Biophysical Chemistry 27 (1987) 97–102
Elsevier

BPC 01160

Fluorescence investigation on conformational state of rabbit muscle aldolase interacting with phosphatidylinositol liposomes

Jan Gutowicz and Anna Kośmider-Schmidt

Department of Biophysics, Academy of Medicine, Chałubińskiego 10, 50-368 Wrocław, Poland

Received 18 August 1986
Revised manuscript received 10 February 1987
Accepted 19 February 1987

Aldolase; Enzyme-lipid interaction; Fluorescence quenching; Conformational change; Temperature dependence

Evidence of conformational changes in rabbit muscle aldolase upon binding to phosphatidylinositol liposomes and the effect of the interaction on the thermal conformational transition are reported. Interaction with phosphatidylinositol liposomes significantly decreases the aldolase tryptophanyl fluorescence and shifts the maximum wavelength to higher values. The dynamic quenching constant for the aldolase fluorescence quenching by acrylamide in the presence of liposomes is much higher than that for unmodified enzyme; this signifies an increase in accessibility of some tryptophanyl residues to small polar molecules. Indirect interaction between single phospholipid molecules, small micelles or any soluble impurities able to penetrate into the protein molecule interior does not seem to be involved in the conformational rearrangement. Native and liposome-interaction-induced conformational states reveal different temperature dependences of the tryptophan residues exposure. The implications of the modification of the conformational state of the enzyme for its function in vivo are discussed.

1. Introduction

Most glycolytic enzymes, including aldolase, have been repeatedly reported to be bound to cell membranes (for a review, see ref. 1) or at least localized close to the membrane [2]. It has been suggested that the binding of aldolase to erythrocyte membranes occurs via one of the intrinsic proteins: band 3 protein [3–6]. In muscle cells, F-actin [7] and troponin-tropomyosin [8] have been found to be adsorptive proteins for the enzyme. Much less is known about the localization of the enzyme in cells of other tissues. Nevertheless, in all cases of binding of the enzyme to membranes it is affected by the pH, ionic strength and concentrations of ionic metabolites. It is, therefore, generally assumed that the binding is controlled by electrostatic interactions. Hence, phospholipid liposomes as model membranes with well-defined surface charge are convenient systems for studies of the mechanism and implications of such interactions. In addition, we believe that, taking into account the domain structure of membranes, some role of a lipid bilayer in enzyme binding to membranes in vivo should not be neglected.

It has been previously found in our laboratory that phospholipid liposomes, especially those made from acidic phospholipids, can adsorb the enzyme and modify its activity [9].

In this paper we report our attempts to verify the suggestion and studies of the effect of the liposomes-aldolase interaction on the conformational state as well as on its temperature-induced transition. A nondenaturing conformational transition in aldolase at about 26 °C has been postulated by Lehrer and Barker [10,11] and recently

Correspondence address: J. Gutowicz, Department of Biophysics, Academy of Medicine, Chałubińskiego 10, 50-368 Wrocław, Poland.

supported by studies using different methods [12–14].

Measurements of tryptophan fluorescence of the enzyme and its quenching by acrylamide as a nonionic fluorescence quencher were used for monitoring the conformational changes.

2. Materials and methods

2.1. Reagents

Fructose-1,6-bisphosphate aldolase from rabbit muscles was prepared in our laboratory according to Penhoet et al. [15]. The concentration of the enzyme was determined spectrophotometrically using an extinction coefficient of $E_{280}^{1\%} = 0.91$ according to Baranowski and Niederland [16].

A commercial, lyophilized preparation of bovine brain phosphatidylinositols from Koch-Light Laboratories was used without further purification. Its purity was checked by TLC.

Acrylamide used in the fluorescence quenching experiments was obtained from Sigma or from Bruxelles r.b.c, recrystallized from water.

Samples were prepared in 10 mM Tris-HCl/1 mM EDTA buffer (pH 7.5) unless otherwise stated.

2.2. Preparation of liposomes

Liposome suspensions were made by mechanical shaking of the lipid with glass beads in appropriate buffer over 0.5 h at room temperature. This procedure produces multilamellar liposomes with a negatively charged, in this case, surface [17]. The concentration of the phospholipid was calculated from phosphorus determination by the method of Bartlett [18].

2.3. Fluorescence measurements

Fluorescence measurements were performed with a Perkin-Elmer MPF-3L spectrofluorimeter equipped with a thermostatted sample holder. Samples were placed in 4-ml quartz cuvettes. The temperature was monitored using a digital thermometer with the platinum resistance sensor immersed immediately into the sample cuvette. Emission spectra were excited at 290 nm and automatically corrected. This wavelength practically excites only the tryptophanyl fluorescence in proteins. The slit width was 6 nm for both excitation and emission pathways. Background was measured in the control samples of free liposomes and subtracted from the fluorescence spectra. In order to measure the temperature dependence, samples were heated to a given temperature, incubated for approx. 2 min and, after fluorescence measurement, heated to the next temperature value.

3. Results and discussion

The interaction of aldolase with phosphatidylinositol liposomes results in the following modifications of its tryptophanyl fluorescence emission band: decrease in fluorescence intensity, shift of the emission band maximum toward higher wavelengths and some broadening of the band (fig. 1). Neither change in the overall shape nor splitting of the band was observed. The observed effects increase with increase in lipid/protein molar ratio, reaching saturation at a value of approx. 800 [9]. The possible mechanisms of fluorescence modifications upon multifluorophore protein-lipid interaction have been discussed elsewhere [19]. One of the possibilities could be changes in the conformational state of the protein upon interaction. The maximum of the aldolase emission band is strongly shifted to lower values of the wavelength in comparison to that of free tryptophan in aqueous solutions. The value of the maximum wavelength (λ_{max}) is equal to about 320 nm for aldolase, and is among the lowest in multitryptophan proteins.

Burstein et al. [20] used the value of λ_{max} as a criterion for classifying the location of tryptophan residues in the interior of the protein molecule. According to this classification, such a low value of λ_{max} indicates that tryptophan residues of aldolase are buried in apolar pockets, separated from water. Modification of the conformation may lead to a change in λ_{max} and quantum yield of the fluorescence as a result of the alteration in location of tryptophan residues and/or their accessi-

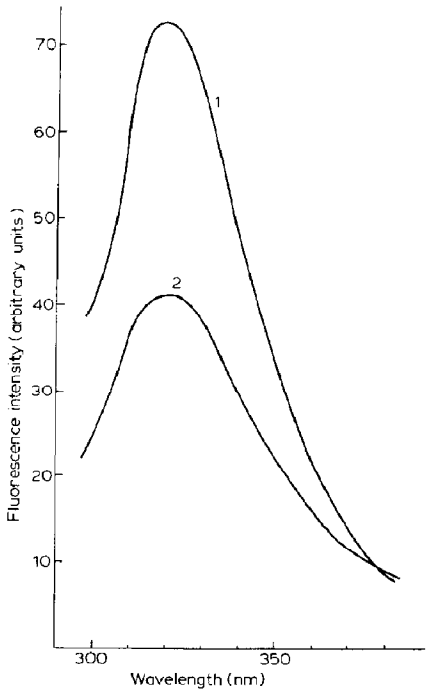

Fig. 1. Influence of phosphatidylinositol liposomes on the fluorescence emission spectra of aldolase. (1) Aldolase, (2) aldolase + phosphatidylinositol liposomes. Enzyme concentration 0.75 μM; lipid/protein molar ratio = 350; room temperature.

bility for interaction with water. In order to verify the hypothesis we applied the method of fluorescence quenching, using the efficient quencher acrylamide as a probe, of protein structure alteration, according to Eftink and Ghiron [21]. If only the collisional (dynamic) mechanism is involved in the quenching of single-tryptophan protein fluorescence it can be described by the simple Stern-Volmer equation: $F_0/F = 1 + K_{SV}[Q]$, where F_0 and F are the fluorescence intensity in the absence and presence of a quencher, respectively and [Q] the concentration of the quencher. When a static mechanism also contributes to the quenching the plots of F_0/F vs. [Q] do not follow the simple equation and exhibit an upward curvature. In multitryptophan proteins the situation is more complicated; the observed fluorescence is an average of the emissions of individual, differently located fluorophores and the occurrence of curvature in the plots also depends on the differentiation in accessibility of tryptophan residues. Nevertheless, Eftink and Ghiron [21,22] have found that acrylamide is predominantly a collisional quencher and the effective quenching constant (K_{eff}), being indicative of the dynamic component of the quenching, is still a measure of the average exposure of tryptophan residues and may be used for monitoring the change in conformation or dynamics of proteins. Furthermore, the native form of aldolase at neutral pH is the case for which linear quenching plots are found [21]. This property can also be seen in our data (fig. 2). It means that the difference in accessibility of various tryptophan residues is rather small in unmodified aldolase.

The fluorescence quenching by acrylamide for native aldolase and that in the presence of a phosphatidylinositol suspension in comparison with that observed for decreasing pH are shown in

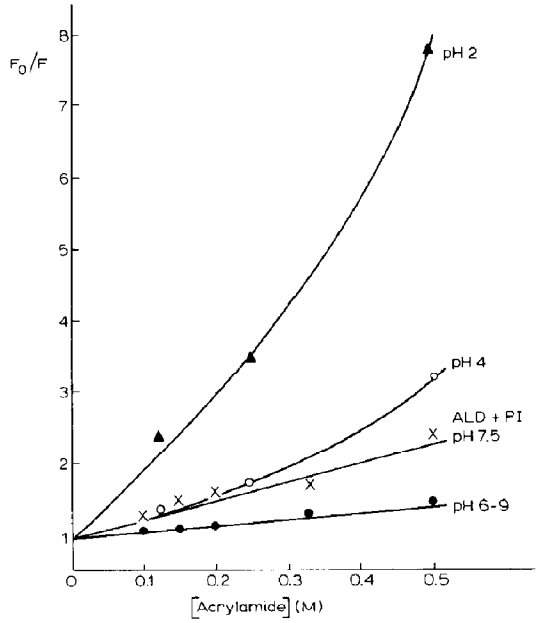

Fig. 2. Stern-Volmer plots of the aldolase fluorescence quenching by acrylamide for the enzyme at various pH values and in the presence of liposomes (●———●) pH 6.0–9.0, Tris buffers; (○———○) pH 4.0, citrate buffer; (▲———▲) pH 2, citrate buffer; (×———×) aldolase + liposomes at pH 7.5, Tris buffer, phosphatidylinositol/aldolase ratio = 350; room temperature. Enzyme concentration in all samples, 0.75 μM.

Table 1

The value of the effective fluorescence quenching constant for aldolase at various pH values and in the presence of phosphatidylinositol liposomes

Conditions: as described in fig. 2

	Quenching constant, K_{eff} (M^{-1})
Aldolase	
pH 6–9	0.8
pH 4	2.6
pH 2	10.0
Aldolase + phosphatidylinositol	
pH 7.5	
Phosphatidylinositol/aldolase = 350	2.6
Phosphatidylinositol/aldolase = 1000	2.8

fig. 2. Effective Stern-Volmer constants were determined from the slope or initial slope of linear or curved plots, respectively. Values of the quenching constant calculated for cases such as those in fig. 2 are listed in table 1. Fluorescence of the unmodified enzyme in the pH range 6–9 was slightly quenched by acrylamide. This indicates that tryptophan residues are nonexposed, with low accessibility for quencher molecules in aldolase at these pH values and is in good accordance with the short-wavelength maximum of the enzyme emission band. Addition of phosphatidylinositol liposomes increased the quenching constant, indicating increased exposure of the tryptophan residues. Similar changes in the quenching constant we observed for decreasing pH. However, an increase such as that at pH 2 could not be reached even for a large excess of the liposomes. In order to ascertain whether the observed effects did not result from the interaction of small structures of the lipid such as micelles, single phospholipid molecules or even soluble impurities, we performed control measurements of the quenching by acrylamide of dialysed samples. A lipid suspension dialysed overnight, subsequently adjusted to appropriate concentrations and then added to aldolase solution gave the same quenching constant as nondialysed samples (data not shown). This result indicates that the interaction of aldolase with large nondialysable liposomes is responsible for changes in the quenching constant. However,

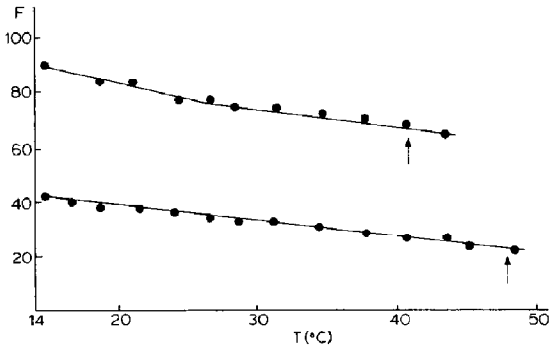

Fig. 3. Temperature dependence of the fluorescence intensity of aldolase in the absence and presence of phosphatidylinositol liposomes. (1) Aldolase, (2) aldolase + liposomes. Enzyme concentration, 2.5 μM; lipid concentration, 250 μM. The straight lines in this figure as well as in fig. 4 were obtained using the method of least squares. Regression coefficients: 0.989 and 0.998 for curve 1 below and above the break, respectively, and 0.998 for curve 2. Arrows indicate the temperatures at which opalescence of the samples appeared.

the small structures and/or soluble impurities likely contribute somewhat to changes in the fluorescence intensity, since the dialysate showed some capability of quenching aldolase fluorescence but without shifting the maximum wavelength and no ability to alter the acrylamide quenching constant.

Next, the temperature dependence of the enzyme fluorescence and its quenching by acrylamide in the absence and presence of phosphatidylinositol liposomes were investigated. The native fluorescence of aldolase decreased with rising temperature and at a temperature of about 40 °C the samples became unstable, opalescent and subsequently turbid (fig. 3). One may observe a break of the plot near 26 °C and changes in its slope. The dependence is nearly identical to that obtained by Heyduk and Kochman [14]. Fluorescence of the enzyme in the presence of the liposomes behaved in the same manner but in this case no break of the plots can be found. In addition, in this case the samples were more stable and their turbidity did not change until 48 °C had been reached (see arrows in fig. 3). The temperature dependence of the fluorescence quenching constant (K_{eff}) appeared to be more sensitive to conformational transitions. The plot of the

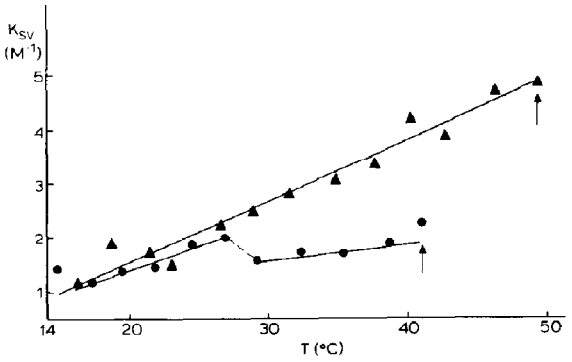

Fig. 4. Temperature dependence of the effective quenching constant (K_{eff}) for aldolase (●) and aldolase in the presence of phosphatidylinositol liposomes (▲). Enzyme concentration, 2.5 μM; lipid concentration, 0.5 mM. Each point represents the K_{eff} value calculated from the initial slope of the Stern-Volmer plots at a given temperature. Regression coefficients: 0.980 and 0.957 for (●) below and above the transition, respectively, and 0.983 for (▲). Arrows as in fig. 3.

quenching constant vs. temperature for unmodified aldolase shows a marked discontinuity near 26 °C and a change in slope of the line (fig. 4). The transition did not occur in the conformational state existing in the presence of the liposomes. Here, the quenching constant monotonically increased with rising temperature. It should be noted that the plot for the enzyme modified by the liposomes has a greater slope, especially in the region 29–50 °C. This means that this conformation is more sensitive to rising temperature in terms of fluorophore exposure but, on the other hand, is more thermostable since aggregation or denaturation of the enzyme occurs at a markedly higher temperature in this case (see arrows in fig. 4).

Since protein molecules are not rigid structures a change in the static conformational state is not the only possible explanation for the data obtained. A quencher of this type is also a sensitive probe for changes in the dynamics of the structure. Fluctuations of the conformation on the nanosecond scale allow the quencher molecules to penetrate into the protein molecule interior and thus quench the fluorescence of even deeply buried fluorophores [21,23–25]. The complex matrix of a protein molecule reveals a number of different types of motions such as conformational fluctuations and segmental, interdomain, intersubunit mobilities (for a review, see ref. 26). Lakowicz et al. [27] have shown that the tryptophan residues in aldolase are relatively immobile. Any changes in these motions due to interaction of the enzyme with membranes can result in modification of the quencher's accessibility to fluorophores or alteration of the environmental conditions of the quenching. Calhoun et al. [28] have presented another interpretation of the quenching. They have postulated the large-amplitude oscillation of local unfolding of polypeptide chain fragments. It is assumed in their conception that 'open' and 'closed' conformational forms can occur in equilibrium. Changes in value of the quenching constant would then mean that a shift in equilibrium occurs. In spite of various possible interpretations of the quenching data, we can generally state that modification of the degree of quenching reflects changes in the static and/or dynamic state of the protein. Exact determination of the contributions of the two mechanisms requires further studies involving dynamic fluorescence methods. Nevertheless, above-described results on fluorescence quenching and differences in temperature dependences between aldolase and aldolase treated with liposomes provide further evidence for the interaction and strongly support the suggestion that modification of its conformation and/or dynamics takes place. We believe that the modification is due to a not very profound change in tertiary structure of the enzyme, since the effects on the enzyme fluorescence spectra appeared to be reversible with factors affecting ionic interactions [9].

Comparison of the conformation alteration with those occurring at low pH allows one to conclude that the denaturation mechanism is not operative here.

The difference between the temperature dependences of the native and modified enzymes is not very drastic but the disappearance of the temperature-induced conformational transition near 26 °C seems to be the most interesting fact.

A number of literature reports have demonstrated that aldolase possesses the ability to bind to membranes as a result of electrostatic interactions. A question that arises from the results ob-

tained concerns the implications for the enzyme function in vivo. In spite of the fact that the activity modification of an enzyme adsorbed on membranes may result from several different effects, the alteration in the conformational state of aldolase may be a crucial factor in the regulation of the enzyme activity.

Since similar changes in structure have been found for glyceraldehyde-3-phosphate dehydrogenase adsorbed on phospholipid liposomes [19], the mechanism of regulation of catalytic properties by adsorption on membranes among the glycolytic enzymes as a more general rule should be taken into account.

Acknowledgements

The authors are very grateful to Mrs. H. Dudziak and Miss M. Brobrowska for helpful technical assistance. This work was supported by research grant C.P.B.P. 04.01. from the Polish Academy of Sciences.

References

1 J.M. Salhany and K.C. Graines, Trends Biol. Sci. 6 (1981) 13.
2 E. Csebe and G. Szabolcsi, Acta Biochim. Biophys. Acad. Sci. Hung. 18 (1983) 151.
3 J.A. Kant and T.L. Steck, J. Biol. Chem. 248 (1973) 8457.
4 J. Yu and T.L. Steck, J. Biol. Chem. 250 (1975) 9170.
5 E. Strapazon and T.L. Steck, Biochemistry 15 (1976) 1421.
6 H.J. Kliman and T.L. Steck, J. Biol. Chem. 255 (1980) 6314.
7 D. Pette, Acta Histochim. Suppl. Bd. XIV (1975) 47.
8 F.M. Clarke and C.J. Masters, Biochim. Biophys. Acta 381 (1975) 37.
9 J. Gutowicz and T. Modrzycka, Biochim. Biophys. Acta 554 (1979) 358.
10 G.M. Lehrer and R. Barker, Biochemistry 9 (1970) 1533.
11 G.M. Lehrer and R. Barker, Biochemistry 10 (1971) 1705.
12 M. Kochman and T. Heyduk, Abstr. 14th FEBS Meet. (1984) 119.
13 A. Kośmider-Schmidt and J. Gutowicz, in: Proc. 7th Sch. Biophys. Membrane Transp. (Agric. Univ., Wrocław, 1984) pt. II, p. 263.
14 T. Heyduk and M. Kochman, Eur. J. Biochem. 151 (1985) 337.
15 E.E. Penhoet, M. Kochman and J. Rutter, Biochemistry 8 (1969) 4391.
16 T. Baranowski and T.R. Niedreland, J. Biol. Chem. 180 (1949) 543.
17 F. Szoka and D. Papahadjopoulos, Annu. Rev. Biophys. Bioeng. 9 (1980) 467.
18 G.R. Bartlett, J. Biol. Chem. 234 (1959) 466.
19 J. Gutowicz and T. Modrzycka, Gen. Physiol. Biophys. 5 (1986) 297.
20 E.A. Burstein, M.S. Vendenkina and M.N. Ivkova, Photochem. Photobiol. 18 (1973) 263.
21 H.R. Eftink and C.A. Ghiron, Biochemistry 15 (1976) 672.
22 H.R. Eftink and C.A. Ghiron, Biochemistry 23 (1984) 3891.
23 J.R. Lakowicz and G. Weber, Biochemistry 12 (1973) 4161.
24 J.R. Lakowicz and G. Weber, Biochemistry 12 (1973) 4171.
25 J.R. Lakowicz, J. Biochem. Biophys. Methods 2 (1980) 91.
26 M. Vijayan and D.M. Saluke, J. Biosci. 6 (1984) 357.
27 J.R. Lakowicz, B.P. Malival, H. Cherek and A. Balter, Biochemistry 22 (1983) 1741.
28 D.S. Calhoun, J.N. Vanderkooi and S.W. Englander, Biochemistry 22 (1983) 1533.