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A STUDY OF THE SURFACE-ACTIVE PROPERTIES OF THE Mg^{2+}-ACTIVATED ATPase FROM CYTOPLASMIC MEMBRANES OF *STREPTOCOCCUS FAECALIS*

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Summary

The surface activity and enzymic properties of the factor F_1, the catalytic moiety of *Streptococcus faecalis* H^+-ATPase, has been studied at the air-water and phospholipid-water interfaces. F_1 does not interact with the monolayer phospholipids, hence its adsorption on a biological membrane must be due mainly to its recognition of proteins of the hydrophobic complex. The dimensions of the F_1 molecule at the air-water interface have been estimated. In the presence of Mg^{2+}, base area is $S = 1.8 \cdot 10^4$ Å^2, height $h = 27$ Å. Bearing in mind the size of a globular subunit, it follows from the measurements that the major F_1 subunits should all lie in the same plane. The ATPase activity of F_1 at the interface is inversely proportional to the monolayer density. With low density monolayer, the specific ATPase activity is higher at the interface than in the bulk of the solution.

Adsorption of F_1 at the interface shifts the isoelectric point of the protein, apparently due to changes in its conformation. The findings are discussed relative to the proton-active transport mechanism.

Introduction

Streptococcus faecalis possesses an active proton transport system [1] involving membrane ATPase of which F_1, the catalytically active factor has

Abbreviation: F_1, this refers to the catalytic moiety of the *Streptococcus faecalis* H^+-ATPase.

now been isolated in a pure state [2,3]. According to its solubilization behaviour [4], this enzyme is a membrane surface protein. We have therefore studied its properties under conditions approaching those of a membrane, i.e. in the air-water and water-phospholipid interfaces.

Materials and Methods

A Teflon trough (Fig. 1) was so constructed as to permit the formation of protein, and mixed protein-phospholipid monolayers. In the first case, one of its two compartments (S = 25 cm^2, V = 12 ml) was filled with the aqueous solution and concentrated protein solution was added through an inlet in the side oriented so that solution could be added or removed without damaging the monolayer. The build-up of the monolayer was followed during the entire period of its formation by measuring the surface pressure (π) and surface potential (ΔV). After the monolayer had formed as judged by surface pressure (π = const.) and surface potential (ΔV = const.) an aliquot of the solution from under the monolayer was pipetted off through the inlet to determine the amount of protein absorbed on the layer. This was assessed from the loss in enzymic activity in the aliquot as compared to the initial solutions

$$C = \frac{E_1 - E_2}{\gamma}$$

where C = amount of protein in the monolayer, E_1 = number of units of activity originally added, E_2 = number of activity units remaining, γ = specific activity of the enzyme. Control measurements showed that factor F_1 was not inactivated during formation of the monolayer.

Adsorption of F_1 on the phospholipid monolayer was studied by filling the trough with buffer solution (see Fig. 1) and applying to the surface the requisite amount of phospholipids dissolved in a 4 : 1 (v/v) mixture of *n*-heptane/ethanol. The phospholipid monlayer was compressed to stretch over only one of the compartments and these were disconnected by drawing off the buffer

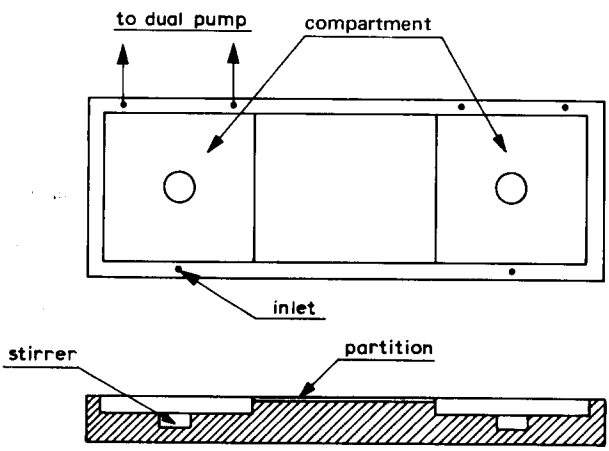

Fig. 1. Trough for measuring protein adsorption.

until below the partition. The ATPase was then introduced under the monolayer and measurements of its adsorption were performed as described in the air-water interface experiments.

To determine specific activity of F_1 in the monolayer the solution under it was replaced with fresh buffer by means of a LKB 4912 A dual pump. One channel of the pump was used for removing the solution from under the monolayer, the other for adding the new buffer. It was found that the amount of protein left in the bulk phase became negligible after washing 5 times with fresh buffer. A concentrated ATP solution was then fed into the lower layer and aliquots were taken at equal intervals to determine the amount of inorganic phosphate formed.

The surface potential was measured by the dynamic capacity procedure [5] and the surface pressure measured as described by Wilhelmy (unpublished). Both parameters were continuously recorded during the experiment. The air-water interface was considered pure if no changes in the surface potential or surface pressure occured in the course of 1 h. The change of buffer under the monolayer did not affect its parameters.

Experimental error: surface potential = ±10 mV; surface pressure = ±0.2 dyne/cm.

Isolation of the enzyme used in this work and its chemical characteristic were described earlier [3]. The specific activity was 40 units/mg. 1 unit of activity is the quantity of ATP in μM, hydrolyzed in 1 min by the enzyme. The ATPase activity was measured at 25°C in a solution containing 5.0 mM ATP, 2.5 mM $MgCl_2$, 5.0 mM Tris-HCl (pH 7.8), by following the changes in inorganic phosphate concentration [6]. Protein concentration was measured according to Lowry et al. [7]. The total phospholipid fraction was extracted from *St. faecalis* cytoplasmic membranes as described by Folch [8]. All reagents used in the experiments were of analytical grade. ATP was obtained from Calbiochem, Tris was from Sigma Chemical Co. Water was double distilled before use.

Results

The protein monolayer is spontaneously formed at the interface by positive adsorption. Fig. 2 shows the changes in the surface potential and surface pressure versus time for varying enzyme concentrations in the subphase (with and without Mg^{2+}). The curves show that F_1 has a considerable surface activity; in the presence of Mg^{2+} π_{max} = 23 dyne/cm (ΔV = 160 mV; the sign of the potential is with reference to the aqueous phase); in the absence of Mg^{2+} π_{max} = 20 dyne/cm (ΔV = 220 mV).

Fig. 3 shows the dependence of the surface concentration of F_1 on its concentration in the bulk solution. Adsorption of the enzyme at the air-water interface is practically irreversible and no desorption of the protein occurred after changing the bulk solution. Fig. 3 shows that the maximum concentration of F_1 on the surface was 0.32 and 0.5 $\mu g/cm^2$ in the presence and absence of Mg^{2+}, in the subphase respectively. Based on these data, it is easy to calculate the maximal area occupied by the enzyme molecule on the surface, if it is taken into account that the molecular weight of F_1 is 360 000 [3]. The value thus

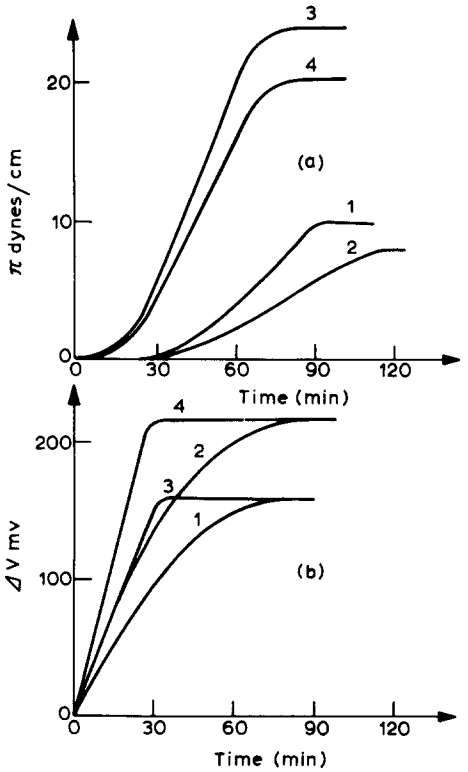

Fig. 2. The time dependence of the surface pressure (a) and surface potential (b) during the adsorption of F_1 at the air-water interface. Bulk solution 5 mM Tris-HCl (pH 7.8), 25°C. 1, 2 = F_1 (1 $\mu g \cdot cm^{-3}$); 3, 4 = F_1 (2 $\mu g \cdot cm^{-3}$); 1, 3 = 2.5 mM $MgCl_2$; 2, 4 = Mg^{2+}-free solution.

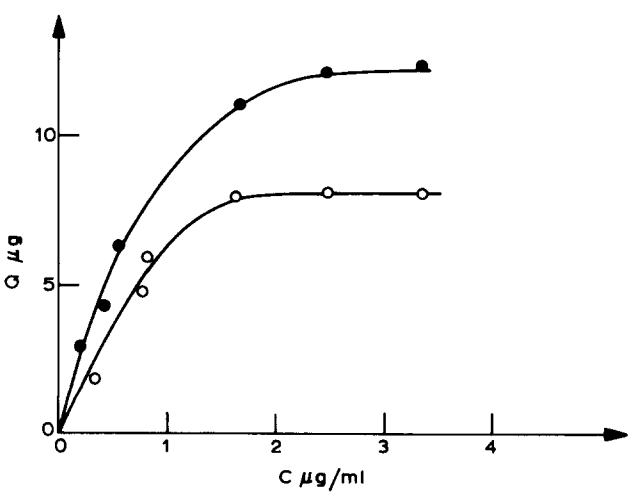

Fig. 3. The dependence of the surface concentration of F_1 on its concentration in the bulk solution containing 5 mM Tris-HCl (pH 7.8), 25°C. The duration of adsorption is 90 min, the monolayer area 25 cm^2. ○, 2,5 mM $MgCl_2$; ●, Mg^{2+}-free solution.

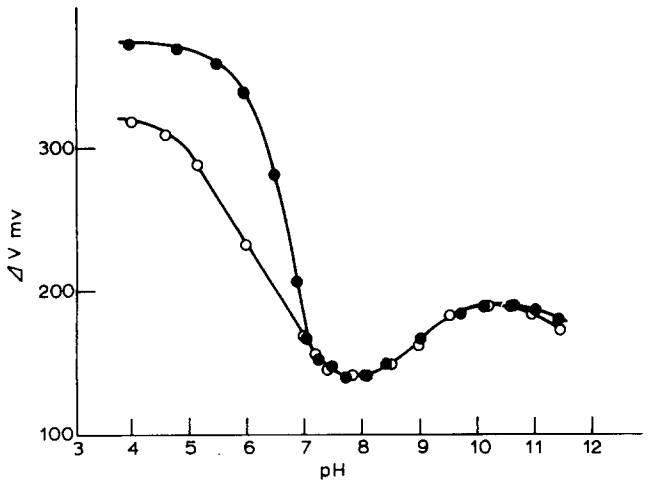

Fig. 4. The dependence of the surface potential of F_1 monolayer on the solution pH at various ionic strengths. Bulk solution 5 mM Tris, 2.5 mM $MgCl_2$, 25°C. Alkylation was performed with NaOH, and acidification with H_2SO_4. •, 100 mM KCl, ○, K^+-free solution.

calculated is $1.8 \cdot 10^4$ Å^2 in the presence of Mg^{2+} and $1.2 \cdot 10^4$ Å^2 in the absence of Mg^{2+} in the subphase. The height of the enzyme molecule can be estimated from the fact that the maximum protein concentration on the surface is approximately equal to the protein specific density multiplied by the monolayer thickness. Assuming the F_1 density to be 1.2 g/cm^3, we obtain a height of 27 Å with Mg^{2+}, and 42 Å without Mg^{2+}.

To determine the conformational changes of the enzyme on entering the air-water interface, we measured the dependence of the surface potential on solution pH at various ionic strengths subphase (Fig. 4). Fig. 4 shows that, in the pH range 7—11, the monolayer has zero net charge (σ). Indeed, the value of the surface potential, ΔV, can be written as follows:

$$\Delta V = V_d + \psi_0(\sigma, C) \tag{2}$$

where V_d = is obtained from the projection of the enzyme dipole moment on to the normal of the interface, ψ_0 = diffusion potential of the electric double layer, C = ionic strength of the subphase.

In our case, ΔV is independent of the ionic strength of the subphase at pH 7—11; therefore, within the given pH range, the enzyme molecule is of zero net charge.

The isoelectric point of F_1 in solution is in the range of pH 4—5 [3]. We can, therefore, definitely say that on adsorption on the air-water interface, the enzyme undergoes considerable conformational change, leading to the change in its isoelectric point.

The interaction between F_1 and phospholipids was studied by measuring its adsorption at the lipid-water interface at various initial densities, π_0, of the phospholipid monolayer. Fig. 5 shows the typical changes with time of the monolayer parameters in the process of adsorption of the enzyme on the monolayer. Fig. 6 shows, how, with increase in density of the lipid monolayer,

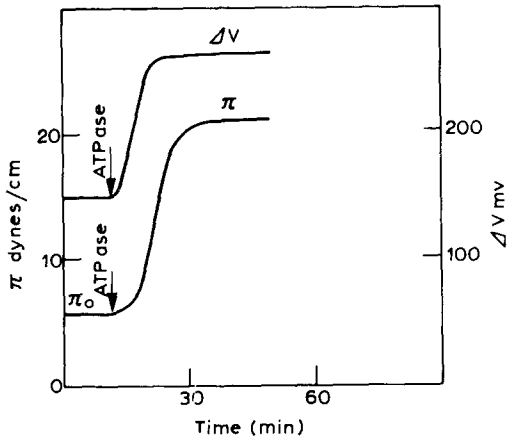

Fig. 5. Typical changes vs. time of the lipid monolayer parameters in the process of F_1 adsorption to the monolayer. Bulk solution F_1 (1 $\mu g \cdot cm^{-3}$), 5 mM Tris-HCl, 2.5 mM $MgCl_2$ (pH 7.8), 25°C.

both the increment, $\Delta\pi$, and the amount of adsorbed protein decrease. It is noteworthy that when $\pi_0 = 23$ dyne/cm (corresponding to π_{max} of the pure F_1 monolayer) adsorption of the enzyme is practically zero. It follows from this that (a) F_1 does not enter into noticeable hydrophobic interaction with the phospholipids, and (b) in its abilities to permeate the phospholipid monolayer at the air-water interface, the enzyme should be classified as a water-soluble protein [9].

The determination of the catalytic properties of an enzyme at a surface boundary should be made of the effect of the unstirred layer which limits the rate of approach of the substrate to the enzyme, i.e. the substrate diffusion rate should not be a limiting step in the ATPase reaction. Therefore,

$$\gamma_s \cdot C_s \leqslant DC/\delta$$

where γ_s = enzyme specific activity at the interface, C_s = enzyme surface con-

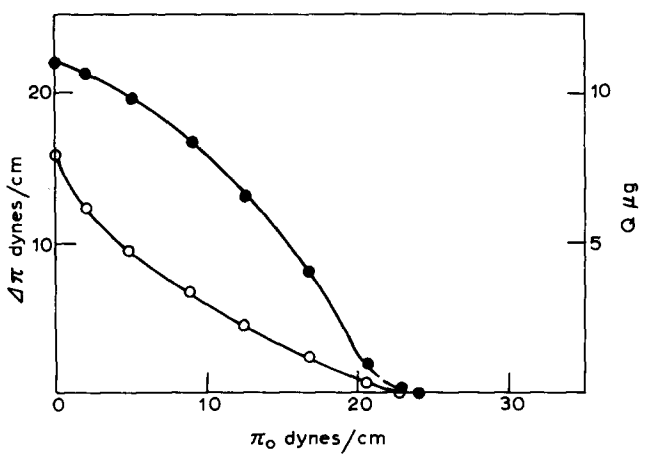

Fig. 6. The dependence of the amount of adsorbed protein (○) and of the final change of the surface pressure (●) on the initial surface pressure of the phospholipid monolayer in the process of F_1 adsorption. Bulk solution F_1 (1 $\mu g \cdot cm^{-3}$), 5 mM Tris-HCl, 2.5 mM $MgCl_2$, (pH 7.8), 25°C. Duration of F_1 adsorption was 90 min, the monolayer area is 25 cm^2.

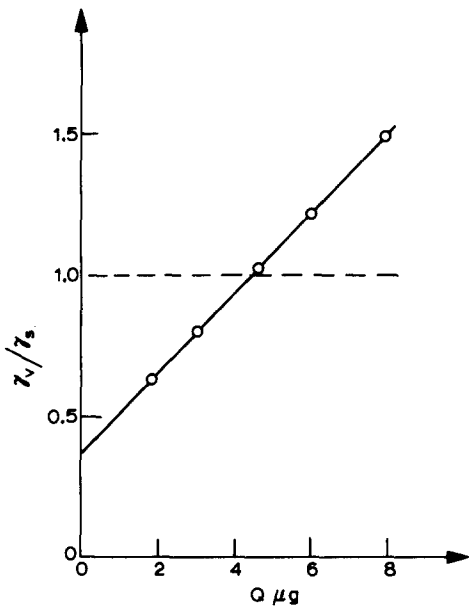

Fig. 7. The ratio between the specific ATPase activities in the bulk solution and at the air-water interface vs. the amount of the adsorbed F_1. ------, specific ATPase activity of F_1 in the water solution. The monolayer area is 25 cm^2.

centration, D = substrate diffusion coefficient, δ = thickness of the unstirred layer. Assuming that $D = 10^{-5}$ cm^2/s, $\delta = 10^{-2}$ cm^2, $C = 5.0$ mM ATP and C_s = 0.32 $\mu g/cm^2$, we obtain $\gamma_s = 1000$ units/mg. The influence of the unstirred layer can, therefore, be neglected under our conditions ($\gamma_s = 200$ units/mg).

Fig. 7 shows the results of measurement of the enzyme activity in the monolayer (unambiguous changes occurred in the surface potential and surface pressure during the enzymic reaction). It can be seen that the specific activity of ATPase at the interface is inversely proportional to the density of the monolayer. The observed change in the specific activity is due to charges in the packing density of the enzyme molecule in the monolayer and not to differences in the adsorption process. In fact, we have carried out the following experiment; a low density monolayer was formed at the interface, and it was then compressed to a higher density, and its activity measured. The specific activity of the compressed monolayer turned out to be the same as that of the monolayer obtained in the usual way. To elucidate the effect of the interface alone on the enzyme activity, we extrapolated the ATPase specific activity to zero protein surface density. As can be seen from Fig. 7, the specific activity of the enzyme at the interface is almost 3-fold greater than that in the bulk solution.

Discussion

It is well-known that the catalytic moiety of *St. faecalis* membrane ATPase, the F_1 factor, can be solubilized by washing the membranes with solutions of low ionic strength in the absence of divalent cations [4]. The Mg^{2+}-mediated

change observed in surface area of the interface occupied by an enzyme molecule suggests the following probably mechanism of detachment of the F_1 molecule from membrane: removal of the divalent cations from the solution induces conformational change in F_1 at the membrane surface. This leads to: (i) weakening of the hydrophobic enzyme-membrane interaction, and (ii) diminution of the ionic strength of the solution which result in enhancement of the electrostatic repulsion between F_1 and the proteins of the hydrophobic complex causing dissociation of the membrane-enzyme complex.

The absence of appreciable F_1-phospholipid interaction is indicative of the negligible role of phospholipids in the mechanism of the membrane adsorption of the enzyme. It is quite possible, however, that the phospholipids participate in formation of the structure of the hydrophobic protein complex. This complex is necessary for transport and synthetase activity of the whole ATPase complex and fulfills the role of receptor of the membrane.

According to the Mitchell hypothesis [10], the molecular mechanism of active proton transport may be represented as follows: The catalytic moiety of the ATPase complex is localized at the inner membrane-solution interface and is contiguous to the hydrophobic protein complex, forming a proton channel in the membrane. As a result of ATP hydrolysis, F_1 injects protons into the channel, along which they pass into the outer solution inducing, thereby an electrochemical H^+ gradient in the membrane.

In order for the ATPase complex to operate effectively by such a mechanism, it is necessary that the membrane-solution interface does not cause denaturation of the enzyme. The results we have presented regarding enzymic activity of F_1 at the interface show that this conditions is in fact fulfilled. The passage of F_1 to the interface causes its activation, apparently as a result of conformational changes at the surface. This is shown by the shift in the isoelectric point of F_1 to the alkaline pH range. The change in the enzyme conformation makes the catalytic site more accessible to the substrate, which is readily explained by the decrease in the electrostatic repulsion (relative to the buffer solution) between F_1 and ATP.

We now consider the molecular dimensions of F_1 in relation to its part as a subunit. Earlier, we showed that one molecule of the F_1 used in our present work consists of four types of subunit [3]: $\alpha = 55\,000$, $\beta = 51\,000$, $\gamma = 34\,000$ and $\epsilon = 13\,500$, in a ratio of 3 : 3 : 1 : 2 [3]. Consideration of the quaternary structure of F_1 leads to the conclusion that all the α- and β-subunits lie in a single plane. In conclusion, it may be said that the relationship found for the catalytic moiety of H^+-ATPase from *St. faecalis* should also hold for other H^+-ATPase (these enzymes have all similar subunit content and the same functions).

References

1 Harold, F.M. and Papineau, D. (1972) J. Membrane Biol. 8, 45–62
2 Scnebli, H. and Abrams, A. (1970) J. Biol. Chem. 245, 1115–1121
3 Babakov, A.V., Gavrilova, E.G., Gevondyan, H.M., Polishuk, G.I., Terekhov, O.P. and Usmanova, R.G. (1979) Bioorgan. Khim. 5, 611–620
4 Abrams, A. (1965) J. Biol. Chem. 240, 3675–3681

5 Babakov, A.V., Myagkov, I.V., Sotnikov, P.S. and Terekhov, O.P. (1972) J. Phys. Chem. USSR 46, 1873—1876
6 Fiske, C.H. and SubbaRow, V.J. (1925) J. Biol. Chem. 66, 375—378
7 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
8 Folch, J., Lees, M. and Standley, G.H.S. (1957) J. Biol. Chem. 226, 497—509
9 Quinn, P.J. and Dawson, R.M.C. (1970) Biochem. J. 116, 671—680
10 Mitchell, P. (1974) FEBS Lett. 73, 189—194