.

on this higher conductance state were intermittent bursts of current with short duration. After approximately 20 min, the current trace returned abruptly to the basal value for the untreated bilayer. The remainder of the record illustrates the random appearance of these current bursts above the basal conductance level of the bilayer. These current fluctuations have been measured in numerous bilayers and with different ATPase preparations. It appears that the most frequently occurring

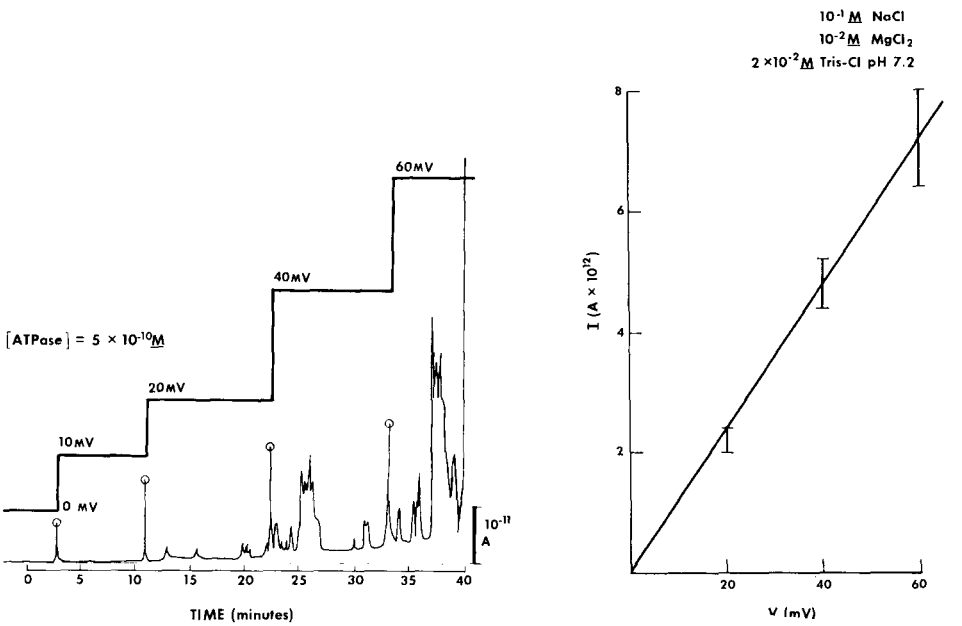

Fig. 7. Typical current response to a stepwise increase in voltage bias on a diphytanoyl–phosphatidylcholine bilayer in the presence of a low concentration of ATPase. The current spikes marked by small circles are partial traces of the switching transients.

Fig. 8. Current–voltage data for most commonly occurring current fluctuations in diphytanoyl–phosphatidylcholine bilayers treated with low concentrations of the soluble ATPase. ATPase concentration about 10^{-10} M.

current blip corresponds to a conductance increment of about $1.2 \cdot 10^{-10}\ \Omega^{-1}$, although half multiples of this value have been observed. It has generally been found that the conductance fluctuations are integral multiples of the lowest common conductance increment.

The effect of changing the voltage bias on the membrane in the presence of a low concentration of the soluble ATPase is illustrated in Fig. 7. It is apparent that increasing the voltage on the membrane increased both the incidence and duration of the current increments, with the result that the integral of the area under the curve was a strong function of voltage.

Fig. 8 is a current–voltage plot constructed from several traces similar to Figs 6 and 7. The data presented depict the most frequently occurring current blips at each voltage. The error bars reflect the variation in magnitude of these fluctuations. These results are reminiscent of the quasi-quantized conducting channels which have been observed in bilayer membranes doped with small amounts of excitability-inducing material[7–9]. Although the magnitude of the conductance of the ATPase "channel" was similar to that reported for the excitability-inducing material system, the conductance of the channel was apparently not voltage-dependent. The aqueous electrolyte surrounding the bilayer membrane has been varied slightly in these studies, but the quasi-quantized current increments have still been observed in most systems when an extremely low concentration of the soluble ATPase was added. Fig. 9 illustrates these phenomena in the electrolyte solution: 10^{-1} M KCl, 10^{-2} M

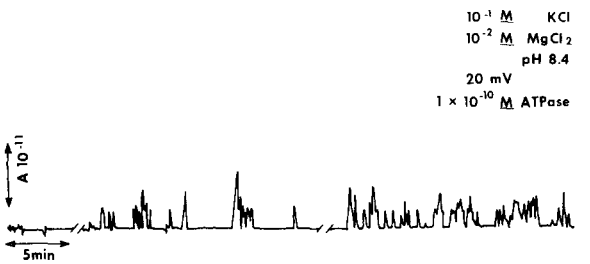

Fig. 9. Current fluctuations for a diphytanoyl–phosphatidylcholine bilayer interacting with ATPase in 10^{-1} M KCl and 10^{-2} M $MgCl_2$, no buffer present.

$MgCl_2$ at pH 8.4 with no buffer present. The most common current increment in this record corresponds to a channel of about $1.4 \cdot 10^{-10}\ \Omega^{-1}$ conductance, similar to the value for the previously described system (Fig. 8). These discrete current increments have been seen with the purest ATPase preparations made, and appear to be an intrinsic property of the bilayer–ATPase interaction. The reason for the shape of the current–voltage curve for the diphytanoyl–phosphatidylcholine bilayers treated with relatively high concentrations of the ATPase (Fig. 4a) may well be the phenomenon of voltage-dependent opening of the conducting channels. This situation is the converse of the voltage-dependent closing of the excitability-inducing material channels which accounts for the negative resistance characteristics of bilayers treated with larger amounts of excitability-inducing material[9].

An investigation of the nature of the current carriers in the conducting channels of the diphytanoyl–phosphatidylcholine bilayers treated with the ATPase has been undertaken. In the first series of experiments, aliquots of HCl were added to the outer

chamber and the pH read directly with a Radiometer pH meter. The differential potential which developed across the bilayer membrane was recorded with the Keithly electrometer at zero current. Addition of the soluble ATPase to the system lowered the membrane resistance, and also caused the membrane potential to decay to zero. In a similar series of experiments, equilibrium membrane potentials were established in response to transmembrane salt gradients and the membrane resistance and membrane potential were recorded. It was found that the addition of the ATPase to the aqueous solution lowered the membrane resistance and changed the membrane potential. In each case, the new membrane potential was identical to the apparent liquid junction potential for the system; no change of potential occurred following breakage of the bilayer.

TABLE I

THE EFFECT OF ATPase ON THE MEMBRANE POTENTIAL GENERATED BY A pH GRADIENT (EXPT I) AND A SALT GRADIENT (EXPT II)

Aqueous phases contain 0.02 M Tris and 0.01 M $MgCl_2$ at 22 °C. The same ATPase preparation was used in both studies.

Expt	*Outside compartment*		*Inside compartment*		*ATPase concn* (*M*)	R_m ($\Omega \cdot cm^2$)	V_m (*mV*)
	Salt	*pH*	*Salt*	*pH*			
Ia	0.1 M KCl 0.1 M NaCl	7.24	0.1 M KCl 0.1 M NaCl	7.24	0	$1.38 \cdot 10^8$	0
Ib	0.1 M KCl 0.1 M NaCl	7.08	0.1 M KCl 0.1 M NaCl	7.24	0	$1.38 \cdot 10^8$	7
Ic	0.1 M KCl 0.1 M NaCl	6.92	0.1 M KCl 0.1 M NaCl	7.24	0	$1.38 \cdot 10^8$	12.5
Id	0.1 M KCl 0.1 M NaCl	6.92	0.1 M KCl 0.1 M NaCl	7.24	$1 \cdot 10^{-9}$	$4 \cdot 10^6$	0
IIa	0.1 M KCl	7.2	0.1 M KCl	7.2	0	$3.6 \cdot 10^8$	0
IIb	1.0 M KCl	7.2	0.1 M KCl	7.2	0	$3.6 \cdot 10^8$	15
IIc	1.0 M KCl	7.2	0.1 M KCl	7.2	$1 \cdot 10^{-9}$	$2.9 \cdot 10^5$	0

The data from representative experiments are listed in Table I. The conclusion drawn from these results is that the bilayer–ATPase interactant system contains channels with the conductance properties of the bulk aqueous solution surrounding the membrane. If such channels exist in the bilayer–ATPase system, it is reasonable to expect an increase in the water filtration coefficient of the membrane. Accordingly, the water permeability of the bilayer was measured under an osmotic pressure gradient. No change in water flux was observed when ATPase was added to systems containing NaCl or urea concentration gradients. However, in several experiments using glucose concentration gradients to create the osmotic pressure differences, a significant increase in water flux occurred when ATPase was added to the system. Typical results are presented in Table II.

TABLE II

THE EFFECT OF ATPase ON THE MEMBRANE OSMOTIC PERMEABILITY

Electrolyte: 10^{-1} M KCl, 10^{-2} M $MgCl_2$, pH 7.5. 1.0 M glucose added to outside compartment at start of experiment.

Temperature (°C)	*ATPase concn* (*M*)	A_m (10^{-3} cm^2)	*Volume flow* (10^{-5} $cm^3 \cdot min^{-1}$)
21.5	0	4.8	1.2
	10^{-9}	4.8	2.6
22.0	0	4.2	1.0
	10^{-9}	4.5	2.5
22.5	0	5.8	1.5
	10^{-9}	6.9	4.2

DISCUSSION

Throughout this study the exact identity of the interactant species which caused an increase in bilayer conductance has been under investigation. The ATPase preparations used in the preliminary study were relatively impure and of low specific activity. Consequently, until the ATPase was purified there existed the possibility that the "electro-active" component was a contaminating protein in the ATPase preparation. It was found, however, that none of the fractions from the Agarose A 1.5 m column (Fig. 1), except those which contain ATPase activity lowered the resistance of the diphytanoyl–phosphatidylcholine bilayer. The purification procedure of Schnebli and Abrams[4] involves a further column step using DEAE 2 cellulose at pH 6.2. ATPase preparations collected from this further stage had a slightly higher enzyme specific activity but no significantly different interaction with bilayer membranes. Furthermore, no material collected from the second DEAE-cellulose column other than the ATPase interacted with bilayers.

It is conceivable that the ATPase contains bound lipid, and this may be the component in the preparation responsible for lowering bilayer resistance. This possibility has been rejected, however, following lipid extraction of the enzyme at −20 °C with ethanol–ether (1:1, v/v). The ATPase which was recovered from this procedure retained its activity on the bilayer and no detectable lipid was found in the organic solvent.

Denaturation of the enzyme by boiling destroyed its capacity to lower bilayer resistance. It is, therefore, probable that the structure of the native enzyme is important for the bilayer interaction. In fact, there appears to be a semiquantitative dependence of the bilayer interaction on the enzymatic activity of the ATPase. It was reported earlier that ATPase preparations which were older than 3–4 weeks progressively lost both enzymatic activity and ability to lower bilayer resistance. Proteolytic destruction of the enzyme also removed the bilayer activity[1]. The addition of 0.1% protease (Sigma) to the buffer solution bathing the diphytanoyl–phosphatidylcholine bilayer prevented the lowering of the membrane resistance by the subsequent addition of ATPase. In contrast, the proteolytic enzyme had no effect

when it was introduced into the system after membrane resistance had been decreased by addition of the ATPase.

Nectin, described by Baron and Abrams[10], is a protein which promotes binding of the solubilized ATPase to the streptococcal membrane. Preparations of ATPase containing nectin and nectin-free both produced a lowering of bilayer resistance.

On the basis of the experimental evidence, it is reasonable to conclude that the molecular species which lowers bilayer resistance is the ATPase and not a contaminating component of the enzyme preparation. However, the molecular state of the protein which interacts with the bilayer is not known, except that a prerequisite for bilayer activity is enzymatic activity. It is conceivable that aggregates of ATPase become incorporated in the membrane and increase membrane conductance by formation of a conducting channel through the bilayer. At low collision frequency these interactions could explain the discrete conductance channels observed. In support of this hypothesis, previous studies have shown that random currents appear at the polarized mercury–water interface in the presence of particulate preparations of ATPase[11,12].

In order to test whether the active species were particulate in nature, the stock solution of enzyme was centrifuged for 1 h at 40000 rev./min in the cold. Subsequent enzyme analysis revealed only a slight decrease in ATPase activity in the supernatant. The extent of interaction between bilayer membranes and ATPase, before and after centrifugation, was quite similar (see Table III). This result would appear to rule out the possibility that large aggregates of the ATPase in the aqueous solution were responsible for the bilayer effect.

The effect of the streptococcal ATPase on the membrane resistance bears some similarity to the interaction between bilayer lipid membranes and the (Na^+, K^+)-

TABLE III

THE EFFECT OF ATPase ON THE MEMBRANE RESISTANCE

Aqueous phases contain 0.1 M KCl and 0.01 M $MgCl_2$ at 22.5 °C. Two batches of ATPase, designated I and II, taken from separate Agarose purification steps were used for these studies.

Enzyme preparation	*pH*	*ATPase concn (M)*	R_m ($\Omega \cdot cm^2$)
ATPase (I)	5.9	0	$3.6 \cdot 10^8$
	5.9	$7.6 \cdot 10^{-9}$	$3.5 \cdot 10^8$
ATPase (I)	8.5	0	$1 \cdot 10^8$
	8.5	$2.3 \cdot 10^{-10}$	$6.6 \cdot 10^5$
ATPase (II)	8.2	0	$1.5 \cdot 10^8$
	8.2	$5 \cdot 10^{-10}$	$2.7 \cdot 10^5$
ATPase (II)	8.1	0	$2 \cdot 10^8$
Centrifuged for 1 h at 40000 rev./min	8.1	$2.6 \cdot 10^{-10}$	$3 \cdot 10^6$
ATPase (II)	7.8	0	$4 \cdot 10^8$
Centrifuged for 1 h at 40000 rev./min	7.8	$7.2 \cdot 10^{-10}$	$5 \cdot 10^5$

ATPase from rat brain, reported by Jain *et al.*[13,14]. However, these authors have observed the production of a short circuit current through the membrane when ATP was added to the chamber containing the ATPase. They concluded that the modified bilayer may contain a reconstituted "cation pump". The asymmetry of the pump with respect to its interaction with substrates and cofactors, together with the elimination of the short circuit current by ouabain, provided strong support for this hypothesis. Jain *et al.*[14] suggested that the asymmetry of the reconstituted cation pump may result from the fusion with the bilayer of membrane-derived vesicles of built-in asymmetry. It is indeed probable that their system consisted of active fragments of the biological membrane incorporated asymmetrically into the lipid matrix of the bilayer, and, consequently is of considerable physiological importance. In contrast, the present work is an investigation of the interaction of a purified membrane protein with the lipid bilayer structure.

In the preliminary investigation, it was found that the soluble ATPase interacts with bilayers formed from the total lipid extract of the streptococcal membrane ghosts to lower the membrane resistance by a factor of 10^3–10^4 (ref. 1). The physical properties of these bilayers were difficult to reproduce, probably due to the complex lipid composition and chemical instability at room temperature[15,16]. It was found, however, that the characteristics of the bilayer–ATPase interaction were similar to those observed with phosphatidylcholine membranes. In the later studies, diphytanoyl–phosphatidylcholine was chosen as the membrane-forming lipid because of the high mechanical stability and reproducible electrical properties of the resulting bilayers[3]. The high specific resistance of these bilayers permits convenient detection of the discrete conductance changes of small magnitude, which appear in the presence of very low concentrations of the soluble ATPase. The first reported observations of discrete conductance changes in lipid bilayers were for the excitability-inducing material system[8,9]; recently it has been found that these phenomena are also produced by low concentrations of certain antibiotics[17,18]. A unique characteristic of the quasi-quantized current changes observed in the bilayer–ATPase system is their relatively long duration, see Figs 6 and 9.

The nature of the bilayer-ATPase interaction is not clear, but the present results indicate the existence of relatively stable conducting channels in the membrane. Furthermore, the increased osmotic permeability and absence of ionic selectivity in the interactant bilayer suggest that the channels may be relatively wide aqueous-filled pores. It is thought that the rise in membrane conductance during the reaction is caused by an increase in the number of the discrete channels, rather than by an increase in the size of the existing conducting pathways. The voltage-dependent appearance of these channels may be indicative of a field-dependent interaction between the bilayer and the ATPase. Whether this is the initial penetration of the ATPase into the bilayer or a subsequent field-dependent transition between non-conducting and conducting conformations[18,19] cannot be resolved. The latter case seems, however, more likely on the basis of the available data. That there is a strong influence of the electric field on the bilayer–ATPase interactant system is indicated by its relatively low dielectric breakdown potential. It also appears unlikely that supramolecular complexes of the ATPase combine in the planar bilayer to form the conducting channel, nevertheless this possibility cannot be excluded.

The soluble ATPase has a molecular weight of 385000 and contains two

kinds of subunits, different in composition, but similar in size, totaling 12 polypeptide chains per molecule[20]. Schnebli *et al.* [20] have proposed a model for the ATPase molecule based on gel electrophoresis and electron microscopy of the purified enzyme. It consists of a planar hexagonal array of 6 globules, each containing one of the α and β subunits; the diameter of each globule is about 40 Å and the longest dimension across the hexagon is about 120 Å. The incorporation of the ATPase molecule into a bilayer membrane of approximately 62 Å thickness[21] would be expected to cause a local perturbation of the passive electrical properties of the lipid bilayer; a single enzyme molecule lying in the plane of the bilayer could almost span the membrane and provide a conducting pathway through the protein.

Abrams and Baron[22] have shown that, when the soluble ATPase was added back to the depleted *S. fecalis* ghosts, the enzyme and membranes recombined. The addition of Mg^{2+} was necessary for full binding activity, and the amounts of bound ATPase in the fully reconstituted and in the native membrane–ATPase complex were the same. From these reconstitution studies it appears that the ATPase may be reinserted into specific sites on the membrane in the presence of Mg^{2+}. While such sites would not exist in the diphytanoyl–phosphatidylcholine bilayers, it is interesting that the increase in membrane conductance in the bilayer–ATPase system exhibits a saturation effect with respect to the concentration of soluble ATPase added (Fig. 3). The ATPase is highly acidic[23] and its incorporation into the bilayer could produce a significant surface charge. Electrostatic phenomena may, therefore, be responsible for the saturation effect at high ATPase concentration.

The bilayer–ATPase interaction does not appear to be strongly dependent on the salt concentration in the range 10^{-2}–10^{-1} M and similar membrane conductances are obtained in aqueous solutions which contain either NaCl or KCl in the Tris–$MgCl_2$ buffer. The pH of the aqueous phase is important, however, and little or no effect of the ATPase on the bilayer conductance can be seen below pH 6 (Table III). The pH dependence of the conductance increase could indicate an electrostatic interaction between the bilayer and ATPase molecules, but this has not been confirmed in parallel studies with the ATPase and uncharged liposomes, generated from hen egg yolk phosphatidylcholine in Tris–$MgCl_2$ buffer. In this system, the binding of the ATPase to the lipid bilayers was not significantly reduced by raising the ionic strength. Electrostatic interactions are, therefore, probably not the predominant forces between phosphatidylcholine bilayers and the ATPase. It is anticipated that the molecular subunits of the ATPase will also interact with the bilayer membrane. Further information regarding the nature of the bilayer–ATPase interaction is expected from this approach and, accordingly, we plan to study the lipid–polypeptide interactions in the bilayer using the enzyme subunits. It will be interesting to compare the results with the preceding data for the enzyme and also the available data for synthetic polypeptides[24].

There is ample evidence that the membrane ATPase of *S. fecalis* is involved in the active transport of monovalent cations and other solutes across the plasma membrane[25–27]. The plan for continued research on this problem includes an investigation of the bilayer–ATPase system for possible excitability characteristics, and a further study of the interesting effect of ATP on the membrane conductance in the interactant system.

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