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The temperature dependence of adenylate cyclase activity solubilised using various Lubrol detergents

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Summary: Arrhenius plots of the fluoride-stimulated adenylate cyclase activity of rat liver plasma membranes are linear. Solubilisation using various lubrol detergents yield adenylate cyclase preparations whose Arrhenius plots reflect the physical properties of the detergent used. This suggests that the detergent is tightly bound to the enzyme and can modulate its activity.

Introduction

The glucagon receptor and the catalytic unit of adenylate cyclase are functionally distinct entities able to undergo free lateral diffusion in the plane of the bilayer; on hormone binding to the receptor, this complex couples with the catalytic unit, activating it and forming a multicomponent system spanning the bilayer (1).

The activity of adenylate cyclase is modulated by the physical properties of the bilayer. In the uncoupled state it experiences lipid phase separations occurring in the inner half of the bilayer, and in the coupled state, activated by glucagon it senses lipid phase separations in both bilayer halves (2,3). These lipid phase separations may be manipulated by lipid fusion and substitution methods (4) or by increasing bilayer fluidity using the neutral local anaesthetic benzyl alcohol (5), and the activity of the enzyme responds accordingly.

A number of studies have focussed on the efficiency of various detergents to yield a solubilised adenylate cyclase (6-8) but

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little is known about the effect, if any, that various detergents have on enzyme activity. In this study we demonstrate that soluble preparations of adenylate cyclase obtained using different lubrol detergents exhibit markedly different Arrhenius plots.

Materials and Methods

Rat liver plasma membranes were prepared and stored as described previously (5). Assays of adenylate cyclase and methods of carrying out Arrhenius plots were as described in full previously (2,5). Soluble preparations of adenylate cyclase were achieved by using various Lubrol detergents, as described by Swislocki et al. (8), fractions were assayed immediately after preparation. Lubrol detergents were a gift from I.C.I. Ltd., Macclesfield, U.K.; creative phosphate, creative phosphokinase, ATP and triethanolamine HCl were from Boerhinger (U.K.). All other chemicals were of A.R. quality from B.D.H., Poole, U.K.

Results and Discussion

Arrhenius plots of the fluoride-stimulated adenylate cyclase activity of native rat liver plasma membranes are linear, as no lipid phase separation occurs in the inner half of the bilayer over the temperature range studies, 3-42^{o}C (3,4,5). However, it is possible to induce one by sequestering the cholesterol present in the membrane using the polyene antibiotic, amphoptericin B (9).

Solubilisation of the fluoride-stimulated adenylate cyclase activity using these four different lubrol detergents, yields enzymes whose activities exhibit markedly different Arrhenius plots (fig.1, table 1). Three of the Arrhenius plots are clearly non-linear with break points occurring at temperatures ranging from 16^{o} to 23^{o}C (table 1, Fig.1). Using Lubrol G to effect solubilisation, a linear result is obtained (fig.1) whose activation energy is markedly different from that exhibited by the native enzyme (table 1). Interestingly the form of the Arrhenius plots appears to be related to the physical properties of the detergents. A number of the detergents have 'melting points' which lie

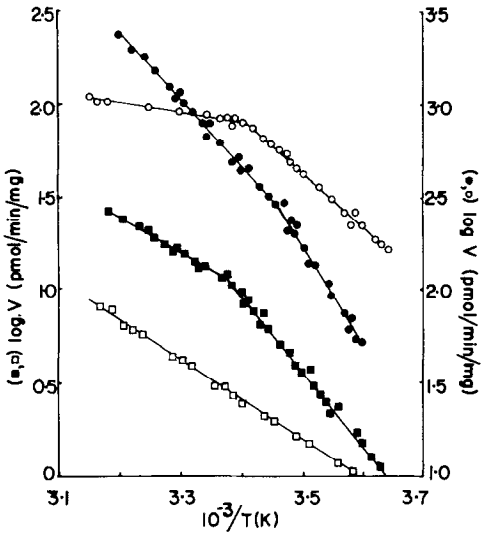

Figure 1. Arrhenius plots of adenylate cyclase activity solubilised using various Lubrol detergents. Lubrol G (□), Lubrol 17A10 (■), Lubrol N13 (○) and Lubrol 17A10 (●). Activity is given in pmols/min/mg protein.

Table 1

Arrhenius plots of Lubrol solubilised fluoride-stimulated adenylate cyclase activity of rat liver plasma membranes

Detergent	'melting point'	Arrhenius plot Break point	Activation energies (KJ mol^{-1}) above break	below break
Native membrane	-	Linear (4)	84.1±3.8	
Lubrol 12A9	19°C	16.2° ± 0.5(4)	67.8±2.5	93.8±3.8
Lubrol G	- 10°C	Linear (3)	40.3±5.8	
Lubrol 17A10	23°C	22.8° ± 2.5(3)	37.0±7.5	75.0±9.4
Lubrol N13	20°C	20.4° ± 2.3(3)	11.4±1.7	51.2±7.3

'Melting points' are from ICI data sheets. The number of determinations is given in parentheses, and errors are S.D.

in the temperature range studied, and there is good correlation with such 'melting point' temperatures and the break point in the

Arrhenius plots (table 1). Arrhenius plots of adenylate cyclase activity solubilised with Lubrol G, which has a subzero 'melting point' yielded a linear result which might be expected by such an interpretation.

This data implies that the detergents are tightly bound to the enzyme, and indeed it is believed that adenylate cyclase is liberated as a detergent-protein complex, where detergent can constitute as much as 10-20% by weight (7,10,11). Tentative calculations for Lubrol suggest that this is sufficient to form a single micelle around the hydrophobic region of the enzyme (10). Thus, changes in detergent or micellar structure that occur at such 'melting points' (5,12) appear to be transmitted to the enzyme and reflected by the Arrhenius plots of the activity so obtained. This may have consequences for studies on solubilised enzyme preparations, and on attempts to reconstitute systems.

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