INTERFACIAL REGULATION: INFLUENCE OF LIPID POLAR GROUP CONFORMATION ON LIPID ACTIVATION OF C_{55}-ISOPRENOID ALCOHOL PHOSPHOKINASE APOPROTEIN

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1. Introduction

The viscosity of the hydrocarbon interior of the membrane lipid phase is of importance for a variety of functions of membrane proteins [1,2]. It was previously observed, however, that a number of 'fluid' lipids failed to activate the lipid-dependent membrane enzyme, C_{55}-IP [3]. This finding has been correlated with the failure of these lipids to bind water, as measured by a newly developed method under experimental conditions identical to those of the enzyme assay [4,5]. The term 'interfacial regulation' has been used to designate the dependence of membrane functions on the amount or structure of bound interfacial water [5].

In the present paper, two independent methods, viz., the addition of detergent, and the acetylation of the primary amino group, have been found to convert the inactive lipid, dilauryl-PE, into an active cofactor of the C_{55}-IP reaction. Based mainly on recent X-ray data [6–8] these results are explained by a consideration of lipid polar group conformations at the lipid/water interface.

2. Experimental

C_{55}-IP apoprotein was purified from membranes of *Staphylococcus aureus*, strain H, through step 6 (chromatography on DEAE-cellulose), as described elsewhere [9,10]. Ficaprenol was prepared from leaves of *Ficus elastica* [11]. Dilauryl-PC and dilauryl-PE were purchased from Calbiochem, Lucerne

Dilauryl-PE was acetylated by the procedure of Knowles et al. [12] until the ninhydrin reaction indicated complete conversion to the *N*-acetyl derivative. The reagents were removed by lyophilization with repeated additions of methanol and chloroform. The product gave a single phosphoruspositive and iodine-negative spot on thin layer plates (Merck, Darmstadt, No. 5554) in solvent (A) (R_f 0.06; dilauryl-PE, R_f 0.14; dilauryl-PC, R_f 0.02) and in solvent (B) (R_f 0.32; dilauryl-PE, R_f 0.52; dilauryl-PC, R_f 0.11). Solvent systems used were (A) chloroform/methanol, 7 : 1 (v/v), and (B) chloroform/methanol/water, 70 : 20 : 0.5 (v/v/v).

Lipid concentrations were determined by phosphorus analysis [13]. The enzyme assay was carried out as previously described [3]. [γ-^{32}P]ATP was kindly given by Drs G. Feix and H. Sano, Freiburg.

3. Results

The present study was carried out with three chemically rather stable ether-lipid derivatives, because acyl-lipids, particularly those containing unsaturated fatty acids, tended to release surface-active degradative impurities like lysolipids and fatty acids (H. Sandermann, unpublished).

When tested in the absence of detergent, dilauryl-PE was virtually devoid of lipid cofactor activity, except for a moderate activity at relatively high lipid concentrations (fig.1A). The addition of the detergent, Triton X-100, gave rise to strong enzyme activation

Abbreviations: C_{55}-IP, C_{55}-isoprenoid alcohol phosphokinase. PE, phosphatidylethanolamine. PC, phosphatidylcholine.

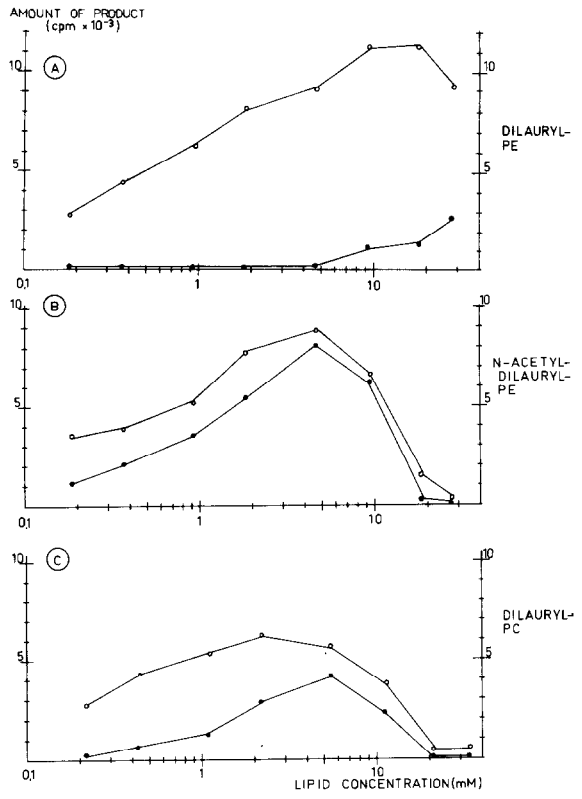

Fig.1. Dependence of C_{55}-isoprenoid alcohol phosphokinase activity on lipid concentration in the absence (●——●) and in the presence (○——○) of 0.3% Triton X-100. The lipids examined were dilauryl-PE (A), N-acetyl dilauryl-PE (B) and dilauryl-PC (C). The assay was carried out in a total volume of 26 μl and at a temperature of 37°C, as described [3]. The amount of [γ-^{32}P]ATP used was 20 nmol (60 000 cpm).

by dilauryl-PE (fig.1A). Triton X-100 by itself was virtually devoid of cofactor activity (cf. [3]).

In contrast to dilauryl-PE, its *N*-acetyl derivative was an active cofactor even in the absence of detergent (fig.1B). The reference lipid, dilauryl-PC, was active in the absence and in the presence of Triton X-100 (fig.1C) although its cofactor activity was lower than that of *N*-acetyl dilauryl-PE. The cofactor activities of dilauryl-PC and *N*-acetyl dilauryl-PE were strongly decreased above lipid concentrations of 10 mM; however, this was not the case with dilauryl-PE. The cofactor activity of 4.6 mM *N*-acetyl dilauryl-PE was decreased by less than 20% in the additional presence of up to 4 mM dilauryl-PE or dilauryl-PC (no detergent present).

4. Discussion

The C_{55}-IP apoprotein appears to have no requirement for a particular chemical structure of the polar group of lipid activators [3]. The present discussion therefore concentrates on the physical properties of the lipids examined: lipid viscosity and interfacial hydrogen bonding. For discussion of the possible role of lipid viscosity, the thermal properties of the lipids employed are considered first.

The thermal phase transition temperatures of dilauryl ether lipid derivatives have apparently so far not been reported. However, estimations can be made from existing data. The thermal phase transition of dilauroyl-PC occurs near 0°C [14]. A 3°C increment has to be added for the replacement of the fatty acyl ester linkages by ether linkages [15], so that the thermal phase transition of dilauryl-PC probably occurs between 0°C and 5°C. When the reported calorimetric phase transition temperatures of 63°C for dipalmitoyl-PE [15–17] and of 48°C for dimyristoyl-PE [17,18] are extrapolated to dilauroyl-PE, and a 5°C increment is added for the replacement of the fatty acyl ester linkages by ether linkages [15,16], one arrives at a probable thermal phase transition temperature between 35°C and 45°C for dilauryl-PE. In analogy to the *N*-methyl derivatives of PE [15,16] the thermal phase transition temperature of *N*-acetyl dilauryl-PE probably is between those of dilauryl-PE and dilauryl-PC. The thermal phase transition at least of synthetic lecithins is largely suppressed and shifted to lower temperatures when 35 mol% of the highly unsaturated ficaprenol is present in the assay system [3,19]. Ficaprenol (0.5 mM) was present in all of the experiments shown in fig.1. It is concluded that the phospholipids studied in fig.1 probably were in a 'fluid' state under the conditions of the enzyme assay (37°C), so that the results obtained cannot easily be explained in terms of lipid viscosity.

Recent X-ray studies [6–8] have indicated that the main feature of the polar group conformation of PE is strong intermolecular hydrogen bonding. The resulting close packing in the plane of the lipid/water interface can be expected to be unfavorable for permeation and lateral diffusion processes and for the insertion of an enzyme protein. Tight intermolecular hydrogen bonding could thus be responsible for the failure of dilauryl-PE to activate the C_{55}-IP apoprotein.

The following two methods are expected to break intermolecular hydrogen bonding:

(i) The addition of Triton X-100 to phospholipids leads to intercalation of detergent between phospholipid molecules [20,21] thus probably loosening the packing of PE molecules and breaking intermolecular hydrogen bonds.

(ii) The acetylation of the primary amino group of PE obviously prevents intermolecular hydrogen bonding. Both of the above methods led to the conversion of dilauryl-PE to an active cofactor, thus providing an additional example for the regulation of the C_{55}–IP reaction by primarily interfacial rather than viscotropic [2] parameters.

It is of interest to compare the present results with those obtained with *N*-acetyl PE by Knowles et al. [12] in the reconstitution of ion pumps. With two of the ATPases tested, *N*-acetyl PE was active only in the presence of a hydrophobic alkylamine. Compared to the reactivation of the C_{55}–IP apoprotein these ATPase therefore appear to have an additional requirement for a positive interfacial charge of the lipid phase. The C_{55}–IP apoprotein has previously been shown not to require a specific surface charge of the lipid phase [3].

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