INFLUENCE OF THE SURFACE POTENTIAL ON THE MICHAELIS CONSTANT OF MEMBRANE-BOUND ENZYMES

Effect of membrane solubilization

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1. Introduction

Detergents used at sufficiently high concentrations solubilize biological membranes, setting free their integral hydrophobic proteins. During this process the proteins may undergo serious alterations (review [1]). Mild detergents, especially those of non-ionic character, interact less drastically and therefore are routinely used for isolation of membrane-bound enzymes [2]. Nevertheless, even in these cases catalytic properties of the enzymes may be changed due to several factors such as alterations of secondary and tertiary structures, depletion of essential lipids, and presence of detergent molecules associated with the enzyme. This paper points to the abolition of the membrane surface potential as a factor influencing the activity of membrane enzymes after solubilization. It is a continuation of our studies in which the effect of the surface potential on Michaelis constants of a series of membrane-bound enzymes has been demonstrated [3–5].

2. Materials and methods

Mitochondria from rat liver were obtained as in [6] and those from the brown adipose tissue of cold-adapted guinea-pigs according to [7]. Microsomes from rat liver and brain were isolated from the post-mitochondrial supernatants as in [3]. The membranes were solubilized by the non-ionic detergent Lubrol-WX used at 1.7%. The concentration of the particles used for solubilization corresponded to ~20 mg protein/ml.

Arylsulphatase C (EC 3.1.6.1) was determined as in [8] as modified in [9] with *p*-nitrophenyl sulphate as substrate. Glycerol-3-phosphate dehydrogenase (EC 1.1.99.5) was assayed with phenazine methosulphate as the primary electron acceptor and 2,6-dichloroindophenol as the secondary acceptor as in [3]. The activity of monoamine oxidase (EC 1.4.3.4) was determined as in [10] with dopamine as substrate by measuring oxygen uptake in the presence of 1 mM KCN. Acetylcholinesterase (EC 3.1.1.7) was determined by measuring liberation of thiocholine from acetylthiocholine [11]. NADPH-cytochrome *c* reductase (EC 1.6.2.4) was measured according to [12]. Glucose-6-phosphatase (EC 3.1.3.9) and the pyrophosphate-glucose phosphotransferase activity of this enzyme were assayed as in [13].

Incorporation of enzymes into liposomal membranes was performed according to [14] with slight modifications [5]. Electrophoretic mobility of microsomes was measured using the microphoretic instrument Parmoquant 2 (Carl Zeiss, Jena).

Lubrol-WX was obtained from Sigma (St Louis MO). Phosphatidylcholine was isolated from rat liver and purified as in [15]. Other chemicals and biochemicals were commercial products of high purity.

3. Results and discussion

Seven enzymic activities associated with various biological membranes were assayed and their kinetic parameters (K_m and V) determined before and after solubilization of the membranes with Lubrol-WX. The results can be summarized as follows (table 1):

(i) Apparent K_m-values of enzymes reacting with anionic substrates were decreased by solubilization to ~1/2;

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Table 1
Effect of solubilization by Lubrol-WX on K_m and V values of membrane-bound enzymes

Enzyme (no. expt)	Material	Substrate	K_m (mM)	K'_m (mM)	K'_m/K_m	V'/V	ψ_s (mV)
Arylsulphatase C (15)	Liver microsomes	Anionic	2.09 ± 0.24	1.31 ± 0.14	0.63	0.99	−12.5
Glucose-6-phosphatase (7)	Liver microsomes	Anionic	1.70 ± 0.28	0.84 ± 0.16	0.49	1.63	−18.4
Glycerol-3-phosphate dehydrogenase (1)	Brown adipose tissue mitochondria	Anionic	3.23	1.39	0.43	1.00	−21.7
Acetylcholinesterase (5)	Brain microsomes	Cationic	0.010 ± 0.001	0.020 ± 0.002	2.00	1.15	−17.7
Monoamine oxidase (4)	Liver mitochondria	Cationic	0.135 ± 0.017	0.339 ± 0.011	2.51	1.00	−24.0
NADPH-cytochrome *c* reductase (2)	Liver microsomes	Cationic (cytochrome *c*)	0.005	0.010	2.00	1.95	−17.7[a]
Pyrophosphate-glucose phosphotransferase (1)	Liver microsomes	Non-ionic (glucose)	51.7	50.7	0.98	2.34	–

[a] Assuming cytochrome *c* to be a monovalent cation ($z = +1$)

K_m and K'_m indicate apparent Michaelis constants in the native membrane and after solubilization, respectively. V'/V is the ratio of maximum reaction rates (at saturating substrate concentrations) after and before solubilization. The values are means ± SEM. ψ_s was calculated from eq. (1) from mean values of K_m and K'_m. The temperature and major electrolytes of the assay media were as follows: arylsulphatase, 37°C, 250 mM Tris–HCl (pH 7.5); glucose-6-phosphatase and PP_i-glucose phosphotransferase, 30°C, 40 mM cacodylate buffer (pH 6.0); glycerol-3-phosphate dehydrogenase, 25°C, 10 mM Tris–HCl (pH 7.4); acetylcholinesterase, 25°C, 2.5 mM phosphate (pH 7.0); monoamine oxidase, 30°C, 10 mM Tris–HCl (pH 7.4); NADPH-cytochrome *c* reductase, 25°C, 20 mM phosphate (pH 7.6)

(ii) Apparent K_m-values of enzymes reacting with cationic substrates were doubled;
(iii) Apparent K_m of an enzyme with respect to a non-ionic substrate remained unchanged;
(iv) Activities at saturating concentrations of the substrates (V-values) were either unchanged or increased, independently of the ionic character of the substrate.

When the solubilized enzymes were incorporated into phospholipid vesicles, their app. K_m-values became dependent upon the composition of the vesicles [5]. However, upon re-solubilization in Lubrol-WX this dependence disappeared (table 2). It is worthy of note that app. K_m-values of the enzymes in re-solubilized vesicles were close to those in solubilized native membranes (cf. tables 1,2; fig.1 in [5]). The effect of surfactants [3] and of the phosphorylation of membrane proteins [4] on K_m-values of membrane-bound enzymes also disappeared upon solubilization of the membranes.

Since most biological membranes have a net negative surface charge, the concentration of anions is lower and that of cations is high in the immediate vicinity of the membrane as compared to the bulk medium. Therefore, app. K_m-values of enzymes reacting with anionic substrates should be elevated and those of enzymes reacting with cationic substrates decreased by the negative surface potential of the membranes. When the membrane is solubilized, its surface potential disappears. Based on this, one may expect that the ratio between app. K_m before and after solubilization can be a measure of the absolute surface potential (ψ_s) according to the formula introduced in [16,17] (see also [3]):

$$\psi_s = \frac{kT}{ze} \ln \frac{K'_m}{K_m} \qquad (1)$$

where k is the Boltzmann constant, T is the absolute temperature, e indicates the electron charge, and z is an integer (positive or negative) designating the number of charges on the substrate molecule; K_m is the apparent Michaelis constant of an enzyme in the native membrane and K'_m is the apparent Michaelis constant in the solubilized material. Calculations made using

Table 2
Effect of re-solubilization on Michaelis constants of enzymes incorporated into liposomes

Enzyme	Composition of liposomes	K_m-values (mM)	
		In liposomes	After solubilization
Arylsulphatase C	Phosphatidylcholine	2.95	1.48
	Phosphatidylcholine 90 mol% + tridecylamine 10 mol%	2.27	
	Phosphatidylcholine 80 mol% + tridecylamine 20 mol%	1.90	
	Phosphatidylcholine 60 mol% + tridecylamine 40 mol%	1.50	1.41[a]
	Phosphatidylcholine 50 mol% + tridecylamine 50 mol%	1.39	
Monoamine oxidase	Phosphatidylcholine	0.170	
	Phosphatidylcholine 70 mol% + phosphatidic acid 30 mol%	0.124	
	Phosphatidylcholine 70 mol% + tridecylamine 30 mol%	0.244	0.357[a]

[a] Pooled liposomes were solubilized as indicated

K_m-values for enzymes incorporated into liposomes are taken from [5]

eq. (1) give values of −12 mV to −24 mV for various membranes under ionic conditions for the enzymic assays employed (table 1).

The question arises as to what extent these values reflect the real absolute surface potential of the membranes. An answer to this may come from electrophoretic studies on membrane fragments, although it should be kept in mind that the electrophoretic mobility reflects the ζ potential which is probably somewhat lower than the surface potential sensed by membrane-bound enzymes. Such measurements were done for rat liver microsomes suspended in 10 mM Tris–HCl (pH 7.4) containing various [KCl]. Assuming that the thickness of the ionic double layer was negligible compared to the particle radius, the ζ potential could be calculated from the Smoluchowski equation:

$$\zeta = \frac{\eta U}{\epsilon_o \epsilon_r} \qquad (2)$$

where η is the viscosity of the medium, U is the electrophoretic mobility, ϵ_o designates the permittivity of vacuum, and ϵ_r is the relative dielectric constant. The values thus obtained amounted to −30 mV, −18 mV, −15 mV and −11 mV for 0, 50 mM, 100 mM and 200 mM KCl, respectively. Similar values have been obtained for cerebral cortex synaptosomes [18] and can also be calculated from the surface charge density of −0.01 C/m^2 reported by various authors for thylakoid membranes (review [19]). Thus, values of the surface potential calculated from changes of app. K_m of membrane enzymes after solubilization (table 1) are in a fairly good agreement with what can be expected for various natural membranes.

This investigation gives further evidence for the controlling role of the surface potential on membrane enzymes. It may also provide an explanation for some observations reported in the literature, e.g., the activation by non-ionic detergents of arylsulphatase [9,20] and the decrease of K_m of glucose 6-phosphatase [21,22]. The latter enzyme was also activated when microsomes were solubilized by high pH [23]. The view presented here is also in line with the observation [22] that the K_m-value of PP_i-glucose phosphotransferase for the anionic substrate, pyrophosphate, was lowered by detergents, whereas K_m for glucose (the non-ionic substrate) remained unchanged (see also table 1). Similarly, K_m of monoamine oxidase for its cationic substrates, monoamines, was increased but K_m for oxygen was unchanged [24].

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