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Phase transition of dimyristoylphosphatidylglycerol bilayers induced by electric field pulses

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The phase transition of dimyristoylphosphatidylglycerol (DMPG) bilayers has been studied by measurements of light scattering under high electric field pulses. Midpoints of phase transitions have been identified by a clear discontinuity of field induced relaxation amplitudes. We show that the phase transition of DMPG suspensions in monovalent salt is virtually independent of the electric field strength up to approx. 35 kV/cm. A shift of the lipid phase by electric field pulses has been observed, however, for DMPG suspensions in the presence of Ca^{2+} ions. DMPG suspensions exhibit a jump of the phase transition temperature from 17°C at Ca/DMPG molar ratios $r < 1/7$ to 32°C at $r > 1/7$. Field pulses of 60 to 100 μs applied to DMPG suspensions with Ca^{2+} at $r > 1/7$ induce discontinuities of relaxation amplitudes in the temperature range 15 to 22°C in addition to the 'standard' one at 32°C, when the electric field strength is above 15 kV/cm. These results indicate that electric field pulses induce a transition from the phase formed at 'high' Ca^{2+}- to the one formed at 'low' Ca^{2+}-ion concentrations. Our results are consistent with a dissociation field effect on Ca^{2+}-lipid complexes which drives the phase transition.

Introduction

The dual function of biological membranes – separation from and communication with the environment – has been studied on various levels [1,2]. Obviously separation can be mainly attributed to the lipid component, whereas communication is usually thought to be under the control of protein components. In general this simple scheme with almost complete disconnection of functions is also believed to hold for bioelectricity. According to this view, lipid bilayers simply serve as electric insulators. However, lipid bilayers have some properties beyond those of simple insulators, which should be very useful for bioelectric communication. Transmission of external electric signals to molecular processes in the cell requires some field induced transition or change of conformation. The requirements for such field induced transitions are well known: large changes of dipole moments and/or changes of the ionization state are necessary [3–8]. It is possible that these conditions are fulfilled by proteins, but lipids may be the essential component as well or at least may contribute.

A most effective transmission of electric signals to molecular processes should be possible by means of lipid phase transitions. The cooperativity of these transitions [9,10] should be extremely useful to induce large changes of 'conformation' by relatively small changes of the electric field strength. In spite of the potential biological importance, the induction of lipid phase transitions by external electric fields did not receive much attention. We have studied the effects of external electric fields on the phase transition of various lipid systems by spectroscopic measurements. Large field induced changes of phase transitions have been found for dimyristoylphosphatidylglycerol in the presence of Ca^{2+}-ions, whereas the phase transition of the same lipid in the presence of monovalent ions remained almost unaffected.

Materials and Methods

The sodium salt of dimyristoylphosphatidylglycerol (DMPG) was obtained from Sigma. DMPG was suspended in a standard buffer containing 3 mM (A) or 5 mM (B) Tris adjusted to pH 8.0 by addition of HCl.

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The samples were shaken vigorously in a Vortex, until the suspensions were homogenous according to visual inspection and then were incubated at 40°C for 10 minutes. After incubation another short period of shaking in the Vortex followed.

The DMPG preparations were analyzed by measurements of the dynamic light scattering using a Polytec laser PL1000K, an Amtec goniometer model SM200 and a Brookhaven correlator BI-8000AT. According to these measurements the average diameter of our DMPG samples was in the range of 50 to 100 nm.

The temperature dependence of the light scattering intensity was recorded in various fluorimeters (Amino SPF500, SLM8000 and a simple self-constructed instrument). In all cases the samples were illuminated at a wavelength $\lambda > 300$ nm and the scattered light was collected at 90°. The temperatures were measured by Pt100 sensors. Phase transitions were also characterized by measurements of the turbidity in a Cary 219 spectrophotometer as a function of the temperature.

The electric field dependence was studied using a pulse generator constructed by Grünhagen [11]. Light scattering of the samples under and after electric field pulses was recorded by an equipment corresponding to that used for fluorescence/light scattering temperature jump experiments [12,13]. The electric field strength and the light scattering intensity as a function of time was transiently stored by a Tektronix 7612D, transmitted to a LSI 11/23 for evaluation of amplitudes by graphic routines and finally transmitted to the facilities of the Gesellschaft für wissenschaftliche Datenverarbeitung mbH, Göttingen, for evaluation of exponential time constants. The samples were subjected to field pulses in cells made from black dynal with optical windows out of quartz suprasil and with Pt-electrodes at a distance of 12.5 mm.

Results

Phase transition temperatures in the presence of $Tris^+$ and Na^+ ions

Electric field jump experiments require samples of relatively low conductivity, in order to avoid large increases of the sample temperature and strong decays of the electric field strength during field pulses. For this reason we have characterized the DMPG phase transition at low salt concentrations. Phase transitions were detected by cooperative changes of the light scattering intensity; transition temperatures T_t were assigned to the midpoint of the change in the scattering intensity. Addition of NaCl to DMPG suspensions in 3 mM Tris-buffer causes first a small decrease of T_t, until a further increase of the Na^+-concentration leads to an increase of T_t (cf. Fig. 1). Our results obtained at high Na^+ concentrations are in agreement with those reported in the literature: we find a T_t value of 23.0 ± 0.3°C at 0.1 M NaCl, which is identical with the corresponding value reported by Cevc and Marsh [14] within the limits of accuracy (cf. also Watts et al. [15]). The minimum of T_t observed at low concentrations seems to be due to the special influence of $Tris^+$ ions. As shown by Wilkinson et al. [16], $Tris^+$ ions lead to chain interdigitation in the closely analogous system of dipalmitoylphosphatidyl suspensions.

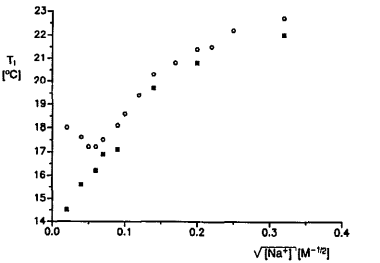

Fig. 1. Phase transition temperature of DMPG suspension T_t as a function of the square root of the Na^+ concentration; T_t values from 'heating' (○) and from 'cooling' (•) curves (580 μM DMPG in 3 mM Tris (pH 8.0), 500 μM EDTA).

In general the T_t values taken from heating curves were higher than those from cooling curves. The difference was maximal at low salt concentration. The difference observed in the absence of added NaCl remained 2.8 ± 0.3 C° even at a heating/cooling rate dT/dt = 0.01 C°/min; the corresponding value at $dT/dt = 0.1$ C°/min was 3.5 ± 0.3 C°.

Jump of the transition temperature upon addition of Ca^{2+}

Addition of $CaCl_2$ to DMPG suspensions did not cause much change of the transition temperature (17 to 18°C; cf. Fig. 2) as long as the Ca^{2+}/DMPG ratio was below $r = 1/7$. However, the change of the light scattering intensity associated with the phase transition decreased upon Ca^{2+} addition and disappeared completely at a ratio $r = 1/7$. When the ratio $r = 1/7$ was exceeded, a different phase transition was observed at a much higher temperature of 32.2 ± 0.3°C, which was associated with an opposite change of the light scattering intensity. Above $r = 1/7$ the light scattering intensity increased, when the phase changed during temperature increase. The transition temperature observed in the range $r > 1/7$ was again almost independent of the Ca^{2+} concentration.

The resulting phase diagram appears to be unique. Apparently a new lipid phase with a different packing pattern is formed at Ca^{2+}/DMPG ratios above 1/7. In some respects our results are analogous to those of

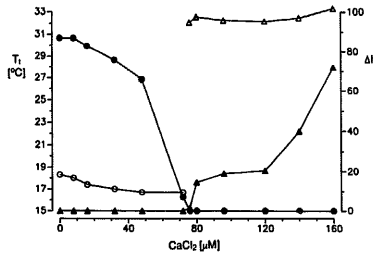

Fig. 2. Phase transition temperature T_t and changes of the light scattering intensity ΔI (in relative units) of DMPG suspensions as a function of the Ca^{2+}-concentration (540 μM DMPG in 3 mM Tris (pH 8.0)); circles represent the 'lower' phase transition, triangles the 'upper' phase transition; open symbols represent the T_t values and filled symbols the ΔI values.

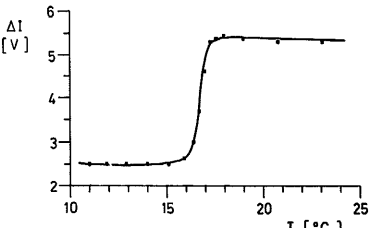

Fig. 3. Light scattering amplitude induced by electric field pulses as a function of the temperature; the temperature at the midpoint of the transition is T_t^E (540 μM DMPG in 3 mM Tris (pH 8.0), 500 μM EDTA; 20.8 kV/cm, 57 μs pulses).

Van Dijck et al. [17]; their measurements performed at higher monovalent salt showed the appearance of a new phase transition at a Ca^{2+}/DMPG ratio ≥ 1.

Effects of electric field pulses: DMPG in the presence of Na^+ ions

Electric field pulses applied to lipid suspensions induce a characteristic relaxation response, which can be easily detected by measurements of the light scattering intensity. In general electric field pulses induce an increase of the light scattering intensity. Apparently there are several contributions to this effect like polarization, deformation and orientation [18–21]; a detailed investigation of these effects for lipid suspensions has not been presented yet. For our present analysis we do not have to know the exact nature of the relaxation effect, but simply use it as an indication for the state of the lipid system. Previous investigations on vesicles formed from dimyristoylphosphatidic acid and from dipalmitoylphosphatidylcholin (Porschke, D., to be published) showed that during phase transitions the field induced relaxation is changed abruptly. We simply use these changes as an indication of phase transitions. For our present purpose we do not have to analyze time constants or individual relaxation amplitudes, but simply use the total light scattering amplitude.

The field-induced light scattering amplitude for DMPG suspensions in buffer A shows a relatively small temperature dependence below and above the phase transition temperature T_t, but changes abruptly at T_t (cf. Fig. 3). The temperature at the midpoint of the amplitude change induced by pulses of a given field strength E is assigned to the phase transition temperature T_t^E at field strength E. Light scattering amplitudes have been scanned through the temperature range of the phase transition with pulses of different field strength.

As expected, T_t^E values measured at low field strengths of e.g. 3 kV/cm are identical with transition temperatures T_t measured in the absence of electric field pulses. However, at higher field strengths the T_t^E values increased above the reference T_t. Because electric field pulses induce some increase of the sample temperature, the T_t^E values have to be corrected for this effect. The correction is based on reference measurements using a fast temperature indication system together with calculation of the temperature increase based on the dissipated electric energy. After correction the T_t^E values still show some increase with the field strength E up to $E \approx 10$ kV/cm and then approach a plateau value (cf. Fig. 4). Due to potential experimental errors together with potential errors in the correction procedure, the accuracy of the T_t^E values is limited. We conclude that the field induced

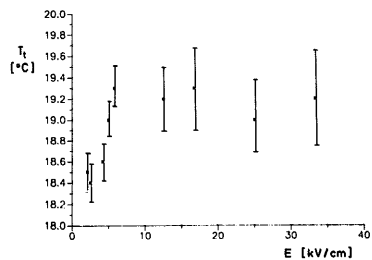

Fig. 4. Phase transition temperature of DMPG suspensions T_t^E as a function of the electric field strength E. The T_t^E values are corrected for the temperature jump effect (cf. text; 3 mM Tris (pH 8.0), 500 μM EDTA).

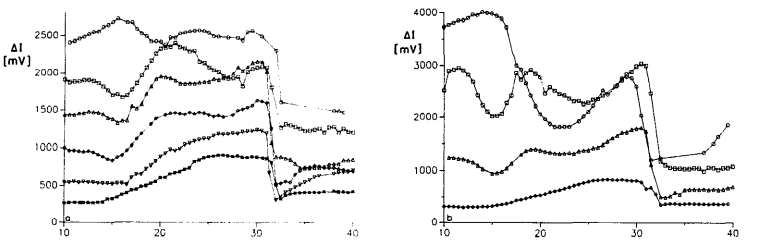

Fig. 5. Light scattering amplitude ΔI of 500 μM DMPG suspensions in the presence of 80 μM Ca^{2+} induced by pulses of different electric field strengths as a function of the temperature (5 mM Tris, pH 8.0): (a) pulse length 63 μs (pulse amplitudes in kV/cm: 7.7 (*), 11.1 (∇), 14.8 ($\diamond$), 18.1 ($\triangle$), 21.8 ($\square$) and 28.8 ($\circ$); (b) pulse length 103 μs (pulse amplitudes in kV/cm: 7.4 ($\diamond$), 12.9 ($\triangle$), 20.8 ($\square$) and 28.3 ($\circ$).

change of the DMPG phase transition temperature in the presence of low concentrations of monovalent ions remains relatively small and is close to the limits of experimental accuracy.

Effects of electric field pulses: DMPG in the presence of Ca^{2+} ions

For an induction of a relatively large change of the lipid phase by electric field pulses, we have selected DMPG suspensions with Ca^{2+} concentrations which are sufficient to suppress the phase transition around 18°C. We used suspensions of 500 μM DMPG in the presence of 80 μM Ca^{2+} and 5 mM Tris, which show a phase transition at 31.2°C in the absence of external electric fields. The relaxation induced by pulses of relatively low electric field strength indeed showed a phase transition at the expected temperature – reflected by a sharp change of the light scattering amplitude (cf. Fig. 5a). When corresponding solutions were analyzed by pulses of high electric field strength, however, the temperature dependence of the amplitudes is much more complex. While the amplitudes increase smoothly with temperature in the range of 10 to 30°C upon application of 'small' field pulses, a clear discontinuity is observed in this temperature range for 'high' field pulses. The discontinuity appears in addition to the 'usual' one at 32°C. Because discontinuous changes of amplitudes are not expected for our system except in the range of phase transitions, we conclude that the electric field pulses induce a partial change of the lipid phase: the 'high-Ca^{2+} phase' is apparently converted to the 'low-Ca^{2+} phase'.

We have studied DMPG-Ca^{2+} suspensions under electric field pulses of different length and found some variation in the phenomenology but nevertheless the same general type of response (Fig. 5b). It is expected that long electric field pulses induce a more extensive shift of the phase equilibrium, which should be reflected by the light scattering amplitudes. However, a more quantitative analysis of these amplitudes requires more information on the nature of the field induced processes in lipid systems.

Discussion

Previous investigations of various field induced reactions have shown that particularly large changes of the equilibrium are induced by electric field pulses for reactions with large changes of the state of ionization [3–8]. Our present observations are consistent with this experience. Lipid particles formed from DMPG are associated with a high charge density, which is partly compensated upon binding of Ca^{2+} ions. Electric field pulses are expected to induce dissociation of Ca^{2+} lipid complexes by a 'Wien' or 'dissociation field' effect [3,4]. Because our lipid-Ca^{2+} system has been studied by light scattering, we could not observe dissociation of Ca^{2+} ions, but only reactions which are coupled to this dissociation. In the suspensions containing 500 μM DMPG and 80 μM Ca^{2+} used in our experiments, binding of Ca^{2+} ions drives the lipid to the 'high Ca^{2+} phase'. When electric field pulses induce dissociation of Ca^{2+} ions, we should expect formation of the 'low Ca^{2+} phase'. Our experiments confirm this expectation. However, we must admit that the light scattering technique used in our investigation does not allow a complete assignment of the lipid phase. Our conclusion on the field induced transition from the 'high' to the 'low-Ca^{2+} phase' is simply based on the discontinuity of amplitudes and the comparison with measurements of light scattering intensities in the absence of electric fields. A more detailed characterization of the lipid

state induced under electric field pulses will be rather difficult, because this state can be maintained only for relatively short times.

If the interpretation of our results obtained for the DMPG-Ca^{2+} system is correct, we should be able to explain the results found for DMPG in the presence of monovalent salt by corresponding arguments. In close analogy to the Ca^{2+}-DMPG system, DMPG particles suspended in a buffer of monovalent ions will attract a large number of positively charged counterions to their surface. Application of electric field pulses will induce partial dissociation of these 'complexes', which is again not detectable by measurements of light scattering. The dissociation process could be detected indirectly, if ion dissociation would lead to a change of the phase transition temperature. However, the 'standard' DMPG phase transition is not very strongly dependent on the degree of monovalent ion binding, as shown by the relatively small increase of the transition temperature with the concentration of monovalent ions. Thus, the results of the electric field experiments are again consistent with expectation.

The light scattering amplitudes ΔI show a rather complex dependence on the temperature and the electric field strength (cf. Fig. 5). It may be suspected that this complexity is due to impurities or due to the presence of stereoisomers [22] in the racemic DMPG mixture used for our experiments. However, our sample proved to be homogeneous according to thin-layer chromatography, and it is quite unlikely that the stereoisomers have a different response to electric field pulses. The phase transitions used for the present investigation are highly cooperative at zero field strength and do not show any indication for the existence of more than a single process contributing to these transitions. Part of the complexity of the field induced transition curves is due to an 'inversion' of the relative amplitudes at pulses of higher field strengths ΔE and/or lengths Δt. This effect is very obvious upon comparison of the $\Delta I = f(T)$ curves for 12.9 and 28.3 kV/cm in Fig. 5b, where the sigmoidal transition curves in the range from 14 to 22°C are in opposite direction. Thus, at certain combinations of ΔE and Δt the field induced phase transition may not be detectable at all from the $\Delta I = f(T)$ curves. This case is found for $\Delta E = 28.8$ kV/cm and $\Delta t = 63$ μs (cf. Fig. 5a), which is just at the transition from the shape observed at 'low' $\Delta E/\Delta t$ values to the opposite shape characteristic of 'high' $\Delta E/\Delta t$ values. The nature of the field induced molecular processes contributing to the change of the light scattering intensities remain to be established.

The interpretation of our results based on the 'Wien' or 'dissociation field' effect is not seriously dependent on the shape of the lipid particles; it is expected to be valid for simple spherical vesicles, multilamellar sheets and other states of aggregation. Nevertheless, it would be useful to know the structure of the DMPG particles in the different lipid phases. Unfortunately the knowledge on the structure of DMPG suspensions is rather limited. As remarked by Epand and Stahl [23] 'the thermotropic behaviour of pure DMPG (in 10 mM sodium phosphate, pH 7) is complex'. According to our results, it is even more complex, when Ca^{2+} ions are added. According to Epand and Hui [24], DMPG forms large multilamellar structures in the presence of high salt, whereas smaller particles of 20–30 nm diameter are formed in the absence of added salt. Epand and Hui [24] suggest that their particles, which have a 'distinct eliptical shape', are not vesicles but half-shells. In addition they observed that the supernate from centrifugation has even smaller particles of 10 mm diameter, which 'may be vesicles'. Probably the latter particles are equivalent to those which result from sonification. We have found that DMPG suspensions in low salt, which have been sonified, do not show any sharp phase transition. Apparently the cooperativity of the phase transition in these particles is reduced due to their high curvature (cf., for example, Ref. 10). For the lipid preparations used in the present investigation we found average diameters in the range of 50 to 100 nm by measurements of the dynamic light scattering. Thus, the diameter of these samples prepared at low salt concentrations (3 to 5 mM Tris) are clearly larger than those obtained in the absence of salt and smaller than the multilamellar structures resulting at high salt concentrations. Obviously the size of the lipid particles is determined by the salt concentration due to electrostatic interactions.

Some features of the DMPG phase diagram characterized in the present investigation are special and should be analyzed in further detail. The initial decrease of the transition temperature T_t upon addition of of Na^+ or Ca^{2+} ions is quite unusual and apparently has not been observed for other comparable lipid systems yet. This effect has been reproducible and seems to be partly due to the presence of Tris ions in the buffer used for the experiments. The transition temperature observed for DMPG at 'high' Ca^{2+} concentrations happens to be rather close to that for one of the metastable states observed by Salonen et al. [22]. A complete phase diagram for DMPG remains to be established. For the purpose of the present investigation knowledge of the complete phase diagram is not required. We have simply selected a set of well reproducible transitions among the various possible ones within the DMPG system and use it as a model which is expected to be characteristic for highly charged phospholipid double layers.

In spite of their potential importance for bioelectricity, lipid phase transitions induced by electric field pulses have hardly been analyzed. Very recently

Antonov et al. [25] measured the phase transition temperature of 1,2-dipalmitoyl-*sn*-glycerol-3-phosphate at different electric field strengths by changes of the conductivity of planar bilayer membranes; they report an increase of the transition temperature with increasing electric field strength. Thus, there is no agreement of our results and those of Antonov et al.; it remains to be established whether the different conclusions are due to differences in the technique, the lipid system and/or other experimental conditions. – Models of phospholipid phase transitions in the presence of electric fields have been developed by several authors [26–28]. However, the 'Wien or dissociation field effect', which appears to be essential for the case of DMPG suspensions, has not been included in these models.

Measurements of the relaxation induced by electric field pulses may not only be used for the identification of phase transitions but also for the characterization of lipid suspensions in general. The observed relaxation times constants are clearly correlated with the size of the particles (cf. Ref. 20 and Porschke, D., unpublished), which is expected for relaxation processes reflecting rotational diffusion. However, field-induced relaxation detected by measurements of light scattering may also be due to field induced deformation [21]. Another process indicated by light scattering is field-induced interaction [29], which can be identified by its concentration dependence. Thus, investigations of lipid suspensions by electric field pulses may provide various useful informations in addition to that described in our present contribution on field-induced phase transitions.

Acknowledgements

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References

1 Gennis, R.B. (1989) Biomembranes – Molecular Structure and Function, Springer, Berlin.
2 Cevc, G. and Marsh, D. (1987) Phospholipid Bilayers – Physical Principals and Models, Wiley, New York.
3 Wien, M. (1931) Phys. Z. 32, 545–547.
4 Onsager, L. (1934) J. Chem. Phys. 2, 599–615.
5 Eigen, M. and DeMaeyer, L. (1963) in Techniques of Organic Chemistry VIII, Part II, Investigation of Rates and Mechanisms of Reactions, pp. 895–1054, Interscience-Wiley, New York.
6 Schwarz, G. and Selig, J. (1968) Biopolymers 6, 1263–1277.
7 Neumann, E. (1981) Top. Bioelectrochem. Bioenerg. 4, 113–160.
8 Porschke, D. (1985) Annu. Rev. Phys. Chem. 36, 159–178.
9 Chapman, D. (1975) Annu. Rev. Biophys. 2, 185–235.
10 Melchior, D.L. and Steim, J.M. (1976) Annu. Rev. Biophys. Bioeng. 5, 205–238.
11 Grünhagen, H.H. (1974) Ph.D. Thesis, Universität Braunschweig.
12 Rigler, R., Rabl, C.R. and Jovin, T.M. (1974) Rev. Sci. Instrum. 45, 580–588.
13 Porschke, D., Meier, H.J. and Ronnenberg, J. (1984) Biophys. Chem. 20, 225–235.
14 Cevc, G., Watts, A. and Marsh, D. (1980) FEBS Lett. 120, 267–270.
15 Watts, A., Harlos, K., Maschke, W. and Marsh, D. (1978) Biochim. Biophys. Acta 510, 63–74.
16 Wilkinson, D.A., Tirell, D.A., Turek, A.B. and McIntosh, T.J. (1987) Biochim. Biophys. Acta 905, 447–453.
17 Van Dijck, P.W.M., Ververgaert, P.H.J.Th., Verkleij, A.J., Van Deenen, L.L.M. and De Gier, J. (1975) Biochim. Biophys. Acta 406, 465–478.
18 Schwan, H.P., Takashima, S., Miyamoto, V.K. and Stoeckenius, W. (1970) Biophys. J. 10, 1102–1119.
19 Tenchov, B.G., Sokerov, S.K.H. and Stoylov, S.P. (1979) Stud. Biophys. 77, 109–110.
20 Ruderman, C., Jennings, B.R. and Lyle, I.G. (1984) Int. J. Biol. Macromol. 6, 99–102.
21 Engelhardt, H. and Sackmann, E. (1988) Biophys. J. 54, 495–508.
22 Salonen, I.S., Eklund, K.K., Virtanen, J.A. and Kinnunen, P.K.J. (1989) Biochim. Biophys. Acta 982, 205–215.
23 Epand, R.M. and Stahl, G.L. (1987) Int. J. Peptide Protein Res. 29, 238–243.
24 Epand, R.M. and Hui, S.W. (1986) FEBS Lett. 209, 257–260.
25 Antonov, V.F., Smirnova, E.Y. and Shevchenko, E.V. (1990) Chem. Phys. Lipids 52, 251–257.
26 Sugar, I.P. (1979) Biochim. Biophys. Acta 556, 72–85.
27 Bhaumik, D., Dutta-Roy, B., Chaki, T.K. and Lahiri, A. (1983) Bull. Mathem. Biol. 45, 91–101.
28 Rabinowitz, J.R. (1984) Int. J. Quant. Chem. Quant. Biol. Symp. 11, 249–256.
29 Porschke, D. (1985) Biochemistry 24, 7981–7986.