Liposome-Interaction Induced Conformation Changes of Glyceraldehyde-3-Phosphate Dehydrogenase

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Abstract. Tryptophanyl emission spectra of rabbit muscle glyceraldehyde-3-phosphate dehydrogenase (G3PDH) were measured after the addition of liposomes prepared of natural phospholipids: phosphatidylinositols (PI), phosphatidylserines (PS) and phosphatidylcholines (PC). The measurings were made for various molar lipid/protein ratios (100—1000). A decrease in the enzyme fluorescence intensity and a "red" shift of the emission band maximum were observed. The susceptibility of the enzyme fluorescence to liposome action strongly depended on the kind of phospholipid and changed in the sequence PI > PS > PC. The presence of liposomes affected the accessibility of tryptophan residues for the fluorescence quencher (acrylamide). The results suggested that interaction induces some specific conformation changes in the enzyme molecules which may be responsible for modification of the enzyme activity. A comparison of the modification in fluorescence characteristics with those observed during denaturation suggested that the denaturation mechanism is not operative. Other possible mechanisms of the interaction are discussed.

Key words: Glyceraldehyde-3-phosphate dehydrogenase — Enzyme-lipid interaction — Conformation changes — Fluorescence quenching — Liposomes

Introduction

It is well known from a number of papers that mammalian erythrocyte glyceraldehyde-3-phosphate dehydrogenase is a membrane bound enzyme (Nilson and Ronquist 1969; Duchon and Collier 1971; Yu and Steck 1975; Green et al. 1965; Mitchell et al. 1965; Shin and Carraway 1973). Detailed studies of the interaction of the enzyme with isolated erythrocyte membranes have shown that it binds to specific sites on cytoplasmic surface of the membranes (Shin and Carraway 1973; McDaniel and Kirtley 1975; Kant and Steck 1973). The binding is affected by environmental conditions, such as pH value, ionic strength, concentrations of metabolites; the enzyme may be displaced from the membranes by treatment with EDTA, detergents or 0.5 mol/l NaCl (Shin and Carraway 1973; McDaniel et al. 1974; Letko and Bohnesack 1974). In addition, the specific activity of glyceraldehyde-3-phosphate dehydrogenase is strongly modified by the binding to the

membranes (Duchon and Collier 1971; Yu and Steck 1975). Studies on the localization of the enzyme in erythrocyte membranes suggest that it is associated with the integral protein — Band 3 (Yu and Steck 1975; Kliman and Steck 1980). Yeltman and Harris (1980) have suggested actin as a possible binding site of G3PDH in erythrocyte membrane. However, the studies by Wilson et al. (1982) do not support this suggestion. They have shown that erythrocyte ghosts can be depleted of actin with little or no effect on the binding of the enzyme. Thus the exact nature of the enzyme binding to the erythrocyte membrane still remains unknown. Moreover, little is known about the enzyme interaction with membranes in cells of other tissues as well as about the significance of the phospholipid fraction for the association. Also, the mechanism of the activity modification has not yet been sufficiently explained.

The present paper describes our attempts to determine whether conformation changes occur in G3PDH as a result of its association with liposomes. To monitor conformation changes tryptophan residues fluorescence spectra were measured and fluorescence quenching by a dynamic quencher was used as a measure of accessibility of the residues to small, polar molecules.

Materials and Methods

Glyceraldehyde-3-phosphate dehydrogenase was prepared from rabbit muscle according to Kochman and Rutter (1968). It was purified by DEAE-Sephadex column chromatography and by subsequent repeated recrystallization from ammonium sulfate solution. The enzyme concentration was determined spectrophotometrically using $E_{280}^{0.1\%} = 1.0$ as extinction coefficient (Dandliker and Fox 1955).

The commercial preparations of bovine brain phosphatidylinositols and phosphatidylserines and yolk phosphatidylcholines (all from Koch-Light Laboratories Ltd) were used. Phospholipid concentrations in samples was calculated from phosphorus determinations by the method of Bartlett (1959).

Phospholipid liposomes were prepared according to Brunner et al. (1976). Dry phospholipids were suspended in 10 mmol/l Tris-Cl, 1 mmol/l EDTA buffer (pH = 7.5) by mechanical shaking with glass beads for 30 min, and then potassium cholate was added to a final concentration of 2%. After overnight incubation the mixtures were passed through a Sephadex G-50 column to remove cholate. The procedure produces single-shelled liposomes of uniform size and the remaining cholate concentration does not exceed 0.02% (Brunner et al. 1976). Control measurements showed no effect on enzyme activity and fluorescence of similar concentrations of potassium cholate.

Fluorescence emission spectra and fluorescence polarization degree were measured on a Perkin-Elmer MPF-3L spectrofluorimeter at room temperature using excitation wavelength 290 nm and automatical correction. This wavelength practically excites only tryptophanyl fluorescence in proteins. Samples were placed in 4 ml quartz cuvettes (1cm × 1cm × 4cm). In order to avoid errors due to possible background from lipids, light intensity of control samples (containing free liposomes) was subtracted.

Absorption UV spectra were determined at room temperature using a Unicam SP-800 spectrophotometer.

In all experiments 10 mmol/l Tris-Cl, 1 mmol/l EDTA buffer (pH = 7.5) was used.

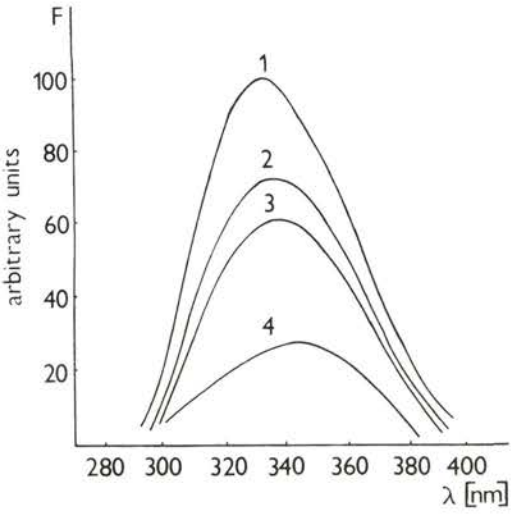

Fig. 1. Effect of phospholipid liposomes on fluorescence emission spectra of gylceraldehyde-3-phosphate dehydrogenase. Concentrations: G3PDH — 1 μmol/l, phospholipids — 0.5 mmol/l, pH = 7.5, volume of samples — 3 ml. Curves: 1 — enzyme without liposomes; 2 — enzyme + PC liposomes; 3 — enzyme + PS liposomes; 4 — enzyme + PI liposomes. Ordinate: spectra related to maximum fluorescence of free enzyme.

Results

The addition of phospholipid liposomes to any G3PDH concentration did not change the UV absorption spectra of glyceraldehyde-3-phosphate dehydrogenase (not shown); however, its tryptophanyl fluorescence decreased significantly (Fig. 1). Half-intensity width of the emission bands were not markedly altered but their maxima were shifted to higher wavelength values. As illustrated in Fig. 2, with the increase of the molar lipid/protein ratio the quenching of tryptophanyl fluorescence increased to reach the maximal value at relatively high ratios. The "red" shift of λ_{max} also increased with the increase in the molar ratio to finally reach values of ~20 nm, ~10 nm and ~6 nm for PI, PS and PC liposomes, respectively (Fig. 3). The fluorescence spectra were not corrected for exciting light scattering by liposomes; some fluorescence decrease may thus have occurred due this effect, however it could not be the main cause of the changes observed in spectra since: — the difference in the intensities of the light scattered at 290 nm between samples of various lipids was small and did not correlate with the differences in ability to affect protein fluorescence: samples with the least light scattering (PI) showed the highest effect; — effects of liposomes saturate themselves at higher lipid protein ratios; it is characteristic of the interaction but not of the scattering effect.

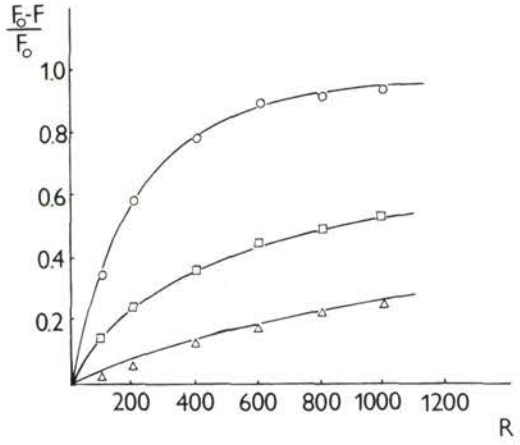

Fig. 2. Relative changes in enzyme fluorescence intensity as a function of molar lipid/protein ratio (R). The enzyme concentration was 1 μmol/l and the concentrations of phospholipids varied, pH = 7.5, volume of samples — 3 ml. F_0 — fluorescence intensity of the native enzyme, F — fluorescence intensity of the enzyme in the presence of liposomes. Fluorescence intensities were measured at emission maximum. (△), PC liposomes; (□), PS liposomes; (○), PI liposomes.

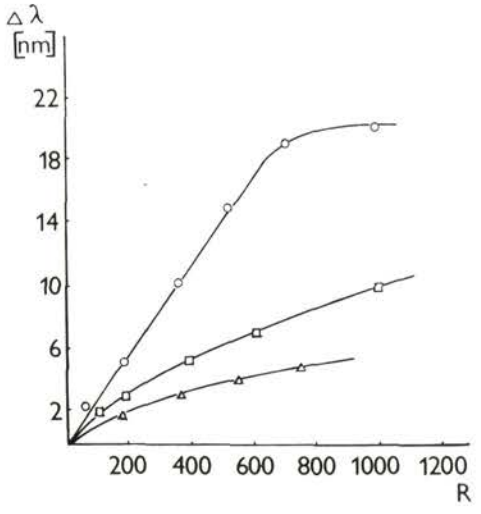

Fig. 3. "Red" shift of λ_{max} as a function of molar lipid/protein ratio (R). For experimental conditions symbols see legend to Fig. 2.

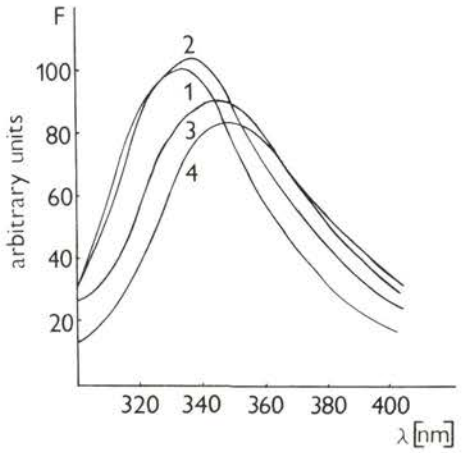

Fig. 4. Effect of urea on fluorescence emission spectra of glyceraldehyde-3-phosphate dehydrogenase. The enzyme concentration in all samples was 1 μmol/l, concentrations of urea: 1 — 0 mol/l, 2 — 2 mol/l, 3 — 4 mol/l, 4 — 6 mol/l. Ordinate: Spectra related to maximum fluorescence of free enzyme.

Since the presence of liposomes substantially modifies the specific activity of G3PDH (Gutowicz and Modrzycka 1978) some conformation changes or even denaturation of the enzyme should be considered. Taking into account that glyceraldehyde-3-phosphate dehydrogenase from rabbit muscles is a multitryptophan protein (it contains 12 tryptophan residues per tetramer — Allison and Kaplan 1964), the observed pattern of fluorescence modification does suggest that some conformational rearrangements occur. According to the classification of Burstein et al. (1973) the wavelength of the emission peak of ~333 nm indicates that most tryptophan residues are located in rather nonpolar regions of the native G3PDH molecule. Therefore, the observed quenching and marked „red" shift of emission maxima can be explained by a conformational rearrangement changing the microenvironment of tryptophan residues. The conformation changes seem to be not very drastic. To compare the observed effect of liposomes on fluorescence spectra with that of unfolding agents we have observed changes in fluorescence spectra in various concentrations of urea (Fig. 4). At increasing urea concentrations the fluorescence initially increased (Fig. 4, curve 2) and then decreased with a simultaneous "red" shift and strong expansion of the band. Drastic changes in protein conformation (unfolding of the polypeptide chain) resulted in small changes in fluorescence intensity (less than 20% for the highest urea concentration). Spectra of unmodified enzyme (curves 1, Fig. 1 and Fig. 4) showed some differences in their shape in the long-wavelength region. These differences were due to different contaminating amounts of denatured protein in various prepara-

Table 1. Polarization degree changes of glyceraldehyde-3-phosphate dehydrogenase tryptophan fluorescence after the addition of phospholipid liposomes and during denaturation. *Enzyme concentration: 1 μmol/l, lipid/protein ratio: 900.*

Modifier	Polarization degree
None	0.10
PC liposomes	0.26
PS liposomes	0.28
PI liposomes	0.29
4 mol/l urea	0.093
6 mol/l urea	0.064

tions of the enzyme; the differences have, however little impact on our considerations since in this experiment, we only wanted to show relative changes in the parameters of the emission band during the protein unfolding. Also, the addition of guanidine hydrochloride resulted first in broadening of the enzyme emission band (Teipel and Koshland 1971). Moreover, a marked decrease in fluorescence polarization degree after the addition of urea was observed while after the addition of liposomes this parameter increased (Table 1). Based on the above results the denaturation mechanism could be ruled out. However, the assumption that the interaction with liposomes could induce a new state of the enzyme structure still seemed to be reasonable. In order to explore the possibility we have used a nonionic fluorescence quencher, acrylamide, as a probe of tryptophan residue exposure (Fig. 5). The efficiency of quenching by a dynamic quencher is a measure of the accessibility of its molecules to fluorogenic residues in protein molecules (Eftink and Ghiron 1976). For some multitryptophan proteins the quenching plots F_0/F vs (Q) do not follow the linear Stern-Volmer equation, revealing upward or downward curving (Efink and Ghiron 1976). Hence the interpretation of such plots for multitryptophan proteins is not simple. Nevertheless, some qualitative information concerning the exposure of fluorophores may be obtained from the initial slope of the plots (K_{eff}). The slight slope of the quenching plot for native G3PDH (Fig. 5) indicates that the tryptophan residues are inaccessible for the quencher, and it supports the conclussion that they are hidden in an apolar interior. The initial slope of the quenching plot increases after the addition of liposomes. The effect of neutral PC is rather small ($K_{eff} \sim 0.3$) but liposomes of acidic PS and PI considerably increase the initial slope of the curves ($K_{eff} \sim 0.85$ $mol^{-1}.l$ and $mol^{-1}.l$ for PS and PI liposomes, respectively). Such an increase in quenching only may be a result of conformation changes which make the tryptophan residues more accessible to the quencher.

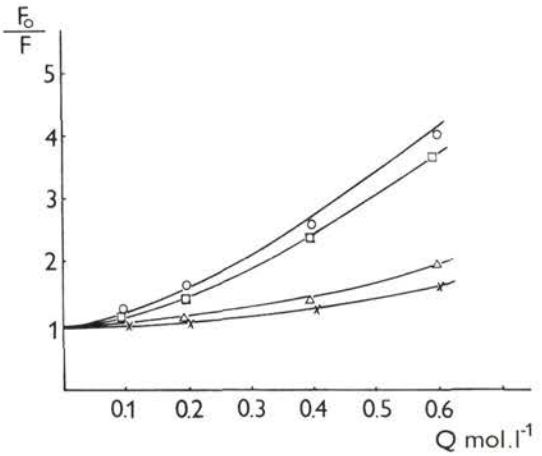

Fig. 5. Stern-Volmer plots of enzyme fluorescence quenching by acrylamide for native enzyme and in the presence of liposomes. The molar lipid/protein ratio was about 400. (×) native enzyme, for other symbols see Fig. 2.

Discussion

Previously, we have found by ultracentrifugation studies that rabbit glyceraldehyde-3-phosphate dehydrogenase can reversibly associate with phospholipid liposomes to different extent depending on the type of phospholipids (Gutowicz and Modrzycka 1978). First of all, results presented above provide additional evidence for the existence of the association. The capacity of fluorescence modification by liposomes depends on the type of phospholipid showing the same sequence as that of association during ultracentrifugation, i. e. PI > PS > PC.

There seem to be several possible mechanisms of fluorescence modification upon multifluorophore protein-lipid interaction: 1) Indirect interaction between single phospholipid molecules or small micelles penetrating the protein molecule interior and fluorophores. In aqueous systems some amount of small micelles and even single molecules of phospholipid exist in equilibrium with liposomes. 2) Association of protein molecules with liposomes inducing no marked changes in their conformation. Then, only long-wavelength fluorescence of the exposed residues could be quenched and an apparent "blue" shift of the maximum would be observed due to the domination of short-wavelength, not quenched fluorescence of hidden tryptophan residues (Eftink and Ghiron 1976). 3) Association wich is accompanied by changes in the protein conformation near the fluorogenic residues at least. 4) Association which results in irreversible unfolding of the polypeptide chain (denaturation).

The observed fluorescence quenching and the simultaneous "red" shift allow to exclude the mechanism 1) and 2). If the mechanisms were involved the wavelength of the emission band maximum would shift to lower values as a result of the quenching of mainly long-wavel ngth fluorescence of more exposed residues. Such effects have been observed for bovine serum albumin in the presence of liposomes (Sidorowicz and Kośmider 1980). A possible direct interaction of tryptophan residues with the apolar part of phospholipid molecules would result in protein fluorescence enhancement. It has been observed for the interaction of phospholipids with hydrophobic apoproteins of blood serum lipoproteins (a review by Morrisett et al. 1977). The conclusion that no direct complex formation occurs between phospholipid molecules and tryptophan residues is also supported by the lack of any changes in UV absorption bands of G3PDH after the addition of liposomes. The denaturation mechanism can be ruled out as well since there is no correlation between modification of the emission spectra or fluorescence polarization degree and those occurring during unfolding by typical denaturants, This conclusion is in agreement with the fact that G3PDH regains its activity after destruction of liposomes by Triton X-100 (Wooster and Wrigglesworth 1976; Gutowicz and Modrzycka 1978). We are not able to interprete the increase in the fluorescence polarization degree exactly since it was determined from total fluorescence of variously situated fluorophores. Nevertheless, we can qualitatively interprete the increase as being a result of changes in mobility and/or intrinsic interactions of the tryptophan residues, the changes being however quite different from those during polypeptide unfolding. Thus, an analysis of the data led us to conclusion that conformation rearrangement must be operative. Data of quenching experiments suffuted this conclusion. During the specific conformation transition some tryptophan residues become more exposed to water. Also, disruption of intrinsic bonds of the residues and the formation of new bonds may contribute to the decrease in fluorescenc intensity and the "red" shift.

On the other hand, the fluorescence quenching method is also sensitive to changes in frequency of conformational fluctuations. Since proteins are not rigid structures and may have flexible regions, the conformational fluctuations allow the quencher molecules to penetrate deeply into the protein molecule interior. Therefore, an increase in G3PDH fluorescence quenching can alternatively be explained as an increase in the dynamic exposure of tryptophanyl residues. This means the formation of new, more flexible regions in the enzyme conformation.

The induction of the specific conformational state upon the interaction with phospholipid bilayer surface may have implications for the enzyme function in vivo. A similar phenomenon, occurring in cells might be a significant factor in the regulation of the enzyme activity and rate of glycolysis.

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