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The effect of ATP on the order and the mobility of lipids in the bovine erythrocyte membrane

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The order and the mobility of the lipids in the membrane were measured by the ESR method for the erythrocytes with both normal and modified intracellular concentration of ATP. The lipid order did not depend on the ATP level, but the lipid mobility was affected by the intracellular ATP concentration. The lipid mobility was higher in the cells with a larger concentration of ATP.

1. Introduction

The intracellular concentration of adenosine 5′-triphosphate (ATP) in erythrocytes influences the morphological and mechanical properties of the cells [1–5]. A long-term storage of red cells, or their metabolic starvation, caused a decrease of the microviscosity of the cell membrane [6–8].

This effect was attributed to a decrease of intracellular concentration of ATP. However, an increase of lipid order and a decrease of lipid mobility during in vivo ageing of erythrocytes were also associated with a decrease of the ATP level [9]. Moreover, a total ATP-depletion of the cells led to irreversible, or only partly reversible effects [7,8]. These inconsistencies between the data and the observed effect of the ATP on critical cell volume of erythrocytes prompted us to do research on a correlation between the dynamical state of membrane lipids and the intracellular concentration of ATP at a physiological level. Apart from the commonly used metabolic starvation of the cells we have also investigated the effect of increasing the ATP level by phosphate activation of glycolysis [10] on the order and the mobility of the membrane lipids. These last parameters were evaluated from the ESR spectra of 5-, 12- and 16-doxylstearic acids placed into the membranes of intact bovine erythrocytes.

2. Materials and Methods

2.1. Erythrocytes

Preparation of erythrocytes, modifications of activity of glycolysis and ATP measurement were described in the accompanying paper [11].

2.2. Cholesterol content

The erythrocyte suspension of 50% hematocrit was mixed with ice-cold butanol in the ratio 100 : 75. The extraction of lipids at 0 °C took 30 min. The suspension was then centrifuged at 20000 × *g* for 5 min [12]. The supernatant was collected and butanol was evaporated under vacuum. The dry residue of lipids was weighted. The quantity of the cholesterol was determined according to Ref. 13 using a standard kit from POCH, Gliwice, Poland.

2.3. Lipid order and mobility

Fatty acid spin labels, 5-, 12- and 16-doxylstearic acids, were purchased from Sigma. The spin labels were added in methanol solution, methanol was subsequently evaporated and the tubes with the dry residue and a highly concentrated erythrocyte suspension were shaken mechanically for 30 min at ambient temperature. An appropriate amount of the spin label was used to maintain the label to lipid molecular ratio 1 : 50.

The ESR spectra were recorded on the SE/X-28 spectrometer manufactured at the Technical University of Wrocław. The field sweep was 100 G, time constant

Abbreviations: ATP, adenosine 5′-triphosphate; Tris, 2-amino-2-hydroxymethyl-1,3-propanediol; ESR, electron spin resonance.

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0.3 s and scan time of 500 s. The temperature of the sample was controlled by a thermocouple. The measure of the lipid order was the order parameter calculated according to Hubbel and McConnell [14] from the spectra of 5-doxylstearic acid. The measure of the lipid mobility was the correlation time τ_c calculated according to Kivelson [15] from the spectra of 12- and 16-doxylstearic acids.

3. Results

3.1. ATP and the order of membrane lipids

The order parameter S was computed from the parameters of the spectra of 5-doxylstearic acid located in membranes of intact erythrocytes. No correlation between the order parameter and the intracellular concentration of the ATP was found ($r = 0.06$, $n = 11$). It is well known, however, that the lipid order depends strongly on the cholesterol in the membrane [16–18]. Such dependence was also found for the red cell from the studied population of animals. The correlation coefficient was $r = 0.89$ ($n = 11$) and it was statistically significant at $P < 0.001$. In order to check if the intracellular concentration of ATP affects the lipid order we compared eight pairs of samples paired according to a similar content of cholesterol. The mean differences of the cholesterol content, of the order parameter and of the ATP concentration between the samples in each pair are shown in Table I. These differences were compared with the value and its standard deviation of the corresponding average parameter in the studied population of animals. There is no statistical correlation between those data.

TABLE I

The effect of the intracellular concentration of ATP on the order of lipids

The mean differences of cholesterol content, of the order parameter and of the ATP concentration were calculated for eight pairs of samples paired according to a similar content of cholesterol. These differences were compared with the value and its standard deviation of the corresponding average parameter in the studied population of animals which were equal (mean ± S.D.): (a) cholesterol content = (25.1 ± 2.6)% (w/w) of total lipids; (b) ATP concentration = 373 ± 86 μM; (c) order parameter $S = 0.616 \pm 0.027$ (at 30 °C for 5-doxylstearic spin label). The number of pairs $n = 8$, the confidence limits are given for the confidence level $1 - P = 0.95$. The correlation coefficient for the relationship between the differences of the ATP concentration and of the order parameter was $r = 0.11$ ($n = 8$) and it was not significant.

Quantity	Mean difference	Mean difference relative to the value of the population's	
		mean	S.D.
Cholesterol content	0.1 ± 0.2% (w/w of total lipids)	0.4 ± 0.8%	3.8 ± 7.6%
ATP concentration	85 ± 42 (μM)	31 ± 15%	98 ± 49%
Order parameter	0.006 ± 0.016	1 ± 3%	22 ± 59%

TABLE II

Effect of modifications of intracellular concentration of ATP on order and mobility of lipids

The number of experiments $n = 6$, the confidence limits are given for the confidence level $1 - P = 0.95$. All values are relative with respect to the control values, which were equal (mean ± S.D.); (a) ATP concentration = 400 ± 40 μM; (b) τ_c at 30 °C for 12-doxylstearic acid spin label (12-DSA) = $(6.8 \pm 1.3) \cdot 10^{-9}$ s; (c) τ_c at 30 °C for 16-doxylstearic acid spin label (16-DSA) = $(2.25 \pm 0.45) \cdot 10^{-9}$ s; (d) S at 30 °C for 5-doxylstearic acid spin label (5-DSA). All differences between the control and the modified samples are statistically significant at $P < 0.01$ (Student's paired statistics t-test).

Sample	%			
Parameter:	ATP concentration	τ_c for 12-DSA	τ_c for 16-DSA	S for 5-DSA
With activated glycolysis	126 ± 15	85 ± 8	88 ± 7	99.6 ± 1.2
Metabolically starved	62 ± 20	135 ± 18	115 ± 8	100.5 ± 1.6

The activation of glycolysis resulted in an increase of the intracellular ATP concentration by about 25%, while the metabolic starvation decreased it by about 40% (Table II). No significant change of the order parameter for confidence level $1 - P = 0.95$ was observed for both ATP changes (Table II).

The lipid mobility was expressed by the parameter τ_c, which for the rapid isotropic motion of the spin-labelled molecule, is related to the rotational correlation time [15]. However, it may also be useful as a measure of the relative lipid mobility when used in the same system and under the same conditions [19]. For the cells studied

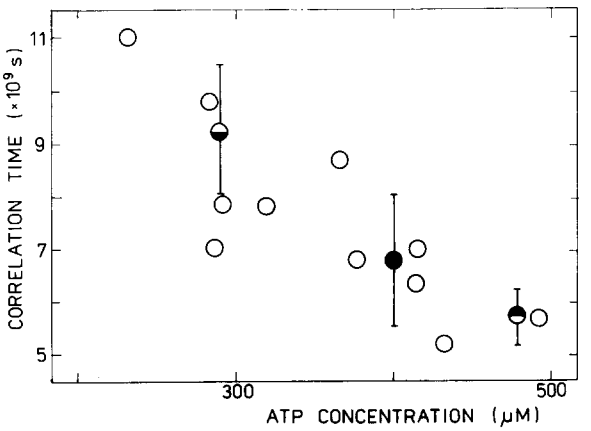

Fig. 1. The effect of the ATP intracellular concentration on the lipid mobility measured from the ESR spectra of 12-doxylstearic acid. Each point (open circles) represents data for erythrocytes from one animal, correlation coefficient $r = -0.81$ ($n = 11$, $P < 0.01$). ◓, ● and ◒, represent average values ± S.D. for erythrocytes with an increased, normal and decreased concentration of ATP, respectively.

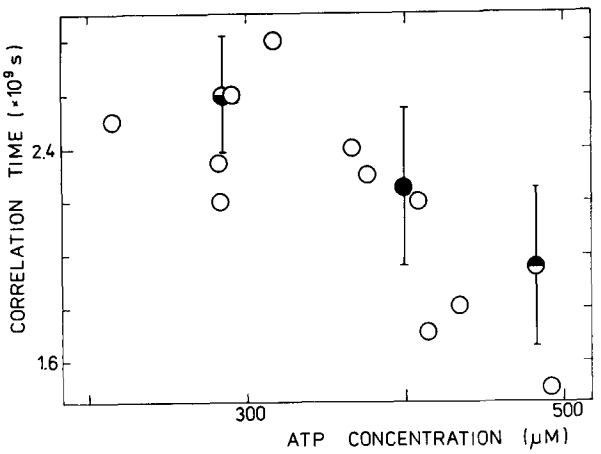

Fig. 2. The effect of the ATP intracellular concentration on the lipid mobility measured from the ESR spectra of 16-doxylstearic acid. Each point (open circles) represents data for erythrocytes from one animal, correlation coefficient $r = -0.70$ ($n = 11$, $P < 0.01$). ◓, ● and ◒, represent average values ± S.D. for erythrocytes with an increased, normal and decreased concentration of ATP, respectively. The mean values overlap due to spread of values of the correlation time, associated with the different individuals whose blood was studied. However, the differences between the values corresponding to the control and modified cells were statistically significant in confidence interval and *t*-paired tests (Table II).

τ_c was calculated from the ESR spectra of 12- and 16-doxylstearic acid incorporated into membranes of intact erythrocytes. The dependences of the τ_c on the intracellular ATP concentration for the cells labelled with 12- and 16-doxylstearic acid are shown in Figs. 1 and 2, respectively. There is a statistically significant correlation between the data. The lipid mobility in membranes of erythrocytes with an elevated level of ATP was increased, with respect to the normal control, while it decreased in the membranes of partially ATP-depleted cells. The results are shown in Table II. The points which represent the mean values and the standard deviations of the changes of both the τ_c and the intracellular ATP concentration are also indicated in Fig. 1 and Fig. 2 showing the plots of τ_c vs. ATP concentration for the whole population of animals.

4. Discussion

4.1. The order and the mobility of membrane lipids

The order parameter S and the correlation time τ_c used here to evaluate the lipid order and the mobility, respectively, are operationally defined parameters and they were not used in their original physical meaning, valid for the isotropic spin probe mobility, fast enough on the ESR time scale [18]. However, since we have studied the same system (the membrane of intact bovine erythrocyte) and under the same conditions throughout the experiments these parameters have given the information about the relative order and mobility of the membrane lipids as previously tested [19].

4.2. Effect of cholesterol and of intracellular concentration of ATP on the lipid order

Both NMR [16] and ESR [17,18] studies clearly indicated that cholesterol molecules affect the lipid order within a model biomembrane.

Such a dependence was also found in the membrane of bovine erythrocytes. In order to find any relationship between the intracellular concentration of ATP and the lipid order we compared the differences between both ATP concentrations and the S parameters in samples paired according to their almost similar cholesterol content. While the difference in the ATP concentration was significant, comparable with the standard deviation of the average ATP concentration in the whole population of animals studied, the mean difference in the S parameter was negligibly small.

Both the decrease and the increase of the intracellular concentration of ATP, caused by a modification of the activity of glycolysis, did not influence the lipid order of the erythrocyte membrane, either. Therefore, the intracellular concentration of ATP does not seem to affect the lipid order of the membrane of bovine red cell.

4.3. Effect of ATP concentration on lipid mobility

The correlation between the intracellular ATP concentration and the lipid mobility was evident in the unmodified red blood cells as well as in the cells with a modified activity of glycolysis. The dependence of the lipid mobility on the intracellular ATP concentration, and its lack for the lipids' order, may seem to be surprising, however, a correlation between the rates of molecular motion and the degree of molecular order in a system such as the erythrocyte membrane is not necessary [18].

The question was what had been the cause and what the effect? The permeability of the membrane for glucose, which is small for bovine erythrocyte [20], could have been a rate-limiting step of glycolysis. Since the glucose permeates the erythrocyte membrane by facilitated diffusion, which may depend on the membrane fluidity [18], the lipid motion could have therefore affected the intracellular concentration of the ATP. The significant changes of the lipid motion after modifications of the ATP level indicated that the dependence was reversed. Our results contradict those obtained upon long-term storage of cells [6,8], or a total ATP-depletion [7]. However, such treatment of the cells leads to a loss of asymmetry of membrane lipids [28] and to vesiculation of the cell membrane [29]. These phenomena may be linked with a total disintegration of the membrane skeleton due to dephosphorylation of its elements [30]. The observed increase of the fluidity of

membrane lipids due to an ATP-depletion resulted from an appearance of a fluid phase which did not exist at the physiological level of the ATP as it had been previously shown by the ESR study [7]. The correlation between the intracellular concentration of the ATP at the physiological level, observed by us, reflects probably a role of the ATP in the regulation of the dynamic properties of the membrane. The increase of the membrane fluidity after a long-term storage [6,8], or a total ATP-depletion [7] seems to be related with structural changes of the cell membrane. A similar duality of the ATP-effect on the shape of erythrocytes has been found [5]. The mechanism of the correlation between the ATP level and the lipid mobility can only be, at present, a subject for speculations. The intracellular level of ATP influences the state of both membrane lipids and proteins [21–27]. The membrane of bovine erythrocyte is relatively rich in polyphosphate phosphatidylinositol [31]. The repulsive electrostatic forces between the negatively charged heads of these lipids could loosen the lipid-protein matrix and thus increase the lipid mobility.

The lipid mobility is also affected by the protein–lipid interactions [32,33]. The possible relaxation of the membrane skeleton after a phosphorylation of band 4.1 protein [22,23] and of ankyrin [21] could, therefore, influence also the lipid mobility.

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