Biochimica et Biophysica Acta, 944 (1988) 155–163
Elsevier

BBA 74133

Critical temperatures for the interaction of free fatty acids with the erythrocyte membrane

Adam Csordas and Maria Rybczynska *

Institute of Medical Chemistry and Biochemistry, University of Innsbruck (Austria)

(Received 29 March 1988)

Key words: Hypotonic hemolysis; Free fatty acid; Thermotropic membrane effect; Critical temperature; Erythrocyte membrane; (Human)

Non-esterified long-chain fatty acids reduce the extent of hypotonic hemolysis at a certain low concentration range but cause hemolysis at higher concentrations. This biphasic behavior was investigated at different temperatures (0–37°C) for lauric (12:0), myristic (14:0), palmitoleic (16:1), oleic (*cis*-18:1) and elaidic (*trans*-18:1) acids. The results are summarized as follows: (A) the fatty acids examined exhibit a high degree of specificity in their thermotropic behavior; (B) oleic acid protects against hypotonic hemolysis even at the highest concentrations, up to 15°C, when it becomes hemolytic, but only in a limited concentration range; (C) elaidic acid does not affect the osmotic stability of erythrocytes up to 20°C, when it starts protecting: above 30°C, it becomes hemolytic at the highest concentrations; (D) palmitoleic acid is an excellent protecting agent at all temperatures in a certain concentration range, becoming hemolytic at higher concentrations; (E) lauric acid protects up to 30°C and becomes hemolytic only above this temperature; (F) myristic acid exhibits an extremely unusual behavior at 30 and 37°C by having alternating concentration ranges of protecting and hemolytic effects; (G) there is a common critical temperature for hemolysis at 30°C for saturated and *trans*-unsaturated fatty acids; (H) the initial slope of Arrhenius plots of percent hemolysis at the concentration of maximum protection is negative for *cis*-unsaturated fatty acids and positive for saturated and *trans*-unsaturated fatty acids.

Introduction

Although the general outline of the cell membrane became clear with the fluid mosaic model suggested by Singer and Nicholson [1], the role of distinct lipid domains and their specific functions in the membrane are still poorly understood and our knowledge of protein–lipid and lipid–lipid interactions is limited.

Based on structural perturbations induced by free fatty acids, the plasma membrane was found to be heterogeneous, consisting of fluid-phase and lipid-phase domains, and it was suggested that *cis*-unsaturated free fatty acids intercalate into fluid-phase domains, whereas *trans*-unsaturated and saturated free fatty acids intercalate into gel-phase domains of the membrane [2,3]. It was also shown that only *cis*-unsaturated free fatty acids can be used to perturb membrane lipid structure and block capping of surface immunoglobulin on lymphocytes [4].

* Permanent address: Academy of Medicine, Department of Biochemistry, Poznan, Poland.

Correspondence: A. Csordas, Institute of Medical Chemistry and Biochemistry, University of Innsbruck, Fritz-Pregl-Strasse 3, A-6020 Innsbruck, Austria.

Moreover, acylated proteins with a specific fatty acid residue suggest that the structure of fatty acids could play an important role in lipid–lipid interactions in the anchoring of proteins to the inner leaflet of the membrane [5–7].

Numerous membrane functions exhibit strong temperature dependence: this is either due to the properties of membrane proteins or to changes in membrane fluidity [8,9]. Therefore, abrupt changes in thermotropic membrane effects can be indicators for specific alterations in the conformation of proteins within the lipid bilayer or, alternatively, for state transitions in lipid domains, and determining such critical temperatures can be useful in attempting to establish links between protein components and the lipid moiety of the membrane.

In a previous study, it was observed that the effects of oleic and elaidic acids on the osmotic resistance of erythrocytes are strongly temperature-dependent [10]. This was especially striking in the case of elaidic acid, which showed no effects at 0°C, protected against hypotonic hemolysis even at the highest concentrations at room temperature and became a potent hemolytic agent at 37°C [10,11]. The purpose of the present study was an exact determination of the critical temperatures for the protecting and the hemolytic effects for various free fatty acids. In addition, the question was raised as to whether, based on their interactions with the erythrocyte membrane, groups of fatty acids can be classified according to structure and configuration, as was found by Klausner and co-workers for other membrane effects [2–4]. Since long-chain fatty acids reduce the extent of hypotonic hemolysis in a certain low concentration range but become hemolytic at higher concentrations [10–13], these distinctly different phenomena were examined in the temperature range from 0 to 37°C at different concentrations of the fatty acids.

The present investigation shows that the temperature-dependent effects on the osmotic stability of human erythrocytes exhibit a very high degree of specificity, as each fatty acid has its individual thermotropic pattern of concentration-dependent interference with the osmotic resistance of erythrocytes.

However, by the common critical temperature at 30°C for *trans*-unsaturated and saturated fatty acids, and furthermore, by a similar trend of the Arrhenius plots of percent hemolysis at the concentration of maximum protection against hypotonic hemolysis, two groups of fatty acids can be distinguished, namely, *cis*-unsaturated on the one side, and *trans*-unsaturated and saturated fatty acids on the other.

Since non-esterified fatty acids exhibit highly specific effects on the erythrocyte membrane, they can be considered as useful probes for the analysis of the microheterogeneity of the lipid bilayer.

Materials and Methods

Materials. Dodecanoic acid (lauric acid, C_{12}), tetradecanoic acid (myristic acid, C_{14}), palmitoleic acid (*cis*-16:1), oleic acid (*cis*-18:1) and elaidic acid (*trans*-18:1) were from Sigma. Heparin was from Immuno (Wien, Austria). The salts used for the buffer solutions were from Merck. All reagents were of analytical grade.

Buffer solutions. Phosphate-buffered saline solution was 150 mM NaCl/50 mM sodium/potassium phosphate (pH 7.4). Hypo-osmotic buffer solution was a 1:3 dilution of the phosphate-buffered saline solution with distilled water.

Erythrocyte preparation. Heparinized blood was drawn from healthy volunteers. The cell pellet was washed three times in 10–20 vol. phosphate-buffered saline (150 mM NaCl/50 mM sodium/potassium phosphate buffer (pH 7.4)) and the buffy coat was removed by aspiration. After three washings with the phosphate-buffered saline (centrifugation for 5 min at 700 × *g* in a Hettich, Roto Silenta/K centrifuge), the erythrocytes were suspended at a hematocrit of 30–40% in the same buffer with 10 mM glucose and energized by incubation for 30 min at 37°C.

Hemolysis assay. Hemolysis was measured after an incubation period of 30 min at the given temperature. The fatty acids were dissolved in methanol and the appropriate molar amounts were applied to the hypotonic buffer solution just prior to addition of the red cells. A 5 ml portion of hemolysis buffer was taken, to which the different molar amounts of the fatty acids, dissolved in methanol, were added in volumes of 5, 10 and 15 µl. Addition of these volumes of methanol did not

affect the osmotic fragility of the erythrocytes. Hemolysis was started by adding 50 µl aliquots of the erythrocyte suspension to the 5 ml volumes of hemolysis buffer containing various concentrations of fatty acids. At the end of the 30 min incubation period, the suspension was centrifuged for 10 min at 700 × *g* with the temperature of the centrifuge adjusted to the incubation temperature of the hemolysis assay. It is important that the centrifugation step follows without delay and at the proper temperature. The absorbance of the supernatant was determined at 540 nm. The highest extents of fatty acid-mediated hemolysis were up to 10% above the absorbance values of 100% hemolysis, which was measured in a 1 : 100 dilution of the phosphate-buffered saline solution.

Results

The critical temperatures of oleic and elaidic acid for altering the osmotic resistance of human erythrocytes

Fig. 1 shows the temperature-dependent effects of oleic and elaidic acids on hypotonic stress of human erythrocytes. As in a previous study, it was found that elaidic and oleic acids are not hemolytic at 0 °C, and elaidic acid is not hemolytic even at room temperature but becomes hemolytic at 37 °C [10]. In the present study, we set out to determine the critical temperatures at which non-esterified long-chain fatty acids have the ability to reduce the extent of hypo-osmotic hemolysis and also the critical temperatures for fatty acid-mediated hemolysis.

Oleic acid shows a strong temperature dependence concerning both the protecting and hemolytic effects. For the sake of clarity, the diagrams in Fig. 1 are in two panels representing a lower and a higher temperature range. At the different temperatures, percent hemolysis is plotted against the concentration of the fatty acid.

Interestingly, in the entire temperature range from 0 to 15 °C, there is protection from hemolysis at the lowest and highest concentrations tested, but at 10^{-4} M, there is a concentration range when the protection against hemolysis becomes marginal or stops altogether. Note the arrows, which indicate the degree of hypo-osmotic hemolysis without addition of the fatty acid. At the

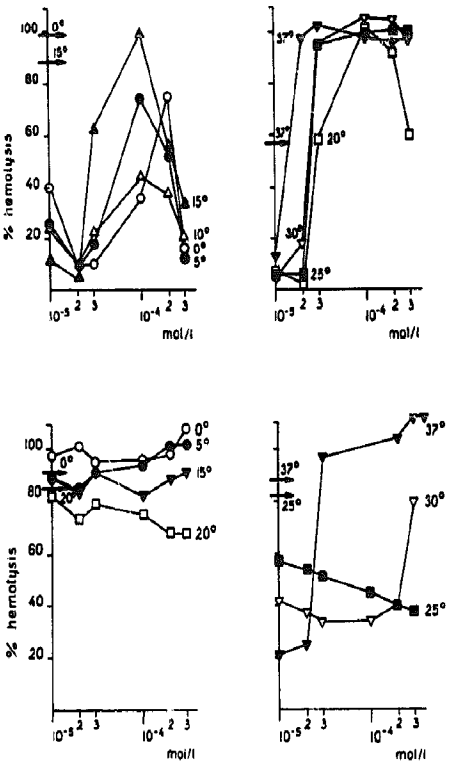

Fig. 1. Temperature-dependent effects of oleic and elaidic acids on the osmotic resistance of human erythrocytes. The extent of hemolysis in the hypotonic buffer solutions was determined at different concentrations of the fatty acids. This is shown in the upper panels for oleic acid (*cis*-18:1) and in the lower panels for elaidic acid (*trans*-18:1). For the sake of clarity, the temperatures at which hemolysis was determined are divided in two groups, representing a lower and a higher temperature range. First, the solution of the fatty acid and then 50 µl of the erythrocyte suspension were added to 5 ml of the buffer solution. After 30 min of incubation, the samples were centrifuged (10 min at 700 × *g*) and the absorbance of the supernatant at 540 nm was determined. The arrows indicate the extent of hypotonic hemolysis at the given temperature without addition of fatty acids.

concentration of 10^{-5} M, oleic acid reduces the extent of hypotonic hemolysis from about 90 to 10% at 15 °C.

Thus, the thermotropic behavior of oleic acid in the lower temperature range (0–15 °C) can be summarized as follows: there is strong protection against hypotonic hemolysis at 10^{-5} M and $3 \cdot 10^{-4}$ M oleic acid, interrupted at 10^{-4} M by a concentration range of minimal protection. At 15

and 20°C, there is no protection at all at the concentration of 10^{-4} M oleic acid. However, it should be stressed that, even at these temperatures, at concentrations higher and lower than 10^{-4} M, oleic acid is an excellent protecting agent. Observing the temperature-dependent behavior of oleic acid from 0 to 20°C, one cannot speak of a sharply defined critical temperature, but rather of a gradual movement from protection to less and less protection. In spite of the gradual changes, one can say that there is a critical temperature at 10–15°C, when an entirely nonhemolytic but protecting oleic acid becomes a hemolytic one, although hemolysis promotion by oleic acid is limited to a very narrow concentration range.

Moving to higher temperatures, from 20 to 25°C, a rather sharp critical temperature can be observed: the protecting effect which is still to be found at 20°C, at the highest concentration of the fatty acid ($3 \cdot 10^{-4}$ M), is not present at 25°C. However, it should be pointed out that at the lowest concentrations the protecting effect persists and oleic acid does protect against hypotonic hemolysis at all temperatures from 0–37°C in this low concentration range (10^{-5} M).

Fig. 1 also shows the temperature-dependent interference of elaidic acid with hypotonic hemolysis. It is evident that the thermotropic behavior of elaidic and oleic acids is quite different. As depicted in Fig. 1, elaidic acid has no effect on the osmotic stability of erythrocytes up to 20°C, when a moderate protection starts. However, at 25°C, it becomes an efficient protecting agent against hypotonic hemolysis. Note the arrow which indicates the extent of hypotonic hemolysis without addition of elaidic acid at 25°C. There is efficient protection at 25°C against hypotonic hemolysis, and the extent of protection keeps increasing with increasing concentrations. Thus, there is a critical temperature for the onset of the protecting effect of elaidic acid around 20–25°C. It should be emphasized that at 25°C elaidic acid has no hemolytic effect in the whole concentration range. There is only a small, gradual change in the protecting effect between 25 and 30°C. A further critical temperature with the drastic change of elaidic acid becoming a hemolytic agent is between 30 and 37°C. However, at concentrations of 10^{-5}–$3 \cdot 10^{-5}$ M, it still remains an excellent protecting agent. Note, for instance, the decrease of hemolysis from 80 to 20% at 10^{-5} M at 37°C.

Critical temperatures for the effects of palmitoleic acid on the osmotic resistance of human erythrocytes

Fig. 2 shows the temperature-dependent effects on the osmotic resistance of erythrocytes for palmitoleic acid (*cis*-16:1) in the temperature range 0–37°C. In this experiment, the question was raised as to whether there are any common features in the thermotropic behavior of the interaction affecting the osmotic stability of erythrocytes for *cis*-unsaturated fatty acids with one double bond. In the temperature range 0–25°C, the effects exhibited by palmitoleic acid show a distinctly different pattern when compared with the structurally very close oleic acid, and one has to conclude that the critical temperatures and the pattern of temperature-dependent behavior are individual properties of fatty acids. As shown in Fig. 2, palmitoleic acid is an excellent protecting agent for human erythrocytes against hypotonic hemolysis at a broad concentration range. The striking difference is that oleic acid has no hemolytic effect up to 15°C, and above and below this hemolytic concentration range there are concentration ranges of oleic acid with marked protecting effects. Palmitoleic acid shows the classical biphasic behavior over the whole temperature range from 0 to

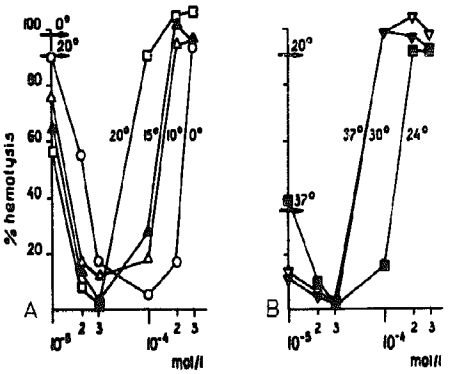

Fig. 2. Temperature-dependent interaction of palmitoleic acid (*cis*-16:1) with the erythrocyte membrane. Percent hemolysis is plotted against the concentration of palmitoleic acid. Experimental set-up was as in Fig. 1. Note the arrows indicating the percent hemolysis in the hypotonic buffer solution without addition of palmitoleic acid. (A) temperature range 0–20°C; (B) 24–37°C.

37 °C, with protection in a certain lower concentration range and hemolysis promotion at higher concentrations. This is also the case with oleic acid at temperatures above 15 °C, with the hemolytic concentration range becoming broader with further increasing temperatures. Thus, whatever structural element of the membrane is responsible for the specific differences in this type of interaction of oleic and palmitoleic acids, it is not effective above 20 °C.

Temperature-dependent effects of lauric and myristic acids on the osmotic resistance of human erythrocytes

In order to see whether there are features of the temperature-dependent behavior characteristic of saturated fatty acids, the concentration-dependent effects on the osmotic resistance of erythrocytes were determined for lauric acid (12:0) and myristic acid (14:0).

As depicted in Fig. 3, lauric acid (12:0) has a rather narrow range of protecting effect at $3 \cdot 10^{-7}$ M, which becomes broader by moving to higher temperatures. Interestingly, at 10 °C, there is a second concentration range of protection, and at higher concentrations, hemolysis promotion. Lauric and myristic acids have a common critical temperature for the hemolytic effect at 30 °C.

Fig. 3 also shows the temperature-dependent effects of myristic acid on hypotonic stress. There is certainly some similarity with lauric acid in the temperature range from 0–20 °C, with the protecting effect being more pronounced. The tendency towards a hemolytic effect at 10 °C at the concentration of 10^{-3} M is a further similarity between the two saturated fatty acids. However, it

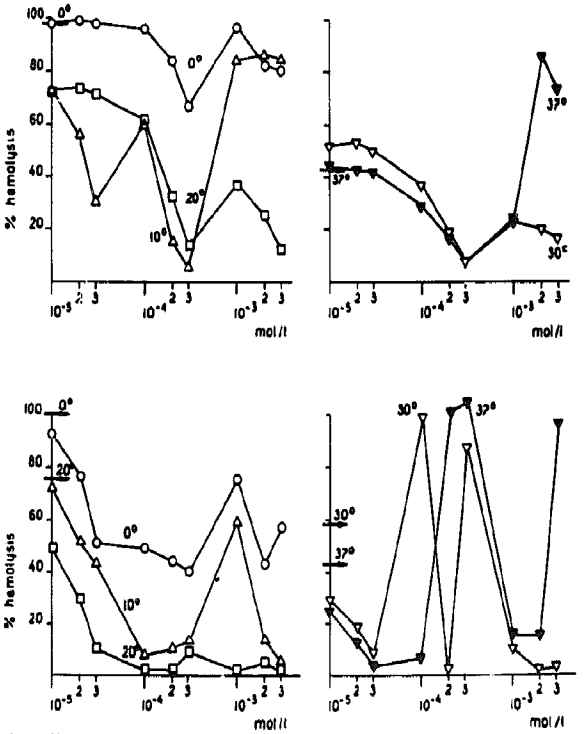

Fig. 3. Temperature-dependent effects of lauric (upper panels) and myristic (lower panels) acids on the osmotic resistance of human erythrocytes. The extent of hemolysis is plotted against the concentration of the fatty acids. For the experimental set-up, see Fig. 1 and Materials and Methods. Note the arrows, which indicate percent hemolysis without addition of the fatty acid at the given temperature. For the sake of clarity, for each fatty acid, the investigated temperature range is shown in two panels. The reproducibility of hemolysis assay was within a range of +5% hemolysis with the same freshly drawn blood samples on the same day. It is essential that the centrifugation step follows without delay and is carried out exactly at the temperature of incubation.

should be pointed out that at 20 °C, myristic acid exhibits the most efficient protecting effect, reducing the degree of hemolysis from 50 to almost 0%. The ability to protect against hypotonic hemolysis at 0 °C seems to be a common property of saturated and *cis*-unsaturated fatty acids, in contrast with the *trans*-unsaturated elaidic acid.

In the higher temperature range, however, the picture becomes completely different for myristic acid. At 30 °C and also at 37 °C, the striking observation of concentration-dependent alternating ranges of protection and hemolysis was made, which is unique for myristic acid. Instead of one concentration range with protection and subsequent hemolysis promotion at higher concentrations, there are three concentration ranges with hemolysis promotion. Paradoxically, at 30 °C, the highest concentrations are not hemolytic but constitute the third protecting range. Thus, myristic acid has a sharp critical temperature at 30 °C, when a fatty acid protecting over the whole concentration range switches in its response to the membrane to a multiphasic behavior. It should be mentioned in this context that it appears unlikely that any of these phenomena exhibited by myristic acid are correlated with its critical micellar concentration, since the critical micellar concentration of myristic acid was found in a similar buffer (pH 7.4) at 37 °C to be $4.6 \cdot 10^{-7}$ [14]. Such a multiphasic behavior was not observed with any other fatty acid. Lauric acid, which is just two C-atoms shorter than myristic acid, has, in the temperature range 30–37 °C, completely different temperature-dependent effects on the erythrocyte membrane, underlining the specificity of free fatty acid interaction.

Arrhenius plots of percent hemolysis at the fatty acid concentration of maximum protection against hypotonic hemolysis

The striking individual differences between the fatty acids are obvious. In an attempt to find common features in the response of structurally different fatty acids to temperature-dependent alterations of the erythrocyte membrane, their thermotropic behavior was compared with the help of Arrhenius plots.

In the Arrhenius plots depicted in Fig. 4 and Fig. 5, the logarithm of percent hemolysis at the concentration of maximum protection of the fatty acid is plotted against the reciprocal absolute temperature (in K). The breaks in the Arrhenius plots do not necessarily mean a critical temperature for hemolysis, but indicate rather abrupt temperature-dependent changes in the membrane structure, resulting in a different response of the fatty acid. Since, for the Arrhenius plots, percent hemolysis is taken at the concentration of maximum protection, the breaks represent either a change in the direction towards more or less hemolysis, or an abrupt change in the same direction. However, it should be emphasized that not only the breaks in the Arrhenius plots, but also their slope and general pattern are relevant in trying to find common features among different fatty acids.

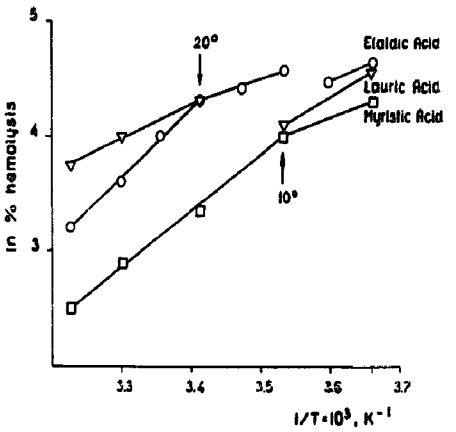

Fig. 4. Arrhenius plots of percent hemolysis at the concentration of maximum protection for elaidic, lauric and myristic acids. The logarithm of percent hemolysis at the concentration of $2 \cdot 10^{-5}$ mol/l of the given fatty acid is plotted against the reciprocal value of absolute temperature (K). The saturated and *trans*-unsaturated fatty acids have positive slopes with a rather similar pattern and some identical breaks in the Arrhenius plots. The diagram of elaidic and lauric acids is interrupted at a temperature range where experimental points were not determined.

Fig. 4 shows the Arrhenius plots for the saturated and *trans*-unsaturated fatty acids, namely for lauric, myristic and elaidic acids, which exhibit as a common feature positive slopes and a similar pattern. Lauric and myristic acids appear to be sensitive to a membrane transition at 10 °C, whereas lauric and elaidic acids respond to a transition at 20 °C.

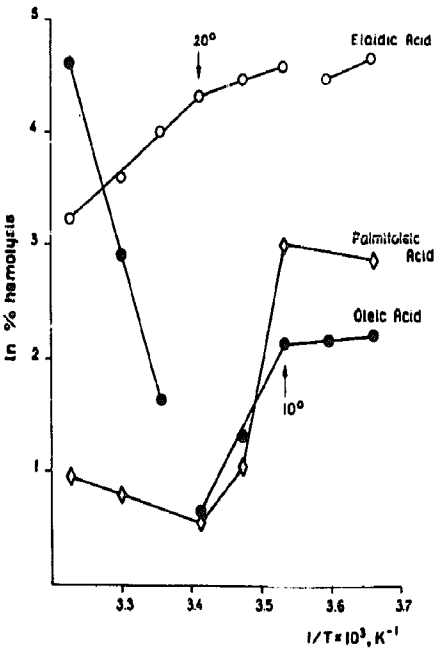

Fig. 5. Arrhenius plots of percent hemolysis at the concentration of maximum protection for elaidic, oleic and palmitoleic acids. Percent hemolysis was taken at $2 \cdot 10^{-5}$ mol/l of elaidic and oleic acids and at $3 \cdot 10^{-5}$ mol/l of palmitoleic acid. The two *cis* unsaturated fatty acids exhibit negative initial slopes, identical break points and a similar pattern in their Arrhenius plots.

In Fig. 5, the Arrhenius plots of the unsaturated elaidic acid (*trans*-18:1), palmitoleic acid (*cis*-16:1) and oleic acid (*cis*-18:1) are compared. The Arrhenius plots of the two *cis*-unsaturated fatty acids are conspicuous by exhibiting negative initial slopes, and as a further common feature, they have identical break points at 10 and 20° C. It is evident that the Arrhenius plots of the *trans*-unsaturated elaidic acid and of the two saturated fatty acids are quite different from those of the *cis*-unsaturated fatty acids. Thus, two groups of fatty acids can be recognized, namely, saturated and *trans*-unsaturated on one side and *cis*-unsaturated fatty acids on the other, as was suggested by Klausner and co-workers [2–4] for other membrane parameters.

However, the pronounced differences in the thermotropic behavior of individual fatty acids, which were observed in addition to the above-mentioned general features, suggest that certain fine structures of the membrane could be selectively recognized by individual free fatty acids.

Discussion

The extent to which substances alter the osmotic resistance of erythrocytes is a parameter of their interaction with the plasma membrane. The extreme specificity of the temperature-dependent fatty acid effects suggests different types of interactions with different domains. With a limited number of sites or domains for the interaction with non-esterified fatty acids in the erythrocyte membrane, one would rather expect the thermotropic effects to show a common pattern for all fatty acids. The results of the present investigation show that this is not the case and suggest that the acyl moiety plays a very specific role in lipid–lipid or lipid–protein interactions.

Based on specific effects exhibited by different classes of free fatty acids on emission polarization of fluorescent probes, and based on investigations concerning the inhibition of capping in lymphocytes, it was suggested by Klausner and co-workers [2–4] that long-chain free fatty acids can be divided in two groups, namely, the *cis*-unsaturated fatty acids on the one hand, and the *trans*-unsaturated and saturated on the other. For this reason, in our study of measuring the temperature-dependent influence of free fatty acids on osmotic resistance, we compared the effects of *cis*-unsaturated fatty acids with those of saturated and of *trans*-unsaturated ones. The results of the present investigation agree with the classification as suggested by Klausner and co-workers [2–4]. However, the strong individual differences in the thermotropic effects of individual fatty acids suggest that within those groups, further distinctions are to be made, and that the individual free fatty acid effects could be indicators for distinct fine structures of the plasma membrane.

The drastic and specific membrane effects exhibited by free fatty acids underline that the heterogeneity of the cell membrane is given not only by its protein content but also by its lipid composition. The association of proteins with lipids in the plasma membrane means that protein–lipid or alternatively, lipid–lipid interactions might be affected by the interference with free fatty acids. In

the effort to distinguish between protein and lipid effects, considerable progress has been made by investigations of the thermotropic effects of the erythrocyte membrane. The osmotic resistance of erythrocytes per se is temperature-dependent and keeps increasing with increasing temperature [15,16]. For the critical temperatures of the free fatty acid effects measured in the present study, the most relevant data are the critical temperatures for hemolysis and resealing of human erythrocytes which were measured without addition of free fatty acids. A common critical temperature for both lysis and resealing processes appears to be at 7–10 °C [17,18]. This critical temperature is also seen in the Arrhenius plots of the fatty acid effects in the present investigation as a break. Forte et al. [19] found, using a spin-labeled stearic acid, that protein 4.1 is involved in a structural thermotropic transition of the erythrocyte membrane, and suggested that interactions of this protein with the membrane contribute significantly to the thermotropic properties of erythrocytes. Moreover, Ca^{2+} caused a disappearance of the 8 °C temperature break and the appearance of two breaks at 32 and 15 °C. These two critical temperatures are also seen in the present study as critical temperatures of some fatty acid effects.

The nature of phase transitions of erythrocyte membrane lipids between 0 and 37 °C is still a matter of discussion. Some investigators reported a phase transition occurring around 20 °C [20–22], but with the technique of deuterium magnetic resonance, no phase transition of acyl chains in the erythrocyte lipids was detected [23]. Structural transitions for plasma membranes of mammalian cells are not well-understood due to the high level of complexity which is reflected in the high level of anisotropy of these membranes. It is not likely that the entire membrane exhibits a phase transition analogous to lipid phase transitions, but interaction between lipids surrounding, for instance, a protein channel and the protein complex could lead also to abrupt changes in membrane permeability. Conformational changes of proteins and cooperative processes are also possible. Several membrane processes have been reported to show discontinuities as a function of temperature [24–26], which could be explained by conformational changes of the membrane proteins induced by a temperature-dependent phase change in the membrane (for reviews, see Refs. 27 and 28).

Structural transitions of the erythrocyte membrane in the 0–50 °C range have been measured by a variety of physicochemical techniques [29–32]. The exact nature of structural changes involved in these transitions has not been elucidated, but it was observed that the lateral mobility of glycoproteins, which changes discontinuously with temperature [29], appears to be controlled by skeletal proteins [33,34], and Minetti et al. [32] found evidence for a role of proteins in these temperature-dependent structural transitions.

Major contributions to our understanding of the thermotropic behavior of the erythrocyte membrane have been made by Morariu et al. [35] with nuclear magnetic resonance techniques. In a study performed with human erythrocytes, a break in the Arrhenius plot of the water exchange time was found at pH 7.4, at 26 °C [35]. Based on this and other observations, it was suggested that conformational changes of proteins, as well as cooperative state transitions of the membrane, are involved in this transport process.

Phase transitions occurring at 19–25 °C were found in erythrocyte membrane parameters, such as microviscosity [21,36], osmotic fragility [15], incorporation of ^{32}P into polyphosphoinositides [37], transport of chloride [38] and exchange transport of glucose [39]. However, from Raman spectroscopical studies, it has been concluded that erythrocyte membranes undergo pH-sensitive cooperative transitions in the physiological temperature range [40]. It should be pointed out that one critical temperature we observed with several fatty acids was at 20 °C, which could be identical with the phase transitions mentioned above, all occurring around 19–25 °C.

The drastic and specific influence of temperature on the interaction of structurally closely related fatty acids with the erythrocyte membrane represents a basis for further investigations, in an attempt to unravel the poorly understood processes involved in the interaction of the lipid bilayer with proteins of the membrane and of the cytoskeleton. Experiments with erythrocytes from which proteins have been selectively removed and with liposomes of defined composition, should enable one

to test which components of the membrane and/or the cytoskeleton are responsible for the observed specific effects of non-esterified fatty acids.

Acknowledgement

This work was supported by Austrian Research Council project Nr.: S-4104.

References

1 Singer, S.J. and Nicholson, G.L. (1972) Science 175, 720–731.
2 Klausner, R.D., Kleinfeld, A.M., Hoover, R.L. and Karnovsky, M.J. (1980) J. Biol. Chem. 255, 1286–1295.
3 Karnowsky, M.J., Kleinfeld, A.M., Hoover, R.L. and Klausner, R.D. (1982) J. Cell Biol. 94, 1–6.
4 Klausner, R.D., Bhalla, D.K., Dragsten, P., Hoover, R.L. and Karnovsky, M.J. (1980) Proc. Natl. Acad. Sci. USA 77, 437–441.
5 Staufenbiel, M. and Lazarides, E. (1986) Proc. Natl. Acad. Sci. USA 83, 318–322.
6 Fujiyama, A., Matsumoto, K. and Tumanoi, F. (1987) EMBO J. 6, 223–228.
7 Kellie, S. and Wigglesworth. N.M. (1987) FEBS Lett. 213, 428–432.
8 Stubbs, C.D. (1983) Essays Biochem. 19, 1–39.
9 Swann, A.C. (1984) Arch. Biochem. Biophys. 233, 354–361.
10 Csordas, A. and Schauenstein, K. (1986) Biochim. Biophys. Acta 856, 212–218.
11 Goodman, D.S. (1958) J. Clin. Invest. 37, 1729–1735.
12 Raz, A. and Livne, A. (1973) Biochim. Biophys. Acta 311, 222–229.
13 Csordas, A. and Schauenstein, K. (1984) Biochim. Biophys. Acta 769, 571–577.
14 Piper, H.M., Sezer, O., Schwartz, P., Hütter, J.F. and Spieckermann, P.G. (1983) Biochim. Biophys. Acta 732, 193–203.
15 Aloni, B., Eitan, A. and Livne, A. (1977) Biochim. Biophys. Acta 465, 46–53.
16 Richieri, G.V. and Mel, H.C. (1985) Biochim. Biophys. Acta 813, 41–50.
17 Minetti, M. and Ceccarini, M. (1982) J. Cell. Biochem. 19, 59–75.
18 Minetti, M., Ceccarini, M. and Di Stasi, A.M.M. (1984) J. Cell. Biochem. 25, 61–72.
19 Forte, T., Leto, Th.L., Minetti, M. and Marchesi, V.T. (1985) Biochemistry 24, 7876–7880.
20 Johnson, R.M. (1975) J. Membrane Biol. 22, 231–253.
21 Zimmer, G. and Schirmer, H. (1974) Biochim. Biophys. Acta 345, 314–320.
22 Bieri, V.G. and Wallach, D.F.H. (1976) Biochim. Biophys. Acta 443, 198–205.
23 Davis, J.H., Maraviglia, B., Weeks, G. and Godin, D.V. (1979) Biochim. Biophys. Acta 550, 362–366.
24 Raison, J.K., Lyons, J.M. and Thomson, W.W. (1971) Arch. Biochem. Biophys. 142, 83–90.
25 Lenaz, G., Curatola, G. and Masotti, L. (1975) J. Bioenerg. 7, 223–299.
26 Benga, G. and Strach, S.J. (1975) Biochim. Biophys. Acta 400, 69–79.
27 Lenaz, G. (1973) Acta Vitaminol. Enzymol. 27, 62–95.
28 Van Deenen, L.L.M., De Gier, J., Demel, R.A., De Kruiff, B., Blok, M.C., Van der Neut-Kok, E.C.M., Haest, C.W.M., Ververgaert, P.H.J.Th., Verkleij, A.J. (1975) Ann. N.Y. Acad. Sci. 264, 124–141.
29 Nigg, E.A. and Cherry, R.J. (1979) Biochemistry 18, 3457–3465.
30 Hui, S.W., Stewart, C.M., Carpenter, M.P. and Stewart, T. (1980) J. Cell Biol. 85, 283–291.
31 Galla, H.J. and Luisetti, J. (1980) Biochim. Biophys. Acta 596, 108–117.
32 Minetti, M., Ceccarini, M. and Di Stasi, A.M.M. (1984) J. Cell. Biochem. 25, 73–86.
33 Golan, D.E. and Veatch, W. (1980) Proc. Natl. Acad. Sci. USA 77, 2537–2541.
34 Schindler, M., Koppel, D.E. and Scheetz, M.P. (1980) Proc. Natl. Acad. Sci. USA 77, 1457–1461.
35 Morariu, V.V., Pop, V.I., Popescu, O. and Benga, G. (1981) J. Membr. Biol. 62, 1–5.
36 Feinstein, M.B., Fernandez, S.M. and Sha'afi, R.I. (1975) Biochim. Biophys. Acta 413, 354–370.
37 Buckley, J.T. and Hawthorne, J.N. (1972) J. Biol. Chem. 247, 7218–7223.
38 Brahm, J. (1977) J. Gen. Physiol. 70, 283–306.
39 Lacko, L., Wittke, B. and Geck, P. (1973) J. Cell. Physiol. 82, 213–218.
40 Verma, S.P. and Wallach, D.F.H. (1976) Proc. Natl. Acad. Sci. USA 73, 3558–3561.