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EFFECT OF Mg^{2+} ON MEMBRANE FLUIDITY AND K^+ TRANSPORT IN RAT LIVER MITOCHONDRIA

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Summary

1. The effects of Mg^{2+} on the fluidity and on the transport properties of mitochondrial inner membrane were compared in parallel experiments. The fluidity was measured by intercalated fatty acid spin probes. Valinomycin-induced K^+ uptake was followed using an ion-selective electrode.

2. The rotational diffusion rate of lipids was very slightly affected by Mg^{2+}, whereas the ordering of the probed region of the inner membrane increased considerably above 30°C in the presence of Mg^{2+}. Mg^{2+} strongly inhibited K^+ transport, particularly above 30°C.

3. In the presence of different concentration of $MgCl_2$ (0—30 mM) the order parameter showed no significant variation, whereas the rotational correlation time had essentially biphasic character with a minimum (i.e., faster diffusion rate) at 10 mM $MgCl_2$.

4. We conclude that Mg^{2+} induces structural changes in the mitochondrial inner membrane and concomitant changes in its functional properties. The term 'fluidity' is inadequate for the interpretation of the data, since changes in the order parameter and in the characteristic correlation time of the inner membrane upon addition of Mg^{2+} did not show parallel tendencies.

Introduction

It is generally believed that the physico-chemical state of lipids plays an essential role in modulating the function of biomembranes. Variations in the

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lipid composition or addition of divalent cations change the phase transition characteristics in the membrane [1—4] and these structural changes influence the functional properties (e.g., solute transport) of the membrane [5,6]. In this study the effect of Mg^{2+} upon the fluidity of the lipid layer and the transport of K^+ are compared in mitochondrial membranes.

Mg^{2+} does not penetrate through the inner membrane of rat liver mitochondria [7,8] but it interacts with the membrane surface [9]. The cation transport is markedly affected by Mg^{2+}: Mg^{2+}-depletion (by EDTA) results in increased K^+ and Na^+ permeability [8,10], whereas excess Mg^{2+} inhibits both Ca^{2+} and K^+ transport [11,12]. The same effects were observed in rat heart mitochondria [13], in erythrocytes [14] and in yeast cells [15]. Recently, Carafoli and Crompton [16], generalizing these observations, suggested that Mg^{2+} might play a role in the physiological regulation of Ca^{2+} transport. Unfortunately, this case cannot be settled until the molecular mechanism of natural ion carriers in mitochondria is better understood. Instead, we chose a well-known transport system, the valinomycin-induced K^+ uptake. Valinomycin is the prototype of mobile carriers which forms a complex with K^+ at the lipid/water interface and diffuses through the apolar hydrocarbon barrier by enclosing the polar K^+ in its polar core [17]. The diffusion of valinomycin is strongly dependent on the fluidity of the membrane interior [18]. Since Mg^{2+} inhibits the valinomycin-induced K^+ uptake [19], the questions arise as to: (i) whether this is brought about by altering the fluidity of the membrane, and if so (ii) whether the fluidity data can be correlated with transport data. For measuring the fluidity of the mitochondrial inner membrane we applied spin probes.

Methods

Materials. The spin-labelled fatty acids, 2-(14-carboxytetradecyl)-2-ethyl-4,4-dimethyl-3-oxazolidinyloxyl and 2-(3-carboxypropyl)-2-tridecyl-4,4-dimethyl-3-oxazolidinyloxyl, subsequently referred to as I(1,14) and I(12,3), respectively, according to the commonly used nomenclature [20], were obtained from Syva (U.S.A.). Valinomycin was purchased from Serva (F.R.G.) and rotenone from K and K Laboratories, Inc., Plainview, NY. All the other reagents were of analytical grade purity.

Transport studies. Rat liver mitochondria were prepared as described by Johnson and Lardy [21]. The protein content of the mitochondrial suspension was determined by the biuret method, for calibration bovine serum albumin was used. The rate of K^+ uptake was measured with a K^+-sensitive membrane electrode (Radelkis OP-K 7113D) connected to a pH meter and compensograph (Radelkis OP-205 and OH-214/1). An Ag/AgCl electrode was used as reference. The measurements were performed in a thermostatically controlled glass cell in a final volume of 5.5 ml, continuously stirring the suspension with a magnetic stirrer. The medium contained 246 mM sucrose, 1 mM KCl, 0.5 mM Tris-phosphate, 4 mM Tris-HCl, 1 μM rotenone, and 2.2 mM Tris-succinate; 1 mg mitochondrial protein per ml, the final pH being 7. K^+ uptake was triggered by the addition of 16 ng/mg valinomycin. The electrode calibration was performed with known amounts of KCl.

Preparation of samples for spin-label studies. 1 ml of 100 μg/ml chloroform stock solution of spin-labelled fatty acid was measured into a glass tube and evaporated under an atmosphere of N_2. 100 μl of mitochondrial suspension, containing 12 mg protein, were then added and mixed with a vortex mixer. Mg^{2+} was added as $MgCl_2$, in a small volume, and corrections for constant volume and osmolarity were performed using sucrose. Control samples contained appropriate concentration of Tris-HCl. 25 μM rotenone and, if indicated, 10 mM $K_3Fe(CN)_6$, were also added.

ESR measurements. ESR spectra were recorded with a JEOL (Japan) JES-PE-1X spectrometer using a 100 kHz modulation technique. The micro flat cell (Scanlon, U.S.A.) was inserted into the variable temperature unit and thermostatically controlled by passing cooled or heated N_2 into the sample compartment. The temperature was monitored by a copper-constantan thermocouple immersed into the mitochondrial suspension. The reproducibility of the temperature measurements was better than ±0.5 °C. Each temperature-dependent measurement was recorded during the heating cycle between 0 and 40 °C; the temperature was varied in approx. 2 °C steps. When using the I(12,3) label the order parameter, S_A, was calculated from the position of the inner ($A_\perp$) and outer ($A_\parallel$) splittings and corrected for polarity (Fig. 1B) [22]. In the case of I(1,14) label, the rotational correlation time was calculated by using the approximate formula [23]:

$$\tau = K\left(\sqrt{\frac{h_0}{h_{-1}}} - 1\right) W_0 \qquad (1)$$

where h_0 and h_{-1} denote the intensity of the central and high field line, respectively, W_0 the breadth of the central line and K = 0.65 ns/G (Fig. 1A). The spin-labeling parameters were also checked by computer simulations (Fig. 1).

Results

Effect of Mg^{2+} on fluidity and transport

Two typical spectra obtained with I(1,14) and I(12,3) which probe the membrane either in the hydrophobic interior or close to the interface, respectively, are shown in Fig. 1. Quintanilha and Packer [25] pointed out that the spin probes are reduced by the redox enzymes of the electron transport chain. Consequently, the intensity of the spectra decreased rapidly above 22 °C and temperature-dependence measurements above this temperature required the addition of a respiratory inhibitor (rotenone) and an oxidizing agent ($K_3Fe(CN)_6$) to the mitochondrial suspension. By this means, the temperature range could be extended up to 45 °C. The presence of $K_3Fe(CN)_6$ did not affect the valinomycin-induced K^+ uptake significantly.

By using two different spin probes, two different features of the membrane become accessible. With the I(12,3) label, the ordering (S_A) of the closely packed region near the interface can be measured, while the I(1,14) label is in an almost non-ordered, isotropic environment and thus, its rotational correlation time (τ_c) can be obtained through line-shape analysis. The temperature-dependence of these two parameters (S_A and τ_c) is shown in Figs. 2 and 3. As seen in Figs. 2 and 3, there is a breaking point at approx. 15 °C in each case.

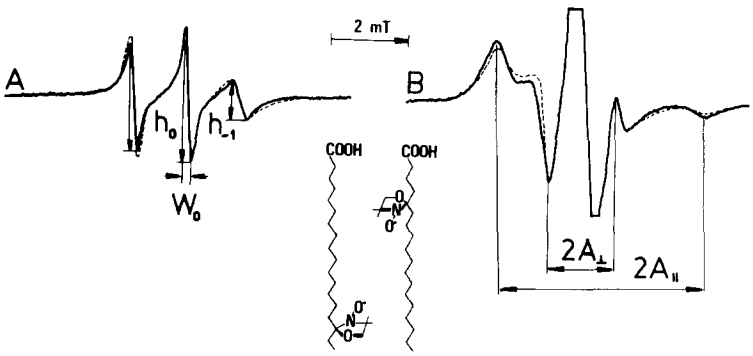


Fig. 1. The molecular structure and ESR spectra of I(1,14) (A) and I(12,3) (B) spin probes in rat liver mitochondria. (———) experimental curves; instrumental setting: microwave power 10 mW, modulation width 1 G, sensitivity 500X. (- - - - -) computer-simulated spectra using the simulation program of Ref. 24; input parameter sets: (A) $S_A = 0$, $\tau = 1.5$ ns, residual linewidth 0.80 G and (B) $S_A = 0.691$, $\tau = 3.8$ ns, residual linewidth 1.20 G. Lettering is explained in Methods.

The effect of Mg^{2+} on τ_c is reflected in a small decrease in motional freedom between 0 and 22°C; at higher temperatures the slopes of the two curves are convergent with an intercept at approx. 30°C (Fig. 2). The order parameter (S_A) exhibits no Mg^{2+} effect below 15°C, but at higher temperatures the two curves separate (Fig. 3), and in the Mg^{2+}-treated samples there is a break at 29°C. Therefore, the effect of Mg^{2+} is most pronounced at physiological temperatures. Statistical analysis indicates that this difference is highly significant; slope values obtained by least-squares fit are —0.0077 (2)/°C and —0.0036 (13)/°C, respectively. Estimated uncertainties in parentheses apply to the last digits and represent S.D. of the fitting parameter. It is interesting that in this temperature region the τ_c values exhibited no Mg^{2+} effect.

Having established the effect of Mg^{2+} on the membrane lipids, we measured

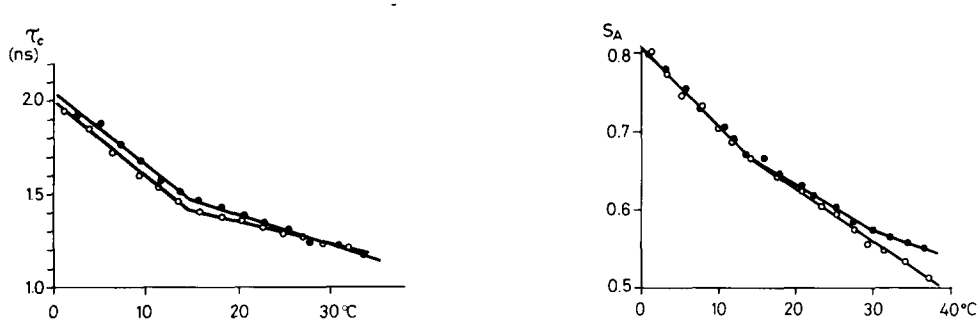


Fig. 2. Temperature-dependence of the rotational correlation time (τ_c) in rat liver mitochondria labelled with I(1,14) spin probe. Each point represents the mean of τ_c values obtained in a range of 2°C (four to six measurements). The straight lines were fitted with the method of least-squares using all the data (and not the mean values). For sample preparation see Methods. 10 mM $K_3Fe(CN)_6$ was present. (○———○) no Mg^{2+} present; (●———●) 5 mM Mg^{2+} present.

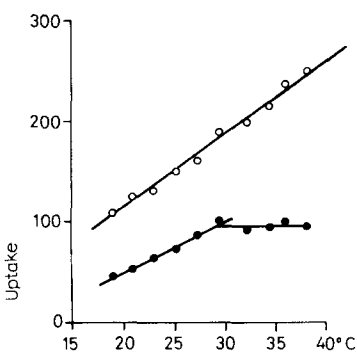
Fig. 3. Temperature-dependence of the order parameter (S_A) in rat liver mitochondria labelled with I(12,3) spin probe. For details of evaluation see Fig. 2. 10 mM $K_3Fe(CN)_6$ was present. (○———○) no Mg^{2+} present; (●———●) 5 mM Mg^{2+} present.

the effect of Mg^{2+} on the valinomycin-induced (active) K^+ uptake at different temperatures (Fig. 4). As shown in Fig. 4, without Mg^{2+} in the medium, the rate of K^+ transport increases with increasing temperature. Mg^{2+} inhibits the K^+ transport throughout the whole temperature range and this effect becomes particularly pronounced above 30°C. At physiological temperature the effect of 5 mM Mg^{2+} is comparable to that of a change of 20°C in temperature. In additional control experiments we found that Mg^{2+} did not inhibit the dinitrophenol- or ADP-stimulated respiration of mitochondria between 20 and 40°C, indicating that it did not interfere with the electron, substrate or phosphate transport. Consequently, we ascribe the inhibitory effect of Mg^{2+} on K^+ transport to structural changes in the membrane.

The effect of Mg^{2+} concentration

So far, in all experiments, the Mg^{2+} concentration was kept close to physiological values (5 mM) [26]. To see whether the observed different trends in the ordering and the motional freedom were significant, we studied in a series of experiments the effect of higher Mg^{2+} concentration at constant temperature. Our choice in temperature was 18°C, since at this point both parameters exhibited slight differences (Figs. 2 and 3), and parallel experiments without $K_3Fe(CN)_6$ also could be done.

The order parameters and rotational correlation times were measured at different $MgCl_2$ concentrations (Fig. 5). The order parameter, S_A, first slightly increased and then decreased with increasing $MgCl_2$ concentration. On the other hand, the τ_c values reach a minimum at 10 mM $MgCl_2$ followed by an



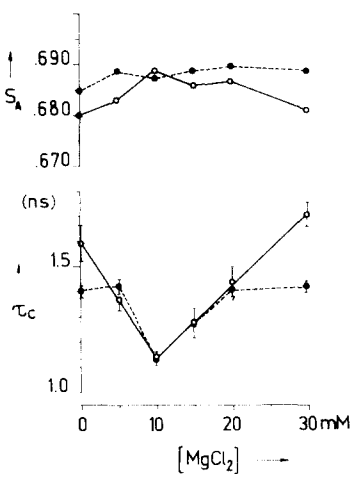


Fig. 4. Temperature-dependence of the rate of valinomycin-induced K^+ uptake in rat liver mitochondria. For the composition of the medium see Methods. K^+ uptake was triggered by the addition of 16 ng valinomycin per mg protein. (○——○) no Mg^{2+} present; (●——●) 5 mM Mg^{2+} present. Uptake expressed as nmol K^+/mg protein per min.

Fig. 5. Rotational correlation time (τ_c) and order parameter (S_A) data as measured with I(1,14) and I(12,3) labels at different $MgCl_2$ concentrations. Data represent the mean ± S.D. values of five parallel measurements carried out on the same mitochondrial preparation. (○——○) without $K_3Fe(CN)_6$; (●- - - - -●) with 10 mM $K_3Fe(CN)_6$.

increase between 10 and 30 mM. Essentially similar effects were obtained in the presence of 10 mM $K_3Fe(CN)_6$ (Fig. 5).

Discussion

From the spectral features of the spin-labelled mitochondrial suspension (Fig. 1) it appears that both fatty acid spin probes are in a relatively ordered hydrocarbon environment. However, their distribution between the two mitochondrial membranes, of which the inner one is of particular interest, cannot be further specified on the basis of spin-label data alone. Unlike as in pure lipid layers the spin probes were readily reduced in the mitochondrial suspension, presumably by the redox enzymes of the respiratory chain [25]. When rotenone, a respiratory chain inhibitor, was added to the suspension the reduction was completely inhibited. Since rotenone is known to affect the redox enzymes of the inner membrane only and not those of the outer one [27], we conclude that the spin probes should be localized largely in the inner mitochondrial membrane.

It is a common practice to identify the transition temperatures of biological membranes from discontinuities in their Arrhenius diagrams. This is warranted when clear correlation exists between structural and functional data [1], although the interpretation of these observations is frequently difficult [28]. In our experiments transitions were found at 15 and 29 °C, respectively (Figs. 2 and 3). The lower transition at 15 °C is close to that observed in the temperature-dependence of ATP synthesis, ADP- and dinitrophenol-stimulated respiration and ATPase activity [29], although our value is somewhat lower than that found in previous spin-label experiments [30,31].

The higher transition temperature (at 29 °C) is of particular interest since this was apparently induced by Mg^{2+}. This value again lies in the range where many transport functions exhibited discontinuities in the presence of Mg^{2+} [29], although in previous studies control data measured in the absence of Mg^{2+} were not available. Comparing Figs. 2—4, it is clear that the correlation between the spin-label and transport data is good. However, it should be noted that the influence of Mg^{2+} on the transport process is more pronounced than the observed structural changes. This is a clear indication that apart from re-ordering of lipids, Mg^{2+} also exerts an electrostatic effect in the interfacial region [11,15] and so it inhibits the formation of K^+-valinomycin complexes. Nevertheless, Mg^{2+} cannot compete with K^+ for the valinomycin binding sites [32].

The spin-label data can also reveal the nature of the structural changes brought about by Mg^{2+}. Such structural changes are often interpreted as changes in the 'fluidity' of the membrane interior. A comparison of Figs. 2, 3 and 5 clearly indicates that the term 'fluidity' is not applicable, since the correlation time and the order parameter did not exhibit parallel trends while varying either the temperature or the Mg^{2+} concentration. Smith and his coworkers have also emphasized that correlation times and order parameters may show independent variations [28], however, this is the first time that such different trends are demonstrated in the same system.

These results indicate that changes in the ordering of the membrane play a

dominant role in the Mg^{2+} effect on K^+ transport, whereas the dynamics of the membrane do not change significantly. To accommodate the bulky valinomycin-K^+ complex, chain defects of more lipids should cooperate and, thus, the rotational diffusion rate of the individual lipids is less informative than the average order, i.e., the average defect content of the acyl chains.

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