Modification of some properties of spherical lipid membranes induced by the association with fructose-1,6-bisphosphate aldolase

M. Langner °+, J. Gutowicz+ and J. Gomułkiewicz°

°*Institute of Physics, Technical University, ul. Wybrzeże Wyspiańskiego 27, 50-370 Wrocław* and +*Department of Biophysics, Medical Academy, ul. Chałubińskiego 10, 50-368 Wrocław, Poland*

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The influence of fructose-1,6-bisphosphate aldolase, as a membrane peripheral protein, on some electrical and transport properties of spherical lipid membranes was investigated. It was found that the association of the enzyme with the membrane did not effect markedly the electrical conductance or capacity of the membrane but decreased the water filtration coefficient and the cationic transferance number. The enzyme association also modifies temperature characteristics of the membrane parameters.

Fructose-1,6-bisphosphate aldolase *Erythrocyte lipid* *Spherical lipid membrane* *Protein-lipid interaction*

1. INTRODUCTION

The supramolecular organization of glycolytic enzymes has been well documented in the literature of the last decade [1–4]. Fructose-1,6-bisphosphate aldolase is one of several glycolytic enzymes that are known to bind reversibly to cell membranes. For example, aldolase has been found to be associated with human erythrocyte and microsomal membrane fractions from rat brain [5–9]. Association of the enzyme with membranes appears to be reversible, dependent on pH, ionic strength of the medium, concentration of substrate, etc. Activity of the enzyme increases on liberation from the membranes. This phenomenon may be important in the regulatory processes of glycolysis in the cell. Therefore, on the basis of present knowledge concerning the association of the enzyme with membranes we can include aldolase in a class of peripheral proteins, but its binding sites on membranes in various cells are not yet known sufficiently. Also, it is not clear how the association influences the properties and functions of cell membranes. Some recent studies have suggested that the enzyme associates with the membranes through other proteins; e.g. through the band 3 protein or actin in erythrocytes [8,10]. However, it seems that the role of the interaction with phospholipids for the binding of the enzyme to membranes is not yet explained sufficiently, and should not be excluded. Using measurements of the change in the tryptophan fluorescence of aldolase it was found that the surface of phospholipid liposomes could reversibly absorb the enzyme. This phenomenon depends on the charge of the lipid polar group, and the ionic strength, and modifies the enzyme's specific activity [11].

The aim of this work is to investigate the influence of the adsorption of aldolase on some properties of the bilayer model membrane. Spherical bilayer membranes made of bovine red cell lipids were used as a model system. Spherical lipid membranes, having a large surface area (about 1 cm^2), allow studies of the electrical and transport properties, which are not possible on other model systems.

+ Address for correspondence

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2. MATERIALS AND METHODS

2.1. *Lipid extraction and formation of spherical lipid membranes*

For extraction of lipids, fresh heparinized bovine blood was used. Plasma and leukocytes were removed by centrifugation. Erythrocytes were then washed 4 times with an isotonic phosphate buffer-NaCl solution, pH 7.4, and suspended in the same solution. The lipids were extracted from erythrocyte ghosts with *n*-butanol at 0°C according to Dodge et al. [12], dried under vacuum and dispersed in a mixture of *n*-decane and *n*-butanol at the ratio 1:1 (v/v) to a final concentration of 10–30 mg/ml.

Spherical lipid membranes were formed at room temperature in KCl aqueous solution (for details see [14]), according to Schagina et al. [13]. The bilayer was formed within 1–3 h after formation of the bubbles. The 'black' membranes were stable for at least 8 h and their surface areas were about 1–2 cm^2. The temperature in the system was stabilized by a water jacket surrounding the chamber.

To investigate the effect of aldolase on lipid bilayer membrane properties, the membranes were formed in an aqueous solution with the enzyme at a concentration 10^{-2} mg/ml.

2.2. *Fructose-1,6-bisphosphate aldolase*

Fructose-1,6-bisphosphate aldolase was prepared from rabbit muscle according to Penhoet et al. [15]. In all experiments, the concentration of aldolase was determined spectrophotometrically using an extinction coefficient $E_{280}^{1\%} = 0.91$ (according to Baranowski and Niederland [16]). The purity of the preparation was checked electrophoretically on a polyacrylamide gel. The electropherogram showed a single band.

2.3. *Measurements of electrical properties and of the water filtration coefficient for lipid bilayer membranes*

In all experiments, the spherical bilayers were observed through a microscope equipped with an eyepiece scale. The membrane area and the bubble volume were determined from the bubble dimensions. The estimated error did not exceed 10%. Ag/AgCl electrodes were employed to apply and record the electric potentials. Electrical resistance of the membrane was calculated from a voltage-current dependence. To assure linearity of the dependence, the external electric potential was not greater than 50 mV. The correlation coefficient was then better than 0.99. The potentials were measured by an electrical system described in [14]. Membrane capacitance was determined from a single impulse relaxation time.

A cationic transferance number, t_+, was calculated from the following equation (according to [17]):

$$V_{diff} = (2t_+ - 1)\frac{RT}{F}\ln\frac{C}{C_o} \quad (1)$$

where V_{diff} is the diffusion potential, C and C_o are the concentrations of KCl outside and inside the bubble, respectively; T is the absolute temperature, R is the gas constant, and F is Faraday's constant. In these experiments membranes were formed in a concentration gradient of KCl, such, that $C/C_o = 10$.

The filtration coefficient L_p was determined by the method proposed in [18], based on the Staverman equation [19]:

$$J_v = L_p(\Delta P - \sigma\Delta\pi) \quad (2)$$

where J_v is the volume flow, ΔP is the difference of the hydrostatic pressure, σ is the reflection coefficient, $\Delta\pi$ is the osmotic pressure difference caused by the KCl gradient. We assume that the membrane is semipermeable ($\sigma = 1$) and that the hydrostatic pressure difference equals zero.

3. RESULTS AND DISCUSSION

3.1. *Electrical resistance*

The electrical resistance determined for 16 membranes formed at room temperature in the presence of aldolase ranged from 10^6 to $10^8\ \Omega\cdot cm^2$. Statistically, these values differ significantly from the resistance of the membranes free of aldolase (table 1). The dependence of the resistance of a representative membrane on temperature in the presence of the enzyme is plotted in fig.1, in comparison with the same dependence for an enzyme-free lipid membrane. As can be seen the presence of the enzyme shifts the temperature break point of the plots to lower values. The break of such a plot indicates that the activation energy determined from the resistance characteristics is

Table 1

Some properties of spherical lipid membrane modified by the association with fructose-1,6-bisphosphate aldolase in comparison to an unmodified membrane

Parameters	Unmodified membrane	Modified membrane
Range of the electrical resistance [$\Omega \cdot cm^2$]	10^6–5×10^7	10^6–10^8
Transition temperature (T_t) determined from temp. characteristic of resistance	36–38°C	32–36°C
Activation energy of ion conductivity [$J \cdot mol^{-1} \times 10^3$]		
(a) below T_t	14.7 ± 2.1	25.07 ± 6.6
(b) above T_t	25.5 ± 2.5	128.1 ± 44.9
Capacitance [$\mu F \cdot cm^{-2}$]	0.41 ± 0.06	0.52 ± 0.12
Transition temp. determined from temp. characteristic of capacitance	36–38°C	not found
Water filtration coefficient [$cm^3 \cdot N^{-1} \cdot s^{-1}$]	$(2.05 \pm 0.39) \times 10^{-7}$	$(2.64 \pm 0.68) \times 10^{-8}$
Transition temperature determined from the temp. characteristic of L_p	34–38°C	not found
Activation energy of water filtration [$J \cdot mol^{-1} \times 10^3$]		
(a) below T_t	17.9 ± 3.2	94.6 ± 26.2
(b) above T_t	114.0 ± 16.0	

The values presented in the table are means ± SD for at least 6 membranes

changed. Table 1 shows an increase of the activation energy.

3.2. *Electrical capacitance*

No change of membrane capacitance was observed upon the addition of the enzyme. For temperature dependence, however, the maximum observed for lipid membranes [14] disappeared after addition of the enzyme. The parameter's value itself did not change markedly with temperature, in the range of 15–50°C.

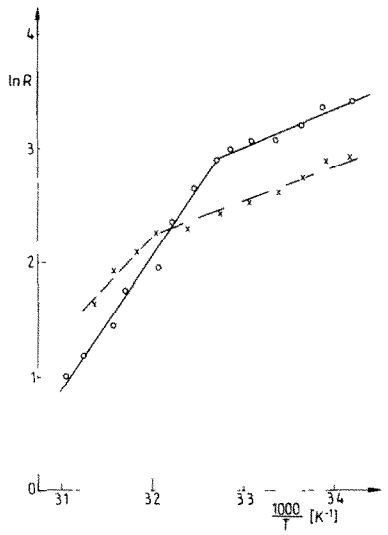

Fig.1. The logarithm of the electrical resistance, R expressed in $10^7\ \Omega \cdot cm^2$, plotted against the reciprocal of the absolute temperature for an enzyme-modified membrane (○). The same dependence for an unmodified membrane is also shown (×).

3.3. *Cationic transferance number*

The dependence of the bulk cationic transferance number on temperature for a single, representative membrane modified by aldolase in comparison with that for the unmodified membrane is shown in fig.2. The association of aldolase decreased the cationic transferance number of the membrane by about 20% at room temperature; at a higher temperature the difference monotonically decreased. The maximum disappeared after addition of the enzyme. At the same time, the cationic transferance value depends only slightly on temperature. The decrease in value of the cationic transferance number indicates that the membrane surface was modified by the aldolase association. On the basis of the previously published results obtained from the fluorescence studies of aldolase-liposome interactions [11], we can interpret such a result as a consequence of the electrostatical interaction between the enzyme and the lipid bilayer.

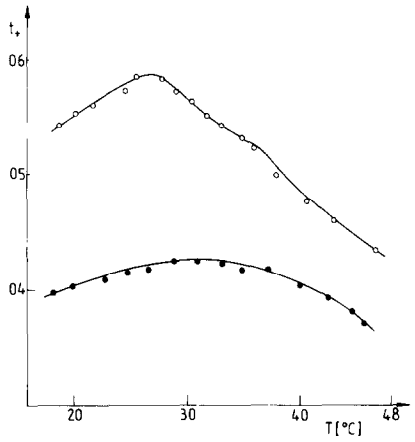

Fig.2. The bulk cationic transferance number for a modified membrane plotted against the temperature (●). The same dependence for an unmodified membrane was shown (○).

3.4. *Filtration coefficient*

At room temperature the filtration coefficient of the modified membranes reaches a value of $2.64 \pm 0.68 \times 10^{-8}$ $cm^3 \cdot N^{-1} \cdot s^{-1}$, which is an order of magnitude smaller than that for an unmodified membrane [18] (see table 1). The membrane becomes less permeable for water. Also, the temperature dependence of the parameter was investigated and no temperature transitions were found. However, due to a large spread of the measured values we cannot exactly interpret this as a lack of sensitivity of the parameter for a structural transition, seen in the temperature dependence of other parameters. For the same reason the activation energy of water could be calculated only approximately. The obtained value of the activation energy was $94.6 \pm 26.2 \times 10^3$ $J \cdot mol^{-1}$ in the temperature range of 15–40°C. This value is by about one order of magnitude greater than that for a free-protein membrane below the transition temperature.

ESR studies have suggested that the observed temperature-dependent transition is a result of some structural transitions in the hydrocarbon chains in the nearest neighbourhood of the polar group layer [14]. The results presented here prove that the peripheral protein, associated with a nonspecifically interacting protein, can influence the structural transitions probably by changing the conformational state of polar groups, hence we suppose that there is no deep penetration of some parts of the protein into the hydrophobic core. As can be seen from the changes of the activation energy of water and ion transport, the state of membrane surface is an important factor for the transport phenomena. Aldolase, and perhaps other peripheral proteins changing the surface state, may influence the transport properties of membranes.

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