Thermodynamical Characteristics and Volume Compressibility of Dipalmitoylphosphatidylcholine Liposomes Containing Bacteriorhodopsin

B. PIKNOVÁ [1], T. HIANIK[1]*, V. N. SHESTIMIROV[2] and V. L. SHNYROV[2]

1 *Department of Biophysics, Faculty of Mathematics and Physics, Comenius University, Mlynská dolina F1, 842 15 Bratislava, CSFR*

2 *Institute of Biological Physics, Academy of Sciences of the U.S.S.R., Pushchino, 142 292 Moscow Region, U.S.S.R.*

Abstract. The effects of bacteriorhodopsin (BR) interaction with large dipalmitoylphosphatidylcholine (DPPC) liposomes (approx. 100 nm in diameter) were examined at various BR/DPPC ratios, using differential scanning calorimetry (DSC) and ultrasonic velocimetry (USV). On DSC, the lipid phase transition temperature, T_c, and the half-width of the phase transition peak, $\Delta T_{1/2}$, showed significant nonmonotonic changes with the increasing BR concentration. Two exponential segments could be distinguished in the dependence of the transition enthalpy change per mol of lipid ($\Delta H/n_L$) on the BR/DPPC ratio: one corresponding to ratios between 0:1 and 1:64, and another corresponding to ratios between 1:44 and 1:16. A maximal value of $\Delta H/n_L$ was observed for BR/DPPC ratio 1:44, probably corresponding to maximal BR-lipid ordering with each BR molecule being surrounded by two layers of lipid molecules. The nonmonotonic changes of thermodynamical parameters suggest long-distance interactions between regions of altered bilayer structure which form around each BR molecule. The results obtained with USV provided support for the above conclusions. The dependence of ultrasound velocity increment A on BR concentration supplies information on relative changes of membrane volume compressibility. Decreasing volume compressibility is reflected in increasing values of parameter A. Within $T < T_c$, the values of A increased with the increasing BR concentration; saturation was observed at BR/DPPC ratio 1:500 ($A = A$(BR/DPPC)). No significant BR-concentration dependent changes of A were observed at $T > T_c$. From these values the average diameter of the distorted region of lipid bilayer was estimated to be approximately 20 nm.

* To whom all the correspondence should be addressed

Key words: Bacteriorhodopsin — Lipid bilayers — Phase transition — Volume compressibility — Lipid protein interaction

Introduction

Protein-lipid interactions play a decisive role in the architecture of membrane structure. They have to be considered whenever explanations are sought for molecular mechanisms of various membrane processes, such as energy transfer and conversion, processes of reception, etc. In this respect, interactions of integral proteins with lipids are of special interest. Integral proteins span the membrane transversely, establishing contacts between their hydrophobic moieties and the hydrocarbon chains of the lipids. Owing to the different geometry of the hydrophobic moiety of proteins and that of lipids, as well as due to the action of electrostatic and elastic forces, regions of altered structure may arise around protein molecules. The formation of similar regions may represent one of the reasons of the occurrence of long-distance interactions in a membrane. Very likely, hydrophobic interactions play the key role in the establishment of links between integral proteins and lipids. Assuming negligible elastic deformation of the lipid bilayer in the environment of the integral protein, the energy of these interactions for low protein concentrations can be estimated from thermodynamical (TD) parameters. The application of the Mouritsen-Bloom mattress model (MB model) (Mouritsen and Bloom 1984) on photosynthetic reaction centers (RC), proteins and antenna proteins (LHCP) incorporated into lipid bilayers showed good agreements with experimental results (Sperotto and Mouritsen 1988). However, the situation is much more complicated at elevated protein concentrations when thermodynamical parameters no more show linear dependence on the relative protein concentration in a membrane. In this case, changes of elasticity of the lipid bilayer in the environment of the integral protein have to be accounted for. *A* relative increase of protein concentration in a membrane may result from phase transition-induced protein aggregation (Chapman 1983), or upon the interaction with a hormone, as it is the case with hormone receptors (Jacobs and Cuatrecasas 1981). In the latter case, substantial changes of mechanical characteristics of the lipid bilayer occur even at physiological concentrations of the respective receptors (Hianik et al. 1988). This presents considerable problems for the interpretation of TD parameters. The same is true for BR which, upon incorporation into a membrane, forms a regular crystal lattice with tightly ordered lipids (Stoeckenius et al. 1979). The crystal ordering of proteolipidic BR domains is not substantially altered upon decreasing the temperature below that of lipid phase transition ($T < T_c$). A widening of the transition can be observed but at elevated BR concentrations (Cortijo et al. 1982). In contrast, at temperatures $T > T_c$, BR is equally dispersed in the lipid bilayer (Lewis and

Engelman 1983a). However, the physical characteristics of membranes with incorporated BR have so far been studied but insufficiently. To obtain quantitative information concerning phase transition of lipid bilayers, the present investigations were focused on the effects of BR on TD and mechanical parameters of unilamellar DPPC liposomes. The thermodynamical parameters were estimated using differential scanning calorimetry (DSC). The effects of BR on volume compressibility of vesicles was also studied using differential ultrasonic velocimetry (DSV).

Materials and Methods

Experiments were performed on large unilamellar liposomes (approx. 100 nm in diameter), prepared with the method of detergent dialysis (see Margolis and Bergelson 1986). Sodium cholate (Spofa) was used as the detergent. It was dissolved in 10 mmol/l Hepes, and 100 mmol/l KCl was added (pH 9.3). Immediately after the detergent was dissolved, the pH of the solution was adjusted to 7.4 with a small amount of 0.1 mol/l HCl, and DPPC (Serva) was then dissolved to give a final detergent/lipid ratio of 7.2:25. The dialysis was performed in two stages. The first stage included dialysis in 10 mmol/l Hepes + 100 mmol/l KCl (pH 7.4) for 12 hours, followed by dialysis in 10 mmol/l Hepes + 10 mmol/l KCl (pH 7.4) for additional 12 hours. Fragments of purple membranes (PM) containing BR isolated from *Halobacterium halobium* (strain 353P) were used and added into the liposome suspension. The concentration of BR in liposome suspension was calculated spectrophotometrically using $\epsilon_{570} = 63,000$ mol^{-1}.l.cm^{-1}. Because PM are easily incorporated into liposomes by means of fusion (Vsevolodov 1988) we assumed the volume concentration of BR to be close to that in liposomes. Moreover, at the maximal BR/DPPC ratio used there was no more than 1 fragment of PM per 1 liposome.The differential methods used allowed us to observe the direct effects of BR on physical properties of lipid bilayer.

PM were kindly donated by L.N.Chekulayeva, Institute of Biological Physics, Academy of Sciences of the USSR, Pushchino. All experiments were done with dark-adapted BR, i.e. the sample was kept in dark throughout the measurements which started 30 min after the chambers had been filled with the liposome suspension; this is an interval sufficient for BR to become dark-adapted (Stoeckenius et al. 1979). Calorimetric measurements were performed on a DASM-4 (Privalov 1980) at a 1 °C/min scan rate. The reference chamber was first filled with the buffer and the measuring chamber with liposome suspension in buffer (chamber volume approx.1 ml each), with the lipid concentration being 4 mg/ml. The change of specific heat capacity ΔC_p , and enthalpy changes per mol DPPC ($\Delta H/n_L$) were determined. Measurements of ultrasound velocity in liposome suspensions were performed using a RADA-S differential ultrasonic scanning velocimeter (Shestimirov 1989). For the USV measurements, the reference chamber was first filled with the buffer and the measuring chamber with the liposome suspension in buffer (chamber volume approx. 0.3 ml each), with the lipid concentration being 4mg/ml. The value of the ultrasound velocity increment, A_0, the relative change in ultrasound absorption per wave- length unit, $\Delta(\alpha\lambda)/\alpha\lambda$, were determined for the system in dependence on temperature for the temperature interval 30-50 °C. The temperature scanning of the samples was performed at a rate of 0.1 °C/min in both directions, i.e. towards increasing T as well as in the opposite dirrection. The curves were taken as reference for final evaluation. Then, the measuring

chamber was filled with the suspension of liposomes with incorporated BR (DPPC concentration 4mg/ml) at various BR/DPPC ratios, the reference chamber was filled with buffer of a molar BR concentration identical with that in the measuring chamber. The temperature dependence of A and $\Delta(\alpha\lambda)/\alpha\lambda$ were determined for each of the BR/DPPC ratios tested. Let us briefly explain the basic principles of the USV method. Due to the formation of standing waves, resonance is produced in the chamber at frequency f_n, at which the distance l between the acoustic transducers in the chamber walls equals the product of halfwaves $\lambda/2$ and integer (acoustic transducers convert high-frequency electrical field (of several MHz) into ultrasound waves). For diluted solutions for which ultrasound velocity, u, does not substantially differ from those measured in the solvent, u_0, as well as for those for which ultrasound absorption α is not too high, holds (Buckin et al. 1979; Sarvazyan 1982)

$$\begin{aligned} \Delta u &= u_0 \frac{\Delta f_n}{f_n} \\ \Delta(\alpha\lambda) &= \Delta\left(\frac{\pi \delta f_n}{f_n}\right) \end{aligned} \tag{1}$$

where f_n is the frequency of the n-th resonance peak measured in the solvent; δf_n is the peak half-intensity width, and it designates the difference of the respective variables. The method of USV is based on the link between density ρ and volume compressibility K, and ultrasound velocity u in a solution. The principal parameter linking compressibility of a solution with its composition, is adiabatic compressibility

$$\Phi_K = (\beta V - \beta_0 V_0)/n \tag{2}$$

where β is the coefficient of adiabatic compressibility

$$\beta = -\frac{1}{V}\left(\frac{\mathrm{d}V}{\mathrm{d}p}\right) = \frac{1}{u^2\rho}$$

where V is the sample volume, V_0 is the volume of the solvent, $n = cV$ are moles of the substance dissolved, and c is the concentration of the latter. The product βV is termed compressibility K. For diluted solutions holds (Sarvazyan and Kharakoz 1977)

$$\frac{\Phi_K}{\beta_0} \approx \frac{M}{\rho_0} - 2(A + B) \tag{3}$$

where $A = \frac{u - u_0}{c u_0}$ is the concentration increment of ultrasound velocity; $B = \frac{\rho - \rho_0}{c\rho_0}$ is the concentration increment of solution density; and M is the molecular mass of the substance dissolved. Taking the measurable parameters, for specific adiabatic compressibility we have:

$$\frac{\Phi_K}{\beta_0} = -2\frac{A}{M} + 0.45 \tag{4}$$

Thus, in the first approximation, parameter A has to be measured to allow the determination of specific adiabatic compressibility.

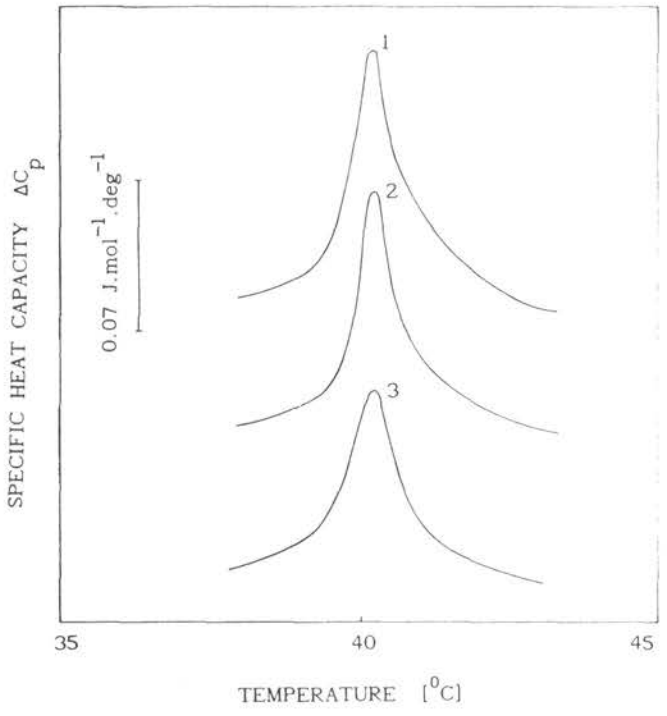

Figure 1. Changes of specific heat capacity ΔC_p for liposomes from pure DPPC and BR/DPPC recombinants. BR/DPPC ratios : $1 - 0$; $2 - 12.5 \times 10^{-3}$; $3 - 1.2 \times 10^{-3}$. Scans have had baselines substracted from the curves.

Results

DSC experiments

Fig. 1 shows changes of specific heat capacity ΔC_p for liposomes from pure DPPC (curve 1) and for those in mixture with BR-containing PM, for various BR/DPPC ratios. It is obvious from the Figure that for liposomes from pure DPPC, phase transition occurs at $T_c = 40.4$°C, which is in good agreement with the data reported by other investigators (Chapman 1983).

BR incorporation into liposomes results in changes of the phase transition parameters. With the increasing BR concentration, both the phase transition temperature T_c (Fig. 2) and the half-peak width $\Delta T_{1/2}$ (Fig. 3), as determined from relationship ΔC_p(T), change. Integrated curves of ΔC_p(T) gave the phase transition enthalpy change ΔH. Fig. 4 shows the dependence of $\Delta H/n_L$ (enthalpy

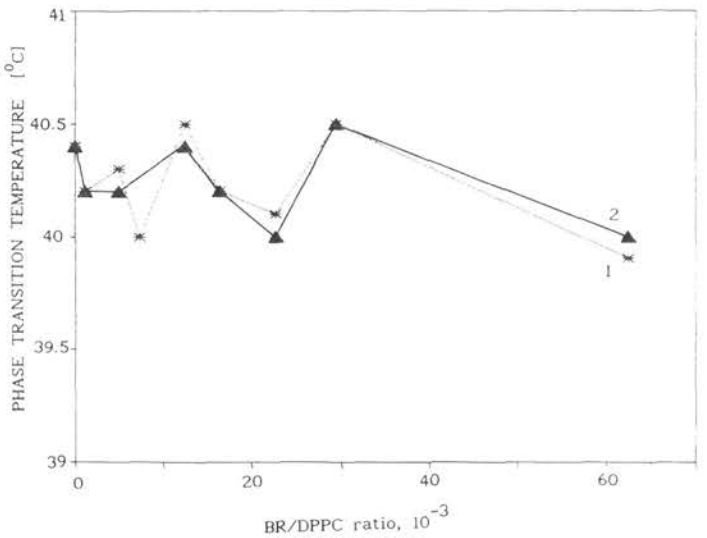

Figure 2. Dependence of phase transition temperature on BR/DPPC ratio for two determinations. (* first scanning, ··· second scanning)

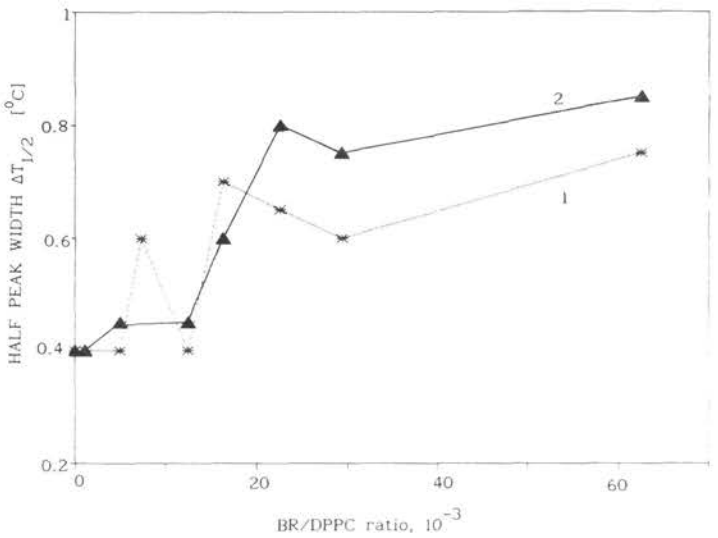

Figure 3. Dependence of half-peak width, $\Delta T_{1/2}$, on BR/DPPC ratio for two determinations. (* first scanning, ··· second scanning)

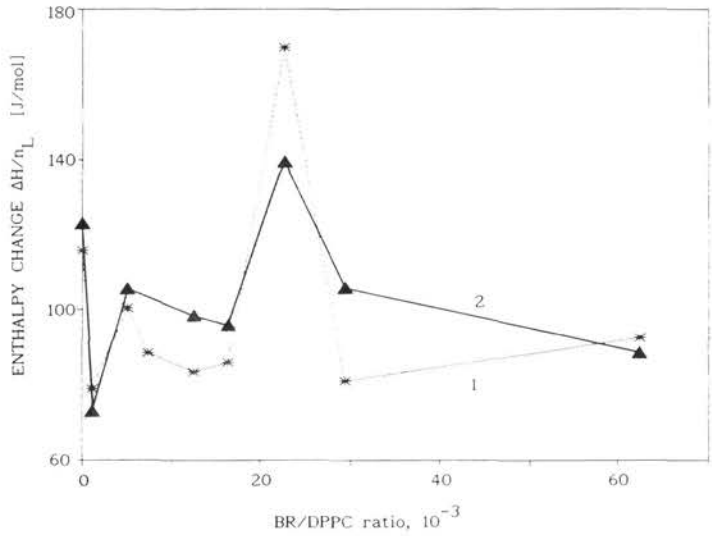

Figure 4. Dependence of enthalpy change per mol lipid $\Delta H/n_L$ on BR/DPPC ratio for two determinations. (* first scanning, ··· second scanning)

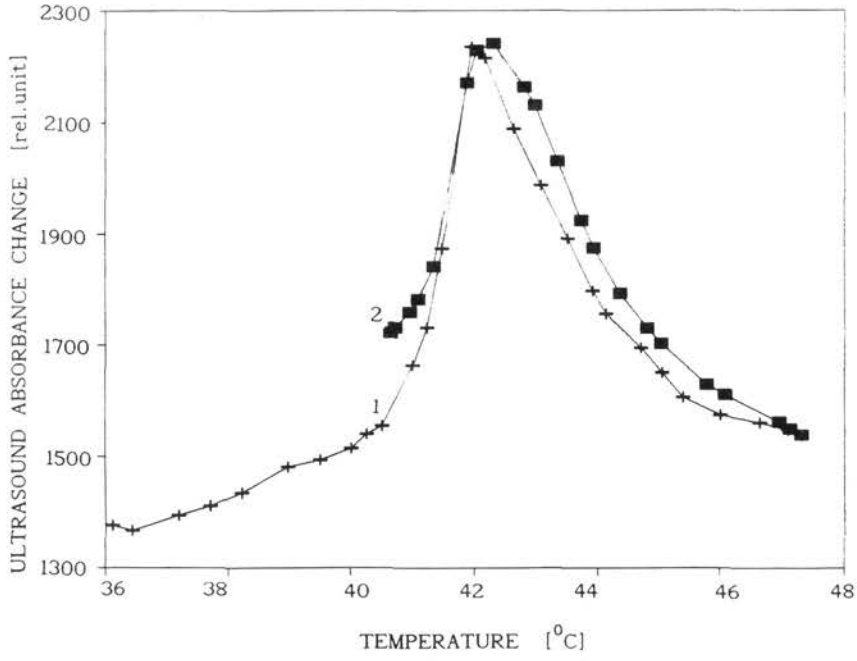

Figure 5. Temperature dependence of relative ultrasound absorbance change of DPPC liposomes. Scanning from low to high (1) and from high to low (2) temperatures.

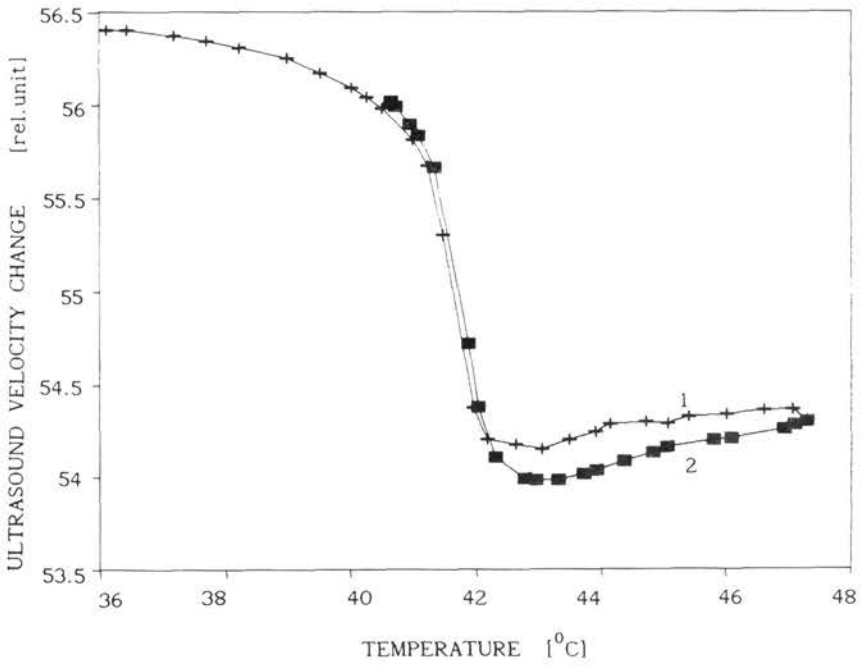

Figure 6. Temperature dependence of relative ultrasound velocity change of DPPC liposomes. Scanning from low to high (1) and from high to low (2) temperatures.

change per mol lipid) on the BR/DPPC ratio for liposome suspension. The dependence illustrated in Figs. 2 and 3, as well as that of $\Delta H/n_L$ (BR/DPPC), have markedly nonmonotonic courses.

USV experiments

Fig. 5 shows the temperature dependence of ultrasound absorption change per wavelength unit $\Delta(\alpha\lambda)/\alpha\lambda$ (curves 1, 2) and Fig. 6 shows the temperature dependence of ultrasound velocity change $\Delta u/u_0$ (curves 1, 2) for liposome suspension from pure DPPC. The Figure shows curves corresponding to temperature scanning in the increasing (curves 1) and decreasing (curves 2) direction. The absorption curve has a marked maximum at $T = 41 - 42$ °C, which agrees well with the results obtained using the same method and small unilamellar DPPC vesicles (Mitaku and Date 1982). Curves of similar shape could be obtained for various BR/DPPC ratios. Strong ultrasound absorption in the phase transition region is of TD nature, and is associated with a strong growth of thermal fluctuations (Mitaku and Date 1982; Michels et al. 1989). The shape of the $\Delta u/u_0(T)$ curve is different. The value

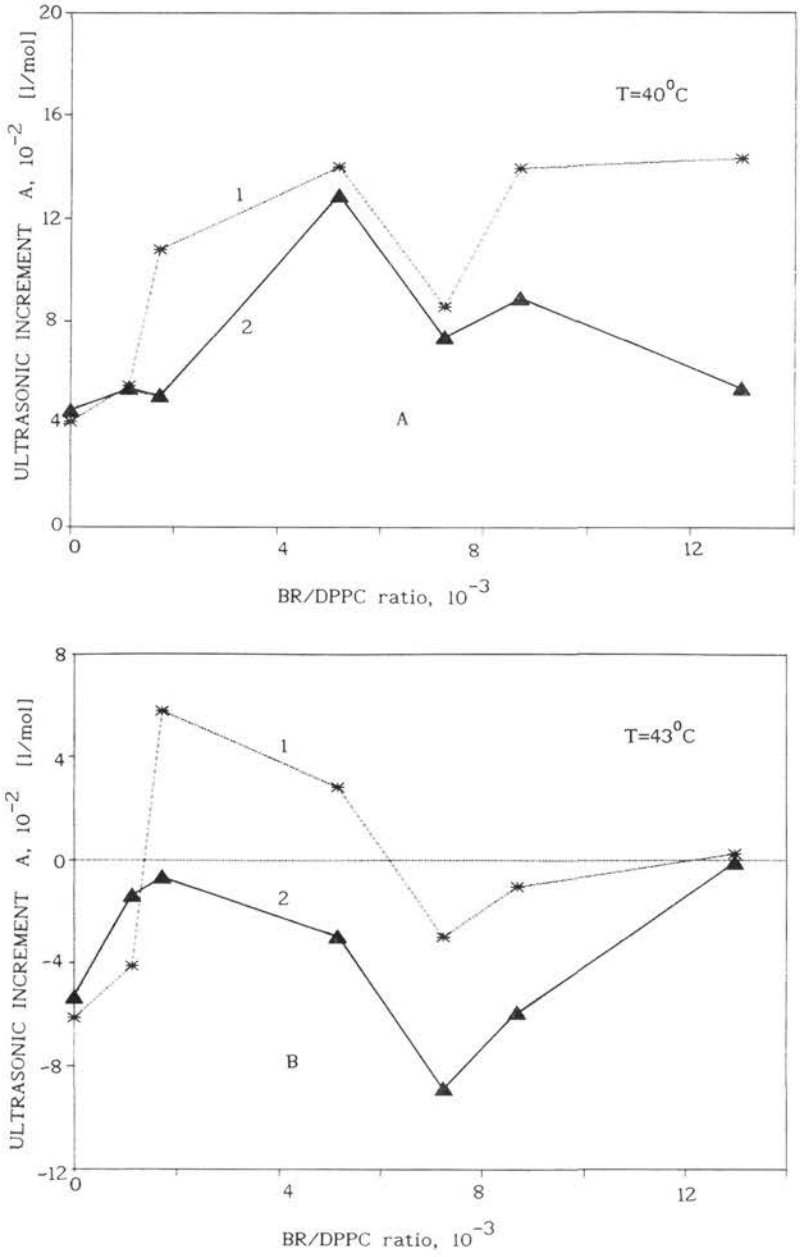

Figure 7. Dependence of ultrasound increment, A, on BR/DPPC ratio; a - 40 °C, b - 43 °C. Scanning from low to high (1) and from high to low (2) temperatures.

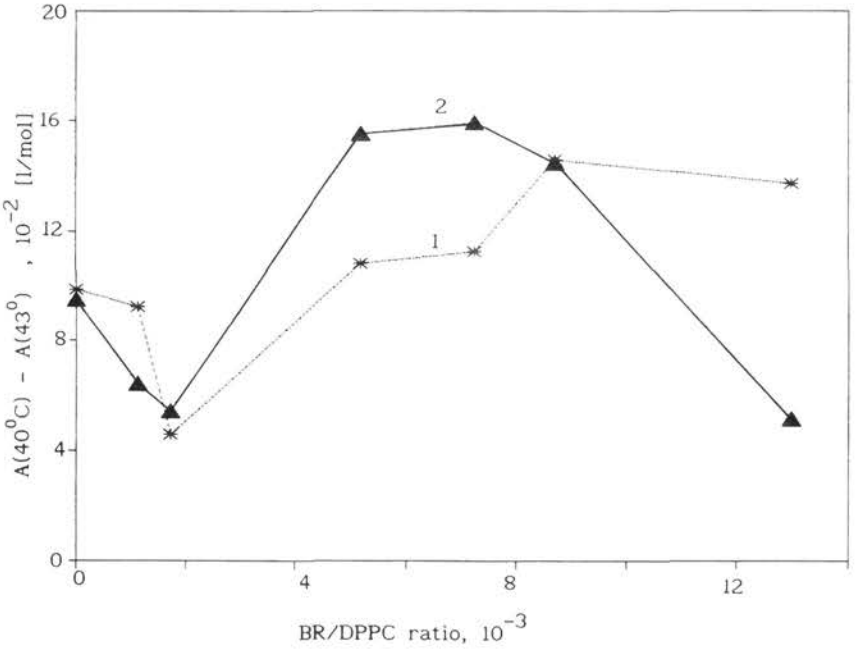

Figure 8. Dependence of ultrasound increment changes $\Delta A = A(40\,°C) - A(43\,°C)$ on BR/DPPC ratio. Scanning from low to high (1) and from high to low (2) temperatures.

of $\Delta u/u_0$ dramatically decreases within the gel-($T < T_c$)-to-liquid-crystal ($T > T_c$) phase transition interval, which is associated with a change of the lipid ordering and thus with different volume compressibility of the liposomes. As mentioned above, the measure of volume compressibility is the ultrasound velocity increment A. Fig. 7*a*, *b* shows the values of A at $T = 40\,°C$ (Fig. 7*a*) and at 43 °C (Fig. 7*b*) for the BR/DPPC ratios tested. Curve 1 was obtained from temperature scanning from low towards higher temperatures, curve 2 is the result of the high-to-low scanning direction. The value of A increases with the increasing BR concentration, and a gradual saturation is observed around the BR/DPPC ratio 1:77. The effects of the structural state of the lipid bilayer on the compressibility of liposomes containing BR are characterized by the dependence of changes $\Delta A = (A_{40} - A_{43})$ on the BR/DPPC ratio in the sample (Fig. 8). Fig. 8 shows that the dependence of ΔA has nonmonotonic character for both techniques of temperature scanning. The value of parameter ΔA decreases within the BR/DPPC ratio interval 0:1 and 1:579 and it increases beyond this interval. Standard errors of the results of all experiments did not exceed 10%.

Discussion

The present experiments showed that DPPC phase transition temperature, peak transition temperature width as well as transition enthalpy change (the latter markedly and nonmonotonically) in dependence on BR concentration. Ultrasonic measurements of A suggest that volume compressibility of vesicles also changes in dependence on BR concentration, i.e. that BR incorporation directly affects the ordering of the DPPC bilayer. Our further analysis of changes of TD and mechanical parameters of lipid bilayers observed will be based on the MB model of proteolipid bilayer as proposed by Mouritsen and Bloom (1984) and further developed by Sperotto and Mouritsen (1988). The basic principle of the model is the formation of a region of changed lipid bilayer structure in the environment of a membrane protein; this region arises due to the hydrophobic protein moiety tending to affect the length of the lipid hydrocarbon chains. The latter changes in dependence on the structural state of the membrane. The length of the protein hydrophobic moiety (h_p) is constant due to the relative stiffness of the latter, whereas membrane thickness may change by 20-30% as a result of phase transition (Evans and Kwok 1982; Inoko and Mitsui 1978). In the gel state (h_g), the hydrophobic membrane part is thicker than when in the liquid-crystal phase (h_f) (Janiak et al. 1976). If $h_p < h_g$, a deformed region of decreased ordering arises in the environment of the protein (Riegler and Möhwald 1986), quantitatively the degree of ordering of this region can be expressed by the parameter of ordering S (de Gennes 1974; Riegler and Möhwald 1986):

$$S = (h_p - h_f)/(h_g - h_f). \tag{5}$$

It can be derived from the Landau-de Gennes theory (de Gennes 1974) that the phase transition temperature will decrease in the presence of a protein if $S < 0.5$ and/or $h_p < 0.5(h_f + h_g)$. This was experimentally confirmed by Riegler and Möhwald (1986): they observed that the protein RC (h_p=2.8 nm) actually decreased the phase transition temperature of DPPC and increased that of dimyristoylphosphatidylcholine (DMPC), a lipid with hydrocarbon chains shorter than those of DPPC. Thus, from the viewpoint of the theory, the BR-induced decrease of the phase transition temperature for BR/DPPC ratios below 1:200 may be interpreted as a decrease of lipid bilayer ordering in the environment of the BR molecules. However, comparing BR-induced changes in DPPC phase transition temperature with those induced by the protein RC (Riegler and Möhwald 1986), the former seem relatively small; practically, this means that the length of the hydrophobic moiety of BR does not differ very much from $0.5(h_g + h_f) \sim 3.0$ nm (h_f=2.6 nm, h_g=3.4 nm; Lewis and Engelman 1983b). This agrees well with the values obtained using X-ray diffraction measurements (Lewis and Engelman

1983b). The decreased lipid bilayer ordering around the BR molecule is thus relatively small. Ultrasound measurements showed a gradual growth of A with the increasing BR concentration (Fig. 7) even in the range of relatively low BR concentrations. Parameter A is linked with the volume compressibility (which can also be interpreted in terms of a growth of membrane ordering). This may be due to the interactions occurring between the regions of changed structure at even relatively low BR/DPPC concentrations, which results in an increase of membrane structure ordering. This dependence shows saturation at relatively low BR/DPPC ratios (below 1:500); this means that the diameter of the changed structure region is larger than $\{(4/\pi).n.s_0 + d_{BR}^2\}^{1/2} \sim 22$ nm (n=500 is the number of the lipid molecules, s_0=0.72 nm^2 is the area per one lipid (Small 1967), $d_{BR} \sim 5.9$ nm is the diameter of one BR molecule (Henderson and Unwin 1975)).

With the length of the hydrophobic moiety of the BR molecule substantially differing from that of the membrane, marked changes of mechanical membrane characteristics may be expected to occur. This was suggested by our previous experiments (Hianik and Vozár 1985) which investigated changes of modulus of elasticity in direction perpendicular ($E_\perp$) to bilayer lipid membranes (BLM) prepared from asolectin (AL) and modified by BR (BR/AL~ 1:500). Parameter $E_\perp = -p/(\Delta h/h)$, where p is the pressure, $\Delta h/h$ is the relative change in membrane thickness, characterizes the degree of BLM compressibility. $E_\perp$ decreases with the decreasing ordering of hydrophobic membrane regions. The value of parameter $E_\perp$ was observed to decrease more than tenfold in average for BLM modified by dark-adapted BR (after approx. 35 min) as compared to those for membrane without BR. The experiments were performed at $T \sim 20$°C, i.e. the asolectin BLM were in liquid crystal phase. However, the thickness of their hydrophobic region (~ 5 nm) exceeded that of the hydrophobic region of the BR, owing to the presence of the hydrocarbon solvent n-heptane.

In the previous analysis, relatively low BR concentrations have been considered. Experiments using the DSC technique showed nonmonotonic changes of TD parameters to occur at higher BR concentrations (BR/DPPC) 1:200). Most markedly, this was reflected in changes of the $\Delta H/n_L$ dependence on the BR/DPPC ratio (Fig. 4): the value of this parameter decreased almost exponentially within the BR/DPPC ratio interval between 0:1 and 1:44. For higher BR contents (1:34 and 1:16), the curves have shapes similar to that for the low BR concentration range. Since obviously different processes occur in the above ranges, the ranges 0:1 to 1:44, 1:44 to 1:16, and 1:44 will be discussed separately. The analysis accounted for the fact that incorporation of PM into liposomes at temperatures $T > 30$°C results in the molecules becoming monomers. However, photoactivity of BR remains unchanged (Heyn et al. 1981).

a) Within the interval of BR/DPPC ratios between 0:1 and 1:44, BR molecules in the DPPC bilayer very probably act as "crystal defects" which facilitate the

decay upon energy (heat) supply. At the same time, as suggested by changes of T_c and the results of measurements of $E_\perp$ (Hianik and Vozár 1985), regions of changed lipid structure arise around the BR molecules. BR molecules may be assumed to move in these regions without directly affecting each other. Likely the effects of one BR molecule on another are mediated by the region of the changed lipid structure only; with the increasing BR concentration these regions start overlapping, which probably results in the formation of a new pattern of ordering.

b) At the BR/DPPC ratio 1:44, a stepwise growth of $\Delta H/n_L$ is observed (Fig. 4). Simple calculations based on the known geometry of the BR and the DPPC molecule show that this point approximately corresponds to the situation of one BR monomer being entoured by two rings of lipid molecules. Probably, in this condition the lipid molecules are tightly bound around the central region of the BR molecule, and phase transition requires high energy to occur. At the same time, this ratio can be expected to be the last one allowing free movements of BR molecules in the membrane without establishing direct contacts.

c) The shape of the DSC curve for BR/DPPC ratios exceeding 1:44 is similar to that obtained for smaller ratios. However, within this concentration range the membrane can be expected to be in a state different from that under a). At the BR/DPPC ratio 1:44, BR molecules form clusters with predominating protein content and the clusters - similarly as in the case described under a) - act as "defects" in the regular ordering of the lipid molecules. The arisal of a new situation in the membrane, due to the increasing BR concentrations over the entire range of BR concentrations studied, is also suggested by the dependence of $\Delta T_{1/2}$ on BR concentration (Fig. 3). Parameter $\Delta T_{1/2}$ is associated with the cooperativity of lipid behavior within the phase transition interval. Within the interval described under a), the transition cooperativity is higher than in the case described under c); during the transition between the two intervals, the cooperativity decreases with the increasing BR concentration in the sample. Interestingly, $\Delta T_{1/2}$ does not change within the interval of BR/DPPC ratio exceeding 1:44. Maybe, only lipids in a phase altered by BR are present at these concentrations, and the value of $\Delta T_{1/2}$ for the BR/DPPC ratio 1:44 is that for the pure altered phase.

The results obtained may be explained based on the phase transition theory and the MB model of protein-lipid interactions. The present investigations show that the results reported by Riegler and Möhwald (1986) for lipid-RC protein mixtures are of universal nature and also hold for another integral protein, BR, at least when tested with DPPC membranes. In contrast to previous reports concerning membrane systems with relatively low concentrations of integral proteins, the present investigations based on the study of membranes with high BR contents tried to point to possible interactions between integral proteins mediated by regions of changed lipid structure, which arise in the environment of protein molecules. The nonmonotonic character of changes of physical membrane parameters represents

one dominant characteristic of these systems at high protein concentrations; this is suggested also by experiments with lipid bilayers with incorporated membrane fragments containing hormone receptors (Hianik et al. 1988). The elastic parameters of BLM showed marked nonmonotonic changes with the increasing concentrations of the hormone receptors studied. This could be brought into association with the interactions between integral proteins which are mediated by regions of changed structure occuring in the lipid bilayer.

References

Buckin V. A., Sarvazyan A. P., Passechnik V. I. (1979): Investigation of lipid membranes by ultrasonic method. Biofizika SSSR **24**, 61-66 (in Russian)

Chapman D. (1983): Biomembrane fluidity: The concept and its development. In: Membrane Fluidity in Biology (Ed. R.C. Aloia) , Vol. 2, pp. 5-42, Academic Press, New York

Cortijo M., Alonso A., Gomez-Fernandez J. C., Chapman D. (1982): Intrinsic protein - lipid interactions. Infrared spectroscopic study of gramicidin A, bacteriorhodopsin and Ca^{2+} ATPase in biomembranes and reconstituded systems. J.Mol. Biol. **157**, 597-618

de Gennes P. G. (1974): The Physics of Liquid Crystals. Oxford University Press, London

Evans E., Kwok R. (1982): Mechanical calorimetry of large dimyristoylphosphatidylcholine vesicles in the phase transition region. Biochemistry USA **21**, 4874-4879

Henderson R., Unwin N. (1975): Three dimensional model of purple membrane obtained by electron microscopy. Nature **257**, 28-32

Heyn M. P., Cherry R. S., Dencher N. A.(1981): Lipid-protein interactions in bacteriorhodopsin-dimyristoylphosphatidylcholine vesicles. Biochemistry USA **20**, 840-849

Hianik T., Vozár L. (1985): Mechanical response of bilayer lipid membranes during bacteriorhodopsin conformation changes. Gen.Physiol.Biophys. **4**, 331-336

Hianik T., Kavečanský J., Zórad S., Macho L. (1988): Changes in mechanical properties of the lipid bilayers modified by liver plasma membrane fragments during insulin action. Gen. Physiol. Biophys. **7**, 191-203

Inoko Y., Mitsui T. (1978): Structural parameters of dipalmitoylphosphatidylcholine lamellar phases and bilayer phase transitions. J.Phys.Soc.Jpn. **44**, 1918-1924

Jacobs S., Cuatrecasas P. (1981): Insulin receptor: Structure and function. Endocrine Rev. **2**, 251-263

Janiak M. J., Small D. M., Shipley G. G. (1976): Nature of the thermal pretransition of synthetic phospholipids: dimiristoyl- and dipalmitoyllecithin. Biochemistry USA **15**, 4575-4580

Lewis B. A., Engelman D. M. (1983a): Bacteriorhodopsin remains dispersed in fluid phospholipid bilayers over a wide range of bilayer thickness. J. Mol. Biol. **166**, 203-210

Lewis B. A., Engelman D. M. (1983b): Lipid bilayer thickness varies linearly with acyl chain length in fluid phosphatidylcholine vesicles.J. Mol. Biol. **166**, 211-217

Margolis L. B., Bergelson L. D. (1986): Liposomes and their Interaction with Cells. Nauka, Moscow (in Russian)

Michels B., Fazel N., Cerf R. (1989): Enhanced fluctuations in small phospholipid bilayer vesicles containing cholesterol. Eur. Biophys. J. **17**, 187-190

Mitaku S., Date T. (1982): Anomalies of nanosecond ultrasonic relaxation in the lipid bilayer transition. Biochim. Biophys. Acta **688,** 411-421

Mouritsen O. G., Bloom M. (1984): Mattress model of lipid-protein interactions in membranes. Biophys. J. **46,** 141-153

Privalov P. L. (1980): Scanning microcalorimeters for studying macromolecules. Pure Appl. Chem. **52,** 479-497

Riegler J., Möhwald H. (1986): Elastic interaction of photosynthetic reaction center proteins affecting phase transitions and protein distributions. Biophys. J. **49,** 1111-1118

Sarvazyan A. P. (1982): Development of methods of precise ultrasonic measurements in small volumes of liquids. Ultrasonics 151-154

Sarvazyan A. P., Kharakoz D. P. (1977): Acoustics investigations of the conformational state of proteins in water solutions. In:Molecular and Cell Biophysics (Ed. G.M. Frank), pp. 93-106 , Nauka, Moscow (in Russian)

Shestimirov V. N. (1989): A temperature scanning ultrasound velocimeter. PhD Thesis, Institute of Biological Physics, Pushchino (in Russian)

Small D. (1967): Observations on lecithin. Phase equilibria and structure of dry and hydrated egg lecithin. J.Lipid Res. **8,** 551-557

Sperotto M. M., Mouritsen O. G. (1988): Dependence of lipid membrane phase transition temperature on the mismatch of protein and lipid hydrophobic thickness. Eur. Biophys. J. **16,** 1-10

Stoeckenius W., Lozier R. H., Bogomolni R. A. (1979): Bacteriorhodopsin and the purple membrane of halobacteria. Biochim. Biophys. Acta **505,** 215-278

Vsevolodov N. N. (1988): Biopigments-Photoregisterings. Photomaterial on Bacteriorhodopsin. Nauka, Moscow (in Russian)

Final version accepted March 26,1991