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# Interaction of cytoplasmic L-glycerol-3-phosphate dehydrogenase with liposomes

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**NAD-linked L-glycerol-3-phosphate dehydrogenase binds to phosphatidylcholine liposomes as shown by the changes in the properties of both the enzyme and the membrane. The surface potential and the fluidity of the liposome membrane (monitored at the 5th C atom depth) change due to the presence of the enzyme, whereas the enzyme is activated by the liposomes. These findings suggest the occurrence of peripheral protein-lipid interactions.**

## Introduction

NAD-linked L-glycerol-3-phosphate dehydrogenase (L-glycerol-3-phosphate : NAD oxidoreductase, EC 1.1.1.8) is present in the particle-free fraction of the tissues [1]. There are experimental results suggesting the presence of a hydrophobic area or pocket on the surface of the enzyme. It has been shown that detergents, below their critical micelle concentration, strongly influence the activity of the purified enzyme [2]. Various fatty acids also interact with the enzyme purified either from liver or from muscle [3].

In order to find out whether this hydrophobic area enables the enzyme to bind to membranes we have studied the interaction of rabbit muscle L-glycerol-3-phosphate dehydrogenase with liposomes by using the ESR technique and fluorescence measurements to monitor the structural features of the membrane, and by measuring the activity of the enzyme.

## Materials and Methods

### *Materials*

Egg yolk phosphatidylcholine was purified according to the method of Wells and Hanakan [4]. Cardiolipin from bovine heart and DMPC were from Serva (F.R.G.), cholesterol from Reanal (Hungary). C-5 and C-16 positional isomers of spin-labelled stearic acid (5-SASL and 16-SASL) as well as 14-PCSL and 14-PASL were from Syva (Palo Alto, U.S.A.).

Rabbit muscle L-glycerol-3-phosphate dehydrogenase was prepared according to Telegdi [5] and was stored in crystal suspension. Ammonium sulphate was removed by gel filtration before the experiments. The following values were used in the calculations: molecular weight 78 000 [6]; absorption coefficient at 280 nm $E^{1\ cm}_{1\ mg/ml} = 1.00$ [5].

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Abbreviations: ANS, 8-anilino-1-naphthalene-sulphonate; DHAP, dihydroxyacetone phosphate; DMPC, dimyristoylphosphatidylcholine; 14-PASL, β-14(4,4-dimethyl-3-oxazolidinyl-*N*-oxy)-stearoyl-γ-acetyl-α-phosphatidic acid; 5-SASL and 16-SASL, stearic acid spin labels with 4,4-dimethyl-3-oxazolidinyl (doxyl) groups attached to the C-5 and C-16 atom, respectively; 14-PCSL, phosphatidylcholine with doxyl group on C-14.

*Methods*

*Enzyme activity measurements.* Forward reaction (NADH oxidation) was measured in 100 mM Tris-HCl buffer (pH 7.5); reaction mixture: $10^{-4}$ M NADH, $10^{-3}$ M DHAP and $(1-3)\cdot 10^{-8}$ M enzyme. Reverse reaction was measured in 100 mM glycine-NaOH buffer (pH 10.0). Reaction mixture: $10^{-3}$ M NAD, $10^{-3}$ M DL-glycerol 3-phosphate, $(1-5)\cdot 10^{-8}$ M enzyme. Specific activities of the different preparations (forward reaction) ranged between 70 and 100 mol NADH/mol enzyme per s.

*Liposome preparation.* Lipids, and 10 $\mu$g/mg of lipid spin probes in the case of spin-labelling measurements were dissolved in chloroform and then evaporated to dryness under nitrogen atmosphere in wide bore sonication tubes. After addition of 100 mM Tris-HCl buffer (pH 7.5), the dry lipid film was hydrated with brief ultrasound irradiation (2 min) so that finally a 20 mg/ml suspension of liposomes was obtained.

*Fluorescence measurements.* 8-Anilino-1-naphthalene sulphonate (ANS, Serva product) was dissolved in 100 mM Tris-HCl buffer (pH 7.5), at 5, 1, 0.5 and 0.25 mM concentrations. To each 3 ml of liposome suspensions 5-$\mu$l aliquots of these dye stock solutions were added and fluorescence intensities were measured with an Opton PMQ II spectrofluorimeter using 365 nm excitation and 460 nm detection. In all experiments liposome, enzyme and dye concentrations were kept sufficiently low (absorbance < 0.1) to ensure that neither light scattering nor concentration quenching affected the detection at 460 nm. Under such conditions always a linear relationship was observed in the double-reciprocal plot of fluorescence intensity vs. dye concentration. The dissociation constant was evaluated from the slope of the straight line. The surface potential change ($\Delta\psi_s$) caused by the enzyme relative to pure lipid liposomes is given by the formula [7],

$$\Delta\psi_s = \frac{RT}{zF}\ln\left(\frac{K_d}{K'_d}\right)$$

where $R$, $F$, $z$ and $T$ have their usual meanings, $K_d$ and $K'_d$ are the dissociation constants of the membrane-dye complex under the two different experimental conditions.

*Spin-labelling ESR measurements.* ESR spectra were recorded on a Jeol Jes-PE-1X X-band spectrometer using 100 kHz modulation technique. The liposome suspension (in the presence or absence of the enzyme) was filled into the micro flat cell (Scanlon, U.S.A.) and thermostatically controlled in a quartz gas transfer line system; sample temperature was independently measured with a copper-constantan thermocouple immersed into the upper portion of the sample solution. Orientational order parameter ($S_{zz}$) was calculated from the splitting of the outer ($2A_{\parallel}$) and the inner (approx. $2A_{\perp}$) extrema after allowing for the apparent shift of the inner features as described in Ref. 8.

$$S_{zz} = \frac{A_{\parallel} - A_{\perp}}{A_{zz} - (A_{xx} + A_{yy})/2}$$

where $A_{xx}$, $A_{yy}$ and $A_{zz}$ denote the single crystal principal values of the hyperfine tensor.

## Results and Discussion

The dependence of enzymatic activity of the forward (NADH oxidation) and the reverse (NAD reduction) reactions of L-glycerol-3-phosphate dehydrogenase as a function of lipid concentration is shown in Fig. 1. Both curves display an initial rise, which is particularly pronounced in the case of the forward reaction, and reach a plateau region. This lipid dependence is similar to that observed for various membrane proteins (for review, see Ref. 9). The saturating lipid-to-protein ratio at the onset of the plateau region is approx. 2000 in our case. The activity increase upon addition of phosphatidylcholine was compared with the increase observed upon addition of a cardiolipin/phosphatidylcholine 1 : 2 mixture (not shown). The lack of any significant difference indicates that there is no selectivity between these two lipids.

The above results are consistent with two alternative structural models of enzyme-lipid/detergent interaction. Firstly, the hydrophobic region of L-glycerol-3-phosphate dehydrogenase can efficiently be sequestered from water by lipid micelles which are formed during protein-membrane collisions; this model is essentially similar to detergent solubilization invoked for the explanation of en-

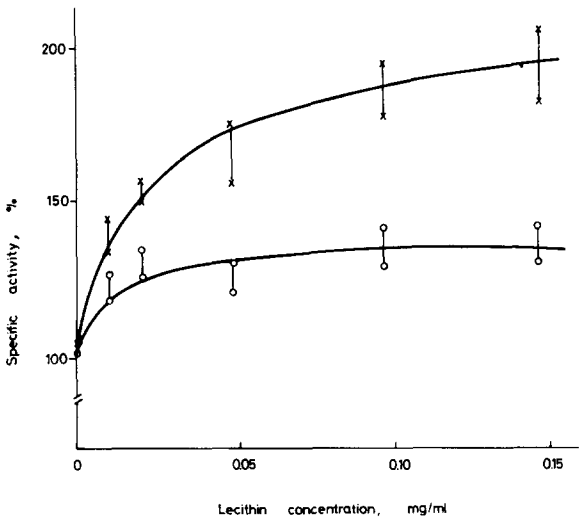


Fig. 1. Effect of phosphatidylcholine liposomes on the specific activity of cytoplasmic L-glycerol-3-phosphate dehydrogenase. ×——× , forward reaction, measured at pH 7.5; O——O, reverse reaction, measured at pH 10.0. Experimental conditions were as described in Materials and Methods.

zyme activation/inhibition in the presence of various detergents [2]. Alternatively, the adsorption of L-glycerol-3-phosphate dehydrogenase to lipid bilayers should be regarded as an interaction of a peripheral membrane protein with its hydrophobic region anchored at the membrane/water interface [10]. The latter model is applicable to several proteins [11–13]. Here we present two lines of experimental evidence for L-glycerol-3-phosphate dehydrogenase membrane interaction which are consistent with this latter model.

The ESR spectra of freely diffusible fatty acid and phosphatidylcholine spin-probe positional isomers provide a wealth of information on the structure and dynamics of these probes in lipid bilayers [14]. Two different positional isomers were applied to obtain information about the membrane at different depths. Stearic acid and phosphatidylcholine labelled at the fifth C atom monitor the structural features near the surface, at the 5th C atom depth. Stearic acid with label at the 16th C atom (16-SASL), as well as the C-14 positional isomers of labelled phosphatidylcholine and phosphatidic acid, on the other hand, monitor structural changes deep in the lipid phase.

The ESR spectra of the C-16 positional isomer in egg phosphatidylcholine liposomes in the presence or absence of L-glycerol-3-phosphate dehydrogenase are shown in Figs. 2A and B. In both cases motionally narrowed spectra were obtained and no measurable fraction of the spin-labelled stearic acid was immobilized. The characteristics of the spectra of the C-14 positional isomers of labelled phosphatidylcholine (14-PCSL) and phosphatidic acid (14-PASL) were the same. These observations rule out the possibility that during enzyme-membrane encounters a significant amount of lipids was removed from the membrane and bound to the enzyme, as in the case of albumin [15], serum lipoproteins [16], and lipid exchange proteins [17]. It should be noted, however, that this result is consistent with the protein adsorption model since the motional properties of methyl terminals are practically uncoupled from any interfacial motional restrictions because of the flexible nature of the acyl chain.

At the 5th C atom depth, on the other hand, the spin probes display spectral anisotropy and, thus, the effects of motional restriction are more difficult to resolve [14]. Best resolution can be expected at higher temperatures because the anisotropic pure lipid spectrum usually displays sharper temperature dependence than the immobilized spectrum component. However, no second spectral component could be found (Figs. 2C and D) even at 40°C; the observed spectral changes were quantitated with the apparent order parameter ($S_{zz}^{app}$) as shown in Fig. 3. Clearly, at any temperature between 10 and 40°C the apparent order parameter both in pure phosphatidylcholine and in DMPC was larger when L-glycerol-3-phosphate dehydrogenase was added (Fig. 3). We can thus concluded that the presence of L-glycerol-3-phosphate dehydrogenase results in an increased molecular ordering which is approximately equivalent with a temperature change of 3 Cdeg. These results can tentatively be explained by lipid condensation brought about by altered lipid/lipid and lipid/protein electrostatic interactions.

The possible effect of L-glycerol-3-phosphate dehydrogenase adsorption on membrane electrostatics was further investigated by following the partitioning of the anionic amphiphilic dye ANS. The dissociation constant of ANS egg phosphatidylcholine liposome complex was determined from

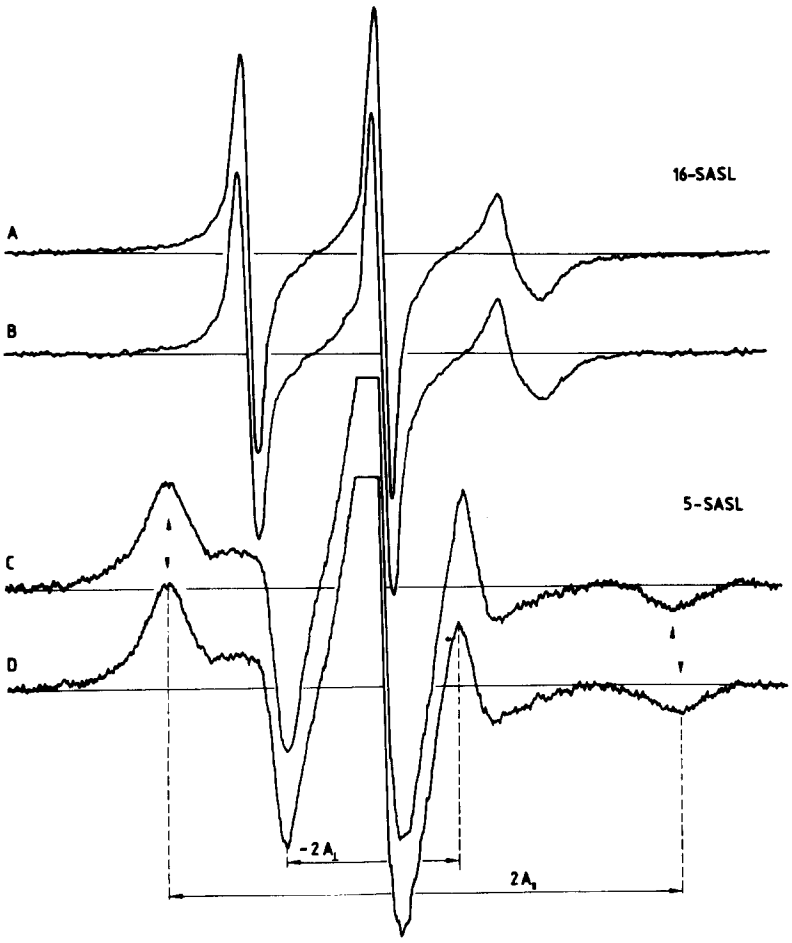


Fig. 2. Spectra of 16-SASL and 5-SASL in phosphatidylcholine liposomes in the presence and absence of cytoplasmic L-glycerol-3-phosphate dehydrogenase. Curve A, 16-SASL in phosphatidylcholine liposomes; Curve B, 16-SASL in phosphatidylcholine liposomes in the presence of L-glycerol-3-phosphate dehydrogenase; Curve C, 5-SASL in phosphatidylcholine liposomes, Curve D, 5-SASL in phosphatidylcholine liposomes in the presence of L-glycerol-3-phosphate dehydrogenase. Lipid concentration, 12 mg/ml; L-glycerol-3-phosphate dehydrogenase concentration, $3.3 \cdot 10^{-6}$ M; temperature, 30°C.

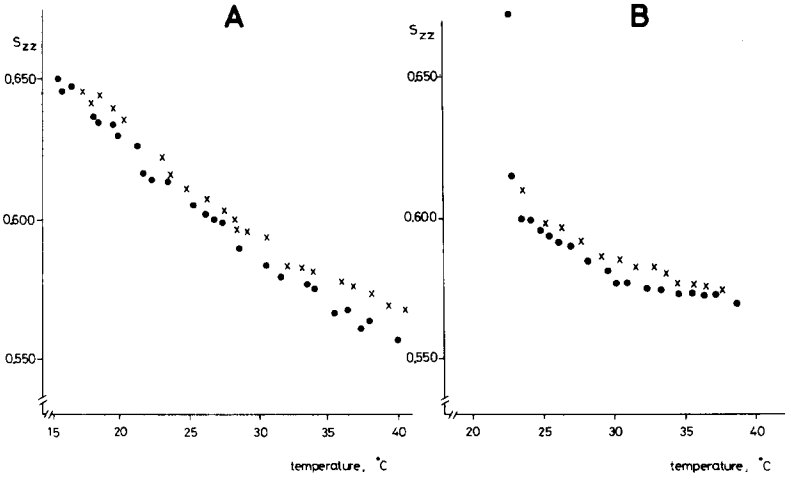


Fig. 3. Effect of cytoplasmic L-glycerol-3-phosphate dehydrogenase on the order parameter of liposome membranes, with 5-SASL as spin label. (A) Phosphatidylcholine liposomes (●), and phosphatidylcholine liposomes in the presence of L-glycerol-3-phosphate dehydrogenase (×). (B) Dimyristoylphosphatidylcholine liposomes (●), and the same liposomes in the presence of L-glycerol-3-phosphate dehydrogenase (×). Concentrations were as in the experiment shown in Fig. 2.

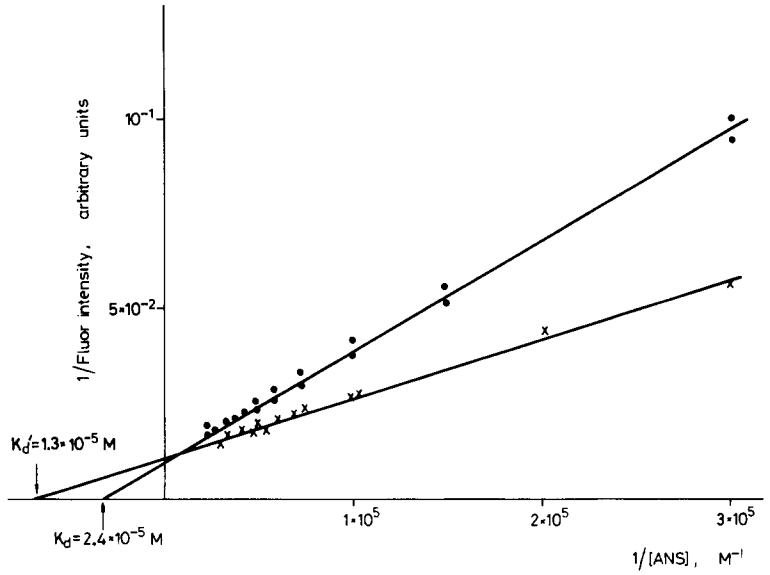


Fig. 4. Effect of cytoplasmic L-glycerol-3-phosphate dehydrogenase on the binding of ANS to phosphatidylcholine liposomes. Titration of egg yolk phosphatidylcholine liposomes (0.5 mg/ml) with ANS (●———●). Titration of the same liposomes in the presence of $6 \cdot 10^{-7}$ M enzyme (×——— ×). Titration of $6 \cdot 10^{-7}$ M L-glycerol-3-phosphate dehydrogenase with ANS under identical conditions gave signal intensities less than 3% of those shown in the figure.

the titration diagram shown in Fig. 4 according to the method of Haynes [7]. As seen from Fig. 4, in the presence of L-glycerol-3-phosphate dehydrogenase the dissociation constant is lowered by a factor of almost 2 as compared to the pure phosphatidylcholine control. From this effect the change in surface charge can be calculated as described in Methods and $\Delta\psi_s = +10 \pm 5$ mV is obtained (this interpretation implies that the enzyme does not bind ANS; this assumption was checked and found to be valid by comparing the ANS-fluorescent intensity in buffer and L-glycerol-3-phosphate dehydrogenase solution). The increasing surface charge density is again consistent with the peripheral protein/membrane interaction, i.e. that L-glycerol-3-phosphate dehydrogenase, while adsorbed to the membrane/water interface, can effectively screen lipid headgroup charges altering thereby the net surface potential.

As for the possible physiological significance of these findings we suggest that L-glycerol-3-phosphate dehydrogenase, a cytoplasmic enzyme, might attach to membrane(s) in vivo under special metabolic conditions, and this dual localization can play a role in the control of its activity. In preliminary experiments the addition of non-respiring rabbit liver or muscle mitochondria, the likely target membrane for L-glycerol-3-phosphate dehydrogenase adsorption, resulted in an increased enzymatic activity.

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## References

1 Young, H.L. and Pace, N. (1958) Arch. Biochem. Biophys. 76, 112–121
2 McLoughlin, D.J., Shahied, I.I. and McQuarrie, R. (1978) Arch. Biochem. Biophys. 188, 78–82
3 McLoughlin, D.J., Shahied, I.I. and McQuarrie, R. (1978) Biochim. Biophys. Acta 527, 193–203
4 Wells, M.A. and Hanakan, D.J. (1969) in Methods in Enzymology (Lowenstein, J.M., ed.), Vol. 14, pp. 179–181
5 Telegdi, M. (1964) Acta Physiol. Acad. Sci. Hung. 25, 177–188
6 Eys, Van Y., Nuencke, B.J. and Patterson, N.K. (1959) J. Biol. Chem. 234, 2308–2313
7 Haynes, D.H. (1974) J. Membrane Biol. 17, 341–366
8 Gaffney, B.J. (1976) in Spin Labeling. Theory and Applications (Berliner L.J., ed.), Vol. 1, pp. 567–571, Academic Press, New York
9 Jost, P.C., Griffith, O.H., Capaldi, R.A. and Vanderkooi, G. (1973) Proc. Natl. Acad. Sci. USA 70, 480–484
10 Singer, S.J. in Structure of Biological Membranes (Abrahamsson, S. and Pascher, I., eds.), pp. 443–461, Plenum Press, New York and London
11 Hudig, H. and Drews, G. (1983) FEBS Lett. 152, 251–255
12 Sixl, F., Brophy, P.J. and Watts, A. (1984) Biochemistry 23, 2032–2039
13 Williams, J.C. and Bell, R.M. (1972) Biochim. Biophys. Acta 288, 255–262

14 Marsh, D. (1981) in Membrane Spectroscopy, Molecular Biology, Biochemistry and Biophysics (Grell, E., ed.), Vol. 31, pp. 51–142, Springer Verlag, Berlin, Heidelberg, New York
15 Morrisett, J.D., Pownall, H.J. and Gotto, A.M., Jr. (1975) J. Biol. Chem. 250, 2487–2494
16 Morrisett, J.D., Jackson, R.L. and Gotto, A.M., Jr. (1975) Annu. Rev. Biochem. 44, 183–207
17 Devaux, P.F., Moonen, P., Bienvenue, A. and Wirtz, K.W.A. (1977) Proc. Natl. Acad. Sci. USA 74, 1807–1810