The Interaction of Fructose-1,6-Biphosphate Aldolase with Liposome Membranes: A Spin Probe Technique Study

M. KOMOROWSKA[1], M. LANGNER[2] and J. GOMUŁKIEWICZ[1]

1 *Institute of Physics, Technical University of Wroclaw, Wybrzeże Wyspiańskiego* 27, 50—370 *Wrocław, Poland*
2 *Department of Biophysics, Medical Academy of Wroclaw, ul. Chałubińskiego* 10, 50—368 *Wroclaw, Poland*

Abstract. Thermotropic properties of liposome membranes prepared of bulk bovine erythrocyte membrane lipids, native, or aldolase-modified, were investigated by the ESR method. Breaks were observed in the log $2T_{\parallel}$ vs 1/T plots for two spin labels: tempopalmitate and 5-doxyl-palmitate methyl ester. These phenomena have been interpreted as reflecting structural changes near the lipid bilayer polar heads region. Upon modification with aldolase, the temperature at which the breaks occurred was decreased for both spin probes.

Key words: Fructose-1,6-biphosphate aldolase — Liposomes — Protein-lipid interaction — Spin labels — Thermotropic properties

Introduction

A recent work of our group dealed with the effects of aldolase on electrical and transport properties of spherical lipid membranes (Langner et al. 1986). Electrical conductance and capacity of membranes were only slightly affected by the association with the enzyme, whereas the water filtration coefficient and the cationic transference number were both considerably decreased. The temperature dependence pattern of these membrane parameters has suggested phase transition to occur in the membrane (Langner et al. 1984).

The present work tries to examine whether the characteristic temperature observed previously was indeed the result of structural changes in the membrane. Unfortunately, microcalorimetry, which is a classical method for studying processes of this kind, could not be applied since mixture of erythrocyte membrane lipids (as used for the present study) produces a very broad endothermic peak resulting from the combination of thermotropic properties of the

various lipid components. Therefore, the spin probe technique was employed. It is sensitive to local structural changes in the regions where the probe is placed (Restall and Chapman 1986). The mobility of the lipids, and thus also of spin probes, decreases with the decreasing temperature. The shape of the spin probe spectra strongly depends on the fluidity of the environment. One of the empirical parameters that characterize the ESR spectra is the distance (in terms of gauss) between the outer hyperfine splitting extremes $2T_{\parallel}$. This parameter also depends strongly on temperature. However this dependence is complex and plots of log $2T_{\parallel}$ vs $1/T$ are often used to illustrate it (Lenaz 1977; Marsh 1981; Smith et al. 1976; Schreier et al. 1978; Le Meste et al. 1985; Gibson Wood et al. 1987; Barnett 1977).

It should be noted that breaks, or discontinuities, of the plots have been reported with many systems studied. The question arised what has been the meaning of the discontinuities. The issue was discussed by numerous investigators (Lenaz 1977; Marsh 1981; Smith et al. 1976; Minetti et al. 1984) who all have interpreted these characteristic temperature points as reflecting thermotropic structural transitions. To avoid artifactual discontinuities the ESR parameters have to be analyzed carefully, and, if possible, also other methods have to be employed to test them (Smith et al. 1976; Schreier et al. 1978).

Materials and Methods

Fructose-1,6-biphosphate aldolase

Fructose-1,6-biphosphate aldolase was prepared at the Department of Biophysics, Medical Academy, Wrocław, according to Penhoet et al. (1969). In all experiments, the enzyme concentration was determined spectrophotometrically using the extinction coefficient $E_{280}^{1\%} = 0.91$ (Baranowski and Niederland 1949).

Purity of the preparation was checked electrophoretically in polyacrylamide gel, and it yielded only a single band.

Lipid extraction and preparation of spin labelled liposomes

Fresh heparinized bovine blood was used. Plasma and leukocytes were removed by centrifugation at 1000x*g* for 10 min. Erythrocytes were washed four times with an isotonic phosphate buffer-NaCl solution, pH = 7.4, and resuspended in the same solution. Lipids were extracted from erythrocyte ghosts with *n*-butanol at 0 °C according to Zahler and Niggli (1977).

The spin probes used in the experiments were 5-doxylpalmitate methyl ester (5 DPM), 12-doxylstearate methyl ester (12 DSM) and TEMPO-palmitate (TP), (University of Łódź, Poland). Appropriate amounts of 2×10^{-3} mol/l ethanolic solution of each spin probe were used to obtain a label — lipid molecular ratio of 1:50. The label solution was mixed with bulk erythrocyte lipids in *n*-butanol, dried under vacuum in a glass tube, and resuspended in 310 mosM TRIS-HCl

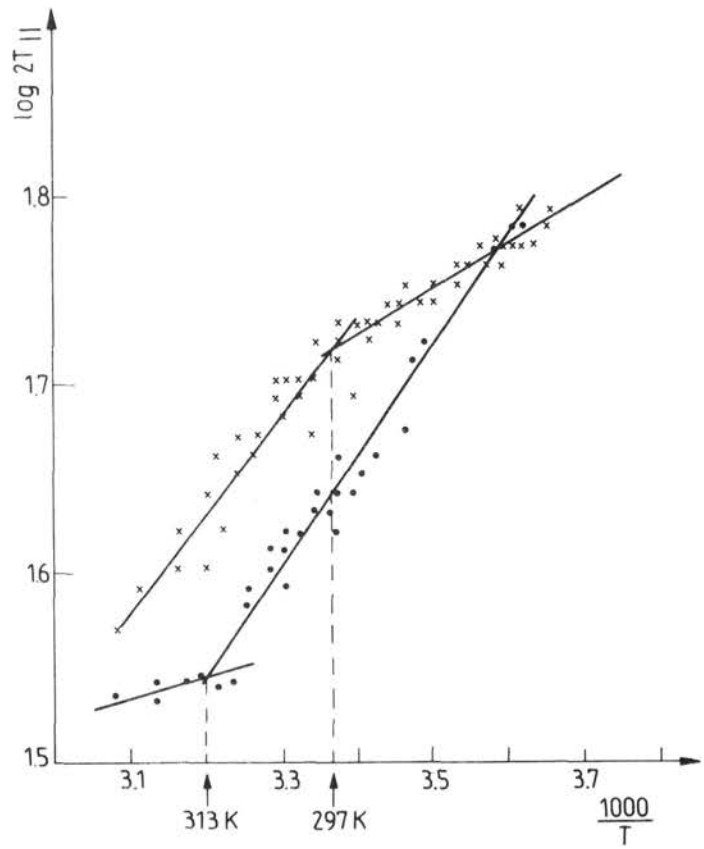

Fig. 1. Semilogarithmic plot of $2T_{\parallel}$ vs $1/T$ for 5 DPM spin label. Circles — liposomes, crosses — aldolase-treated liposomes. The lines were fitted by the least square method.

buffer, pH = 7.4. The mixture was mechanically shaken until a milky suspension was obtained. The lipid concentration was 70 mg/ml.

To investigate the effect of aldolase on lipid bilayer properties, the labelled lipid was shaken in the above buffer with addition of 0.4 μl enzyme in 50% $(NH_4)_2SO_4$ solution, at the concentration of 6×10^6 molecules/ml (10^{-2} mg/ml). Spectra were recorded and plots of log $T_{\parallel}$ vs $1/T$ were constructed for each probe.

The interaction of aldolase with the liposome surfaces was also monitored by measuring the kinetics of ascorbate-induced reduction of the nitroxyl radical of the labels. Spin labelled liposomes were prepared as described above. Ascorbate concentration in the liposome sample was 2.98×10^{-3} mol.l^{-1}, that of the spin label TP was 1×10^{-4} mol.l^{-1}. The time course of the resulting decrease of the ESR middle line height (h_o), was measured.

The spectra were recorded on a Jeol ESR spectrometer. The temperature was varied within 0—45 °C ± 0.1 °C.

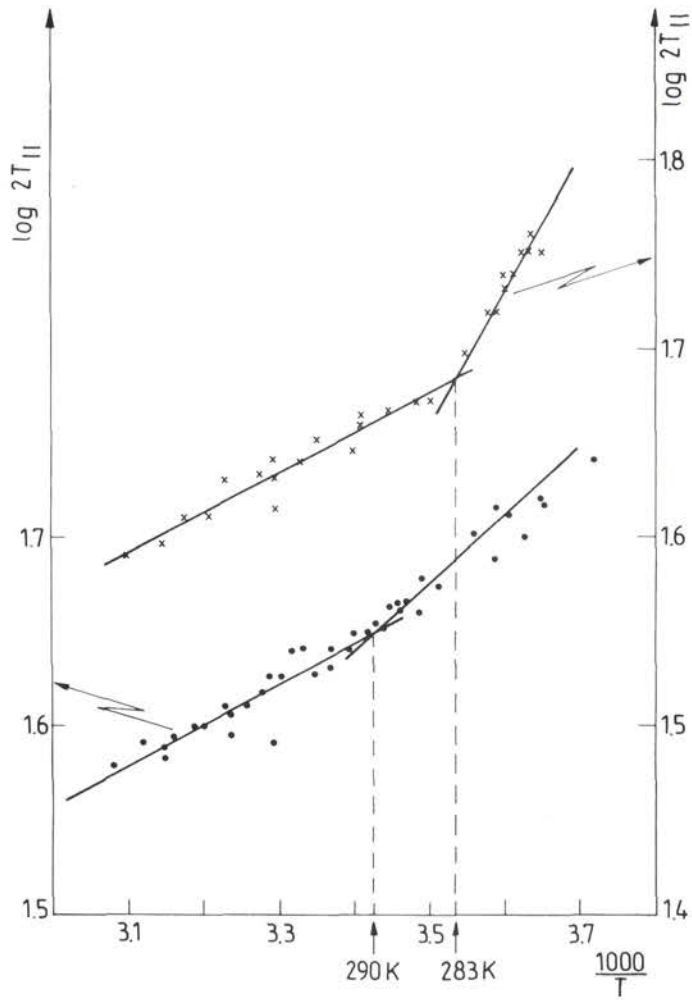

Fig. 2. Semilogarithmic plot of $2T_{\parallel}$ vs $1/T$ for TP spin label. Circles — liposomes, crosses — aldolase-treated liposomes. The lines were fitted by the least square method.

Results

Three types of spin labels were used in the experiments: 12-doxylstearate methyl ester, (12 DSM), 5-doxylpalmitate methyl ester, (5 DPM); and TEMPO-palmitate (TP).

The presence of aldolase was associated with broadening of the spectra of 5 DPM and of TP, but did not affect the 12 DPM spectra. This suggested a

Table 1. Transition temperatures for modified and control liposomes

Spin probe	Control liposomes	Aldolase-treated liposomes
SDPM	313 ± 4 K	297 ± 4 K
TP	290 ± 4 K	283 ± 4 K

Maximal errors are shown calculated from linear regression analysis.

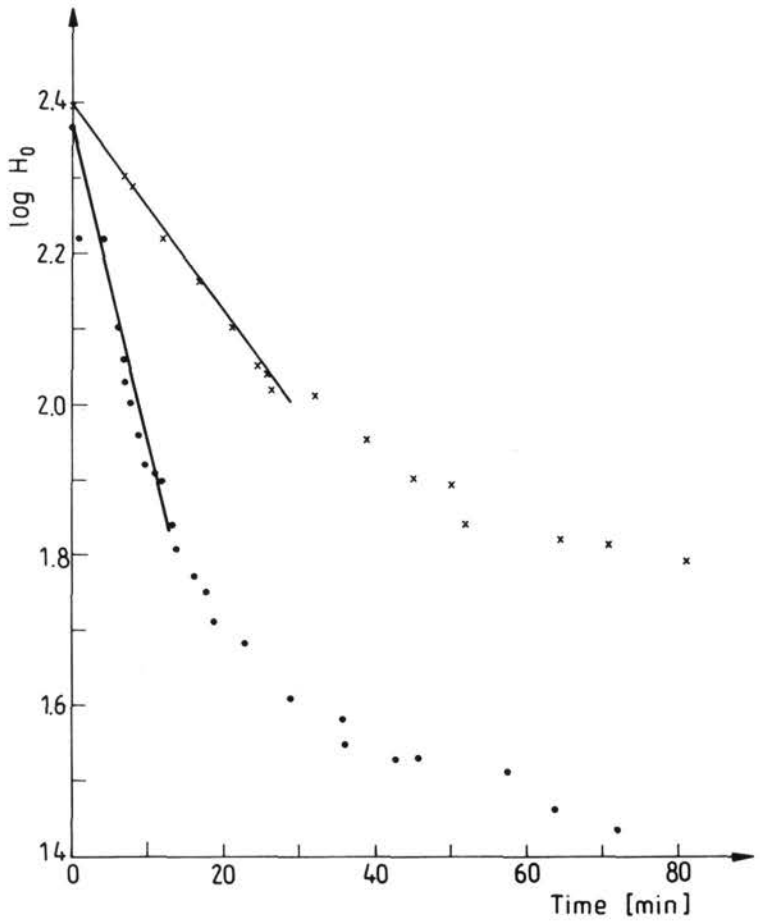

Fig. 3. The time course of the middle peak intensity in TP spectra in the presence of ascorbate at room temperature. Circles — liposomes, crosses — aldolase-treated liposomes. The lines were fitted by the least square method.

decreased mobility of the TP spin label incorporated near the surface of the lipid bilayer polar heads, and a similar effect on the motion of the 5 DPM label located at the depth of the 5th carbon in the hydrocarbon chain.

The plots of log $2T_{\parallel}$ against the reciprocal temperature for TP and 5 DPM are shown in Figs. 1 and 2 respectively. The breaks are clearly visible. The respective transition temperatures are summarized in Table 1. In the presence of aldolase, the break points for both labels were shifted to lower temperatures. The shifts were 7 *K* for TP and 16 *K* for 5 DPM. Such a change of the plot slope (Figs. 1 and 2) is suggestive of a phase-transition-like process.

Fig. 3 shows the change of TP concentration following the reaction of ascorbate with the nitroxyl radical of the label incorporated in the liposome membrane. The time course of the intensity of the central peak in the ESR spectra of the TP label is complex (Fig. 3). The nitroxide groups of the spin probe are reduced by ascorbate. This process can be schematically represented as

$$\rangle N - O^{\cdot} + \text{ascorbate} \rightleftharpoons \rangle N - OH + \text{dehydroascorbate}.$$

This reaction finds significant employment in the experiments designed to test the accessibility of the reporter group (Marsh 1981). The reduction rate is determined by the rate of translocation of the spin probe and of ascorbate. For an excess of ascorbate, the dependence of logarithm of the chosen spin probe line height against time indicated that it was a pseudo first-order kinetics (Schreier-Mucillo et al. 1976).

To decrease the speed of quenching, there was an excess of spin label and deviations from linearity were observed. The change in the slope of this plot for unmodified liposomes, and for liposomes with aldolase, showed a decreased reduction rate in the presence of aldolase. The slopes of the initial part of the curve: log H_0 vs. time for this reaction were (-0.047 ± 0.002) for liposomes, and (-0.014 ± 0.001) for liposomes with aldolase.

Discussion

Following conclusions can be drawn based on our experimental results:

(i) Thermally induced structural changes were shown to occur in the lipid membrane polar region and at the depth of the 5-th carbon. The transition temperatures were 290 K at the surface, and 313 K in the 5-th carbon region. No changes could be detected in the region adjacent to the 12th carbon in the hydrocarbon chain.

(ii) The presence of aldolase resulted in a decrease of membrane fluidity. The broadening of the ESR lines suggested changes in the organization of the lipid

bilayer to occur in the region studied. The transition temperature was decreased for the hydrophilic surface and the hydrophobic 5-C region.
(iii) Aldolase adsorption was found to disturb the reaction of the spin label with ascorbate. This was consistent with conclusion (ii). It can be assumed that aldolase adsorbed to the surface region of liposomes, and decreased the rate of ascorbate diffusion to the proximity of the nitroxide radical.

These results confirmed earlier suggestions based on studies of the thermotropic properties of electrical behavior of spherical lipid membranes (Langner et al. 1984, 1986).

The break temperature, determined from the temperature characteristics of resistance and of the filtration coefficient, was 36—38 °C for unmodified membranes, while it was shifted to 32—36 °C after modification by aldolase. Values, obtained by the spin probe method, were 40 °C for unmodified liposomes and the hydrophobic region, and 17 °C for the polar head region; the respective values following aldolase treatment were 24 °C and 10 °C.

The differences between the values obtained by both methods, may be associated with differences between the systems investigated (spherical lipid membranes and liposomes). Moreover, membrane regions which influence the transport properties (electric conductance, water permeability) of the membranes, may be different from those where the spin probes 12-DSM, 5-DPM, and TP, were located. The differences in the transition temperatures and of their changes observed between TP and 5-DPM probes, can in our opinion, be explained by different localization of the probes in the membranes.

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