Low-energy Mg^{2+}-induced temperature transitions in liver microsomes

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The differential scanning microcalorimetry and fluorescence methods, using probes ANS and pyrene, have been employed to study thermotropic behaviour of rat liver microsomes in the presence and absence of Mg^{2+}. Addition of Mg^{2+} yields three partially reversible phase transitions at 18, 27 and 32°C, respectively. A character of Mg^{2+}-induced rearrangements in a membrane and their relation to a catalytic function of a cytochrome P-450-dependent enzymatic system is discussed.

Rat liver microsome *Mg^{2+}* *Differential scanning microcalorimetry* *Fluorescent probe*

1. INTRODUCTION

A membrane of mammalian liver microsomes is characterized by the variety of protein and lipid components and heterogeneity of their organization [1–4]. Heterogeneity of a phospholipid component in a membrane may manifest itself when studying cytochromes of the P-450-dependent enzymatic microsomal monooxygenation system and be the reason for discrepancy in the results produced by different methods and under various conditions [5–11]. It is not excluded that the organization of phospholipids is changed due to the compounds that, strictly speaking, are not the components of a biological membrane. Thus, for instance, the bivalent metal ions–negatively charged phospholipids interaction is responsible for an increase in a phase transition temperature, lateral phase segregation, appearance of non-bilayer structures both in model systems and in a mitochondrial membrane [12–17].

This paper aims at exploring the effect of Mg^{2+} on thermotropic behaviour of rat liver microsomes. Mg^{2+} is known to be an effector of intracellular enzymes [18] and often used as a component in a buffer solution when investigating the enzyme activity in vitro.

Abbreviation: ANS, 1-anilinonaphthalene-8-sulphonate

2. METHODS

Microsomes were prepared from rat liver as in [19]. Control preparations of the microsomes were dialysed against 500 vol. 0.1 M Tris–HCl buffer (pH 7.4) containing 1 mM EDTA for 15 h. The total fraction of microsomal lipids was isolated as in [20]. Concentration of lipids in the assay was determined by phosphorus content 21. After Mg^{2+} addition, the assays were subjected to incubation for 30 min at 25°C. Then, the preparations were examined in a differential scanning microcalorimeter, model DASM-IM (USSR) [22]. The spectrum-luminescence study of ANS and pyrene was carried out as in [23,24] using a spectrofluorimeter, model Fica 55 (France).

3. RESULTS

Thermotropic properties of microsomes have been investigated by differential scanning microcalorimetry in the temperature range 10–80°C. Fig.1a presents the heat absorption curve of predialysed microsomes (the low-

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temperature range). Temperature-induced changes are observed only within the temperature range 37–80°C (fig.1e). The heat absorption domain consists of a number of partially overlapping peaks.

It is known that at these temperatures the irreversible denaturation of protein components proceeds [5–7].

Indeed, with repeated heating of preparations, no heat absorption was observed (not shown). Further experiments were not concerned with detailed investigations in a high-temperature region.

After incubation of microsomes in a buffer containing 10 mM $MgCl_2$, a thermogram showed 3 low-energy transitions, with maxima at 18, 27 and 32°C and transition enthalpy, ΔH, being about 0.1 kcal/mol each in terms of total content of phospholipids (fig.1b, upper curve). The results obtained were reproducible by using the same preparation of microsomes. However, the temperatures, at which Mg^{2+}-induced transitions were observed, varied within ± 2°C, depending on the preparation used. Repeated scanning of a sample after its heating to 80°C decreased heat absorption at 18 and 27°C and shifted a peak from 32°C to 34°C with increasing ΔH up to about 0.2 kcal/mol (fig.1b, lower curve). An increase in the phase transition temperature with repeated heating of microsomes was observed in [6] as well as by the authors of the present paper on a model system consisting of cytochrome P-450 and synthetic phospholipids [19]. The above changes in heat absorption within the range 10–37°C, after repeated heating, are attributed to structural rearrangements of the system due to denaturation of protein components [6].

When investigating total fraction of microsomal lipids, we have not detected phase transitions on a heat absorption curve in the temperature range 10–80°C (fig.1c).

The addition of Mg^{2+} gives a peak at 18°C that does not change practically upon repeated scanning (fig.1d).

Fig.2a,b presents Arrhenius plots of the ratio of luminescence intensities of pyrene eximers and monomers (F_e/F_m). Addition of 10 mM $MgCl_2$ to microsomes produces a temperature breakpoint in the vicinity of 25°C (fig.2b). Arrhenius studies of temperature-dependent ANS fluorescence intensities in microsomes in the presence of Mg^{2+} give

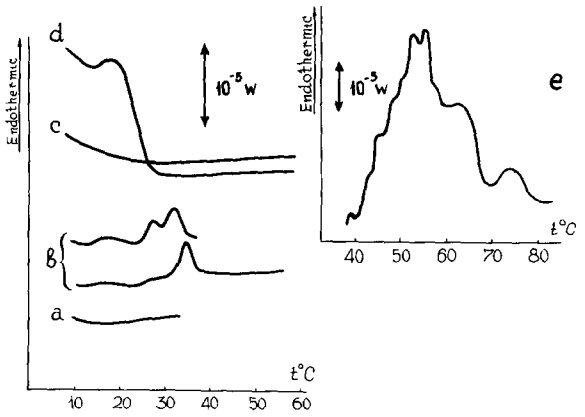

Fig.1. Microcalorimetric curves of thermotropic behaviour of microsomes and liposomes: (a) microsomes after dialysis; (b) microsomes in the presence of 10 mM $MgCl_2$ (upper curve) and second scan, the same sample after heating to 80°C (lower curve); (c) liposomes from total fraction of microsomal lipids; (d) liposomes in the presence of 10 mM $MgCl_2$; (e) the high-temperature domain of sample 1a scanning. Heat absorption intensity in the figure was twice decreased as compared to the original curve. 0.1 M Tris–HCl buffer (pH 7.4). Content of phospholipids in microsomes, 1 mg/ml; in liposomes 10 mg/ml. Scanning rate, 1 K/min.

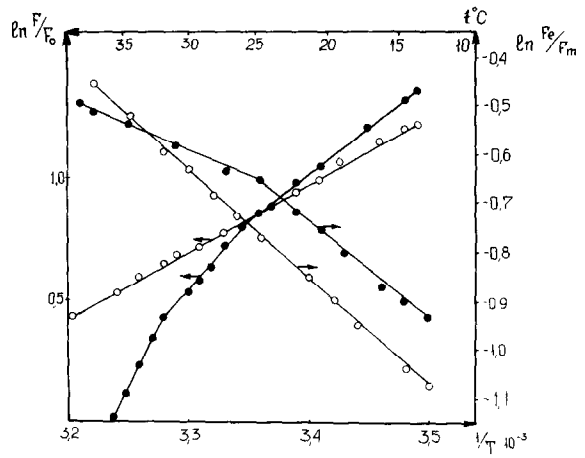

Fig.2. Arrhenius plots of the dependence of ANS and pyrene fluorescence in microsomes. Temperature dependence of F_e/F_m for pyrene in microsomes: (a) after dialysis; (b) with addition of 10 mM $MgCl_2$; change in ANS fluorescence intensity in microsomes; (c) after dialysis; (d) in the presence of 10 mM $MgCl_2$. F_e/F_m was measured at 395 and 475 nm, respectively. Concentrations of ANS and pyrene used were up to $1 \cdot 10^{-6}$ M.

Table 1

Temperature dependence of the fluorescence parameters of ANS and pyrene in microsomes

Preparation	Probe	Breakpoint °C	Apparent energy of activation, ΔE kcal/mol		
Microsomes after dialysis	ANS	None	6.3 ± 0.4		
Microsomes + 10 mM $MgCl_2$	ANS	25;32	Below 25°C 7.6 ± 0.5	25–32°C 11.1 ± 0.4	Above 32°C 19.3 ± 0.5
Microsomes after dialysis	Pyrene	None	4.6 ± 0.5		
Microsomes + 10 mM $MgCl_2$	Pyrene	26	Below 26°C 4.1 ± 0.3		Above 2.2 ± 0.5

two breakpoints at 26 and 32°C, respectively (fig.2d). Arrhenius plots of the control preparations are linear in the temperature range 13–35°C (fig.2c).

In table 1 the values of thermodynamic parameters characterizing microsomal behaviour both in the presence and in the absence of Mg^{2+} are presented.

It should be noted, that the activation energy values obtained from the study of catalytic activity of rat liver microsomal cytochrome of the P-450-dependent enzyme system using different substrates and methods, vary in the interval of 21.0 ± 3.5 kcal/mol and 10.0 ± 2 kcal/mol below and above the break point, respectively [8–11].

4. DISCUSSION

Thus, the data of this study testify that the addition of Mg^{2+} to microsomes results in structural rearrangements of a membrane which are exhibited by 3 low-energy transitions on microsome melting thermograms at 18, 27 and 32°C, respectively, and by the breakpoints in the Arrhenius plots of fluorescent probes in the same temperature range. The observed effect bears evidence of, at least, two domains developing in microsomes, that differ in the structure and composition of membrane components. A peak at 18°C is likely to display a phase transition of phospholipids not participating in the interaction with proteins since it is detected upon liposome scanning from total fraction of microsomal lipids.

Along with lipids and Mg^{2+}, the proteins may also contribute to formation of intramembrane structures with heat absorption maxima at 27 and 32°C. It is known that Mg^{2+} and Ca^{2+} increase essentially a phase transition temperature, T_c, of negatively charged phospholipids [15,16]. T_c increases also with incorporation of some proteins into liposomes [2]. All this allows the conclusion that the peaks at 27 and 32°C are due to the interaction of Mg^{2+} with negatively charged microsomal phospholipids that are the surroundings of protein components. Comparison of temperature dependences of fluorescent probes, ANS and pyrene differing in their localization in a membrane, with microcalorimetric data points to the fact that heat absorption at 27°C is attributable to the changes in organization of hydrophobic sites of a membrane. The peak at 32°C may indicate growing mobility in more polar sites of a microsomal membrane. As follows from table 1 the addition of Mg^{2+} leads to changes in activation energy values of fluorescent probes ANS and pyrene in microsomes. Different localization of these probes in the membrane allows us to assume that Mg^{2+} affects the organization of both hydrophobic and slightly hydrophilic 'sites' in the membrane. In conclusion, it should be noted that when studying catalytic activity of the monooxygenase enzymatic microsomal system in the presence of Mg^{2+}, in some cases [10] changes in the enzyme activity have been detected at 21.3 ± 5.1°C while in another [9] at 32°C.

Comparison of these results with the data here suggests that one of the ways of regulation of monooxygenase activity involves the interaction of Mg^{2+} with phospholipids of microsomes.

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