ELSEVIER

Biochimica et Biophysica Acta 1280 (1996) 115–119

BBA Biochimica et Biophysica Acta

Influence of the redox state of ubiquinones and plastoquinones on the order of lipid bilayers studied by fluorescence anisotropy of diphenylhexatriene and trimethylammonium diphenylhexatriene

Marek Skowronek, Małgorzata Jemioła-Rzemińska, Jerzy Kruk, Kazimierz Strzałka *

Department of Plant Physiology and Biochemistry, The Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31-120 Kraków, Poland

Received 29 June 1995; revised 26 September 1995; accepted 1 November 1995

Abstract

The measurements of diphenylhexatriene (DPH) and trimethylammonium diphenylhexatriene (TMA-DPH) fluorescence anisotropy in egg yolk lecithin (EYL) and of DPH anisotropy in dipalmitoylphosphatidylcholine (DPPC) liposomes containing different concentrations of oxidized and reduced ubiquinone (UQ) and plastoquinone (PQ) homologues have been performed. All the oxidized UQ homologues strongly induced ordering of EYL membrane structure, whereas in DPPC liposomes, above the phase transition temperature, the most pronounced effect showed UQ-4. PQ-2 and PQ-9 were less effective than the corresponding ubiquinones in this respect. The reduced forms of UQ and PQ homologues increased the order of membrane lipids to a smaller extent than the corresponding quinones both in the interior of the membrane and closer to its surface. Nevertheless, the investigated prenylquinols showed stronger increase in the membrane order than α-tocopherol or α-tocopherol acetate, which could be connected with binding of prenylquinol head groups to phospholipid molecules by hydrogen bonds. The strong ordering influence of ubiquinones on the membrane structure was attributed to methoxyl groups of the UQ quinone rings.

Keywords: Ubiquinone; Plastoquinone; Ubiquinol; Plastoquinol; Fluorescence anisotropy; Diphenylhexatriene

1. Introduction

Ubiquinone-10 (UQ-10, Coenzyme Q) and plastoquinone-9 (PQ-9) are essential components of the respiratory and photosynthetic electron transport chains, respectively, transferring electrons between protein complex and protons across the membranes [1,2]. Ubiquinone homologues with the shorter side chain (e.g., UQ-9) are found in bacteria, fungi, invertebrates and certain plant species [3,4]. UQ-10 and PQ-9 occur in the membranes in a state of equilibrium with their reduced forms (UQH_2-10 and PQH_2-9, respectively). Their function as an efficient electron shuttle and a proton pump requires both high lateral and transverse mobility in the membranes. For understanding the molecular mechanism of prenylquinones action in natural membranes it is necessary to determine their localization, orientation, mobility and influence on the order of surrounding membrane lipid molecules.

Hitherto accumulated results obtained by different physical techniques, like differential scanning calorimetry (DSC) [5–7], NMR [8,9], infrared spectroscopy [10,11], fluorescence quenching and anisotropy measurements [6,12–14], together with the monolayer technique [15,16] indicate that the molecules of short-chain prenylquinones are located relatively close to the membrane surface and probably oriented parallel to membrane lipid acyl chains having their quinone head groups exposed to the membrane polar regions. On the other hand, long-chain UQ-10 and PQ-9 molecules reside mainly in the fluid bilayer interior and are arranged more or less parallel to the membrane surface but they also have the possibility of diffusion towards the membrane/water interface where the redox changes of these molecules take place. Moreover, the proportions of prenylquinones in the bilayer midplane region and at the membrane surface may change upon their concentration [16,17].

* Corresponding author. Fax: +48 12 336907; e-mail: strzalka@mol.uj.edu.pl.

SSDI 0005-2736(95)00264-2

The hydrogen carriers in the electron transfer chains are the reduced, quinol forms of the prenylquinones whose properties in relation to the quinone forms have not been thoroughly examined. The available data indicate a more polar character of the quinols enabling them to occupy regions closer to the membrane surface than the corresponding quinones [8,16,18–20].

Among numerous physical methods like DSC [7,20–22], NMR [8,9], EPR [23], infrared spectroscopy [10,21,24], dilatometry [25] and turbidity measurements [20], fluorescence spectroscopy [13,14,22,25,26] is one of the most widely used methods for the determination of the influence of different lipid soluble molecules on the membrane phase transitions or membrane order and dynamics. Because of its high sensitivity, it requires low sample concentrations and low proportions of fluorescence probes added to the membranes, usually in the order of 0.1 mol% which does not perturb the bilayer structure [25,27].

To determine the anisotropy, a sample containing fluorescent probe is illuminated with monochromatic polarized light and the fluorescence intensities parallel ($I_{\parallel}$) and perpendicular ($I_{\perp}$) to the polarization direction of the excitation light are measured. The anisotropy is determined from the following equation:

$$r = \frac{I_{\parallel} - I_{\perp}}{I_{\parallel} + 2I_{\perp}}$$

The anisotropy value shows the extent of probe motion during its excited lifetime. A high anisotropy value means that the motion is limited by an ordered environment of the probe [27].

Using the fluorescence anisotropy method, we tried to examine the influence of ubiquinones and plastoquinones with different side-chain length, both in the reduced and oxidized state, on the order of liposomal lipid bilayers.

The fluorescent membrane probes used in our study, diphenylhexatriene (DPH) and trimethylammonium diphenylhexatriene (TMA-DPH) which locate in the bilayer interior and near the membrane surface, respectively, are monitoring changes in the order of surrounding lipid molecules [27]. Since DPH moiety is hydrophobic like surrounding lipid matrix, any specific direct molecular interactions between the probes and relatively apolar prenylquinone(ol) molecules should not take place.

2. Materials and methods

Ubiquinone and plastoquinone homologues were a kind gift from Hoffmann-La Roche (Switzerland). They were purified by TLC on silica gel plates (Merck) using chloroform as an eluent. The prenylquinols were obtained by reduction of the corresponding quinones with $NaBH_4$ in methanol. Dipalmitoylphosphatidylcholine (DPPC) and egg yolk lecithin (EYL), type V-E, were purchased from Sigma. The fluorescence probes, DPH and TMA-DPH were from Sigma and Molecular Probes, respectively. Small unilamellar liposomes were prepared by injecting 25 μl of ethanol solution of appropriate compounds into 1.975 μl of water under continuous magnetic stirring. To avoid quinol autooxidation, water and ethanol was bubbled with nitrogen. The final DPPC or EYL concentration was 0.5 mM, phospholipid/DPH ratio of 2000, phospholipid/TMA-DPH ratio of 500, and quinone or quinol content in liposomes varied between 1 mol% and 10 mol%. Liposomes were prepared at room temperature for EYL and at 50°C for DPPC. Anisotropy measurements were performed on Perkin-Elmer LS-50 fluorimeter equipped with a thermostated cuvette holder and stirrer. The anisotropy values were corrected for the instrument response and were the average from several independently prepared samples. The error bars did not exceed the size of data points given in the figures. Quinone and quinol incorporation into liposome membranes was determined by hexane washing method [28] and it was found to be better than 95%.

3. Results

The anisotropy of DPH and TMA-DPH in EYL liposomes at different concentrations of oxidized and reduced short-chain ubiquinones is shown in Fig. 1. The most striking feature of these plots is the high difference in DPH anisotropies for the oxidized and reduced ubiquinones, especially at high $UQ(H_2)$ content. Moreover, the ubiquinols which are supposed to locate closer to the membrane surface, influence the membrane lipid order to a

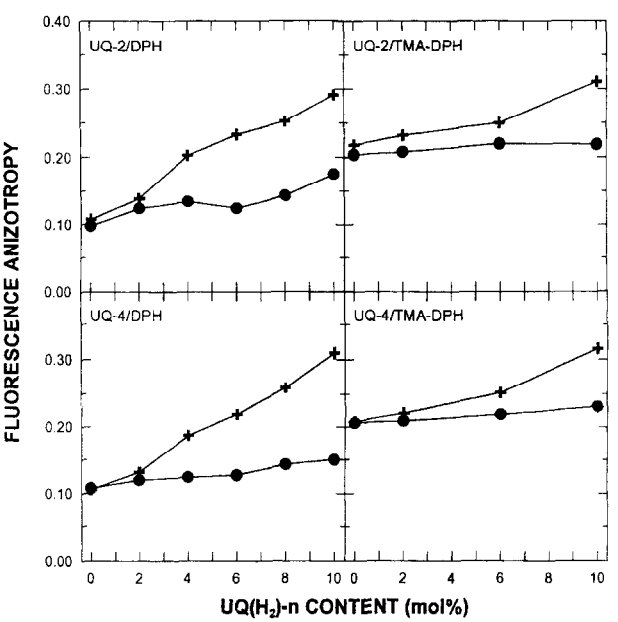

Fig. 1. Fluorescence anisotropy of DPH and TMA-DPH in EYL liposomes containing oxidized (+) or reduced (●) UQ-2 and UQ-4 at different molar percentages (t = 21°C).

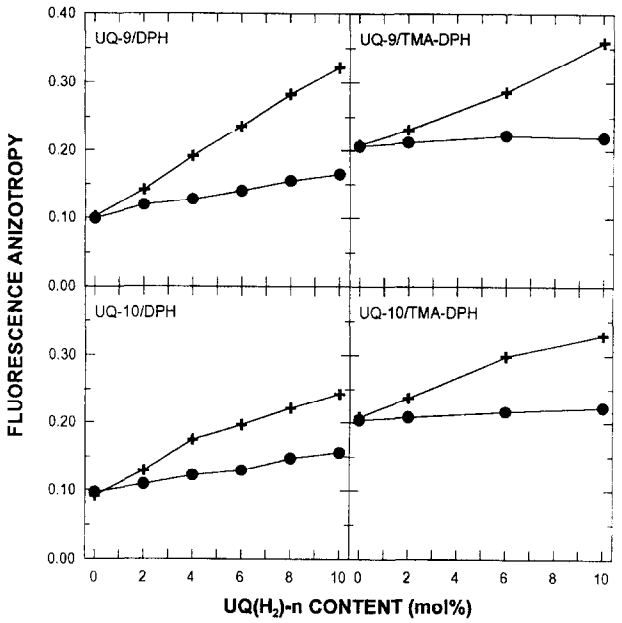

Fig. 2. Fluorescence anisotropy of DPH and TMA-DPH in EYL liposomes containing oxidized (+) or reduced (●) UQ-9 and UQ-10 at different molar percentages ($t = 21°C$).

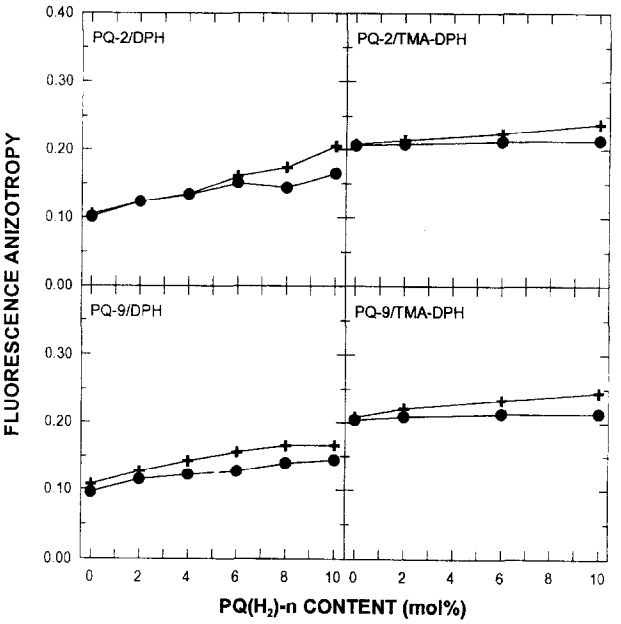

Fig. 3. Fluorescence anisotropy of DPH and TMA-DPH in EYL liposomes containing oxidized (+) or reduced (●) PQ-2 and PQ-9 at different molar percentages ($t = 21°C$).

much smaller extent than the ubiquinones, even close to the lipid/water interface where TMA-DPH resides. Similar dependence is observed for long-chain ubiquinone(ol)s (Fig. 2), although for the UQ-10/UQH_2-10 couple, the difference is lower. Interestingly, the increase in TMA-DPH anisotropy caused by UQ-9 or UQ-10 is even higher than in the case of UQ-2 and UQ-4. The changes in DPH anisotropies induced by different ubiquinols are of similar magnitude, as well as the changes of TMA-DPH anisotropies, although considerably smaller than in the former case. In the case of plastoquinone(ol)s (Fig. 3), the difference between oxidized and reduced forms is small both for DPH and TMA-DPH. The anisotropy increase for plastoquinols is similar to that of ubiquinols, but the influence of plastoquinones on the probes anisotropy is much smaller than in the case of ubiquinones. The tendency of the oxidized forms to increase the membrane order to a higher extent than of the reduced quinones is still retained. The dependence of DPH fluorescence anisotropy on increasing prenylquinone(ol)s concentration in DPPC liposomes, above the phase transition temperature, is shown in Fig. 4. The ordering effect of oxidized ubiquinones is here more dependent on their side-chain length which is strongest for UQ-4 and least pronounced for UQ-10. These differences might be caused by different depth in the membrane at which the ubiquinones localize [17]. The short-chain UQ-2 which is located close to the membrane surface does not penetrate interior regions of the membrane where DPH resides, whereas UQ-4 molecules corresponding with their length to the half of the bilayer thickness are able to reach DPH 'sensitive' zone effectively. Long-chain ubiquinones, especially at

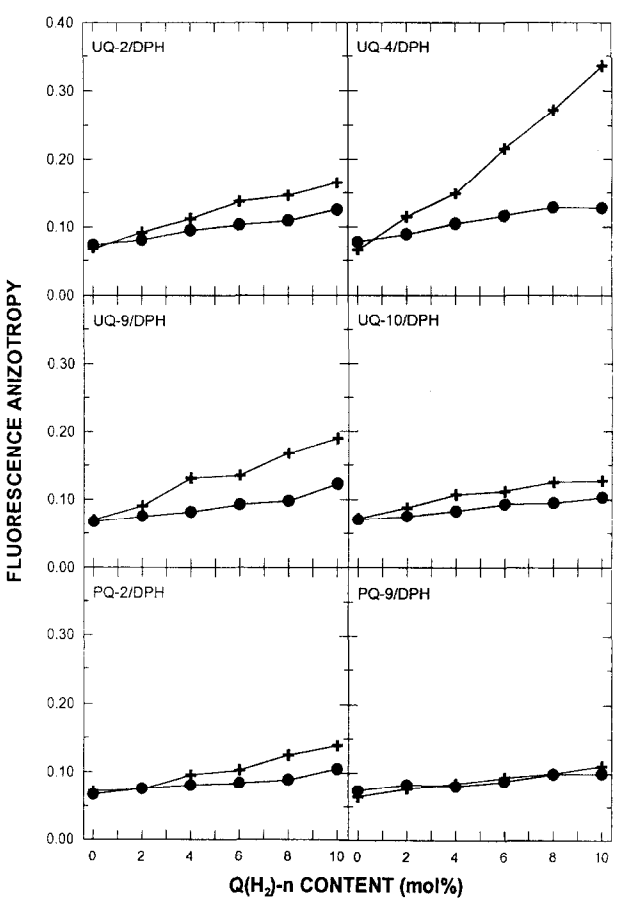

Fig. 4. Fluorescence anisotropy of DPH and TMA-DPH in DPPC liposomes containing oxidized (+) or reduced (●) UQ-2, UQ-4, UQ-9, UQ-10, PQ-2 and PQ-9 at different molar percentages ($t = 50°C$).

higher concentrations would accumulate within the hydrophobic midplane region of the membrane probably 'below' DPH molecules, therefore causing also less perturbation of the membrane lipid arrangement than UQ-4. In the case of EYL liposomes where the anisotropy differences for different UQ homologues are small, probably because of the unsaturated character of the membrane lipids, the transversal diffusion of different UQ-homologues is more effective, leading to similar penetration of the DPH region by different UQ homologues.

Different ubiquinol homologues in DPPC liposomes (Fig. 4) seem to influence the membrane order to similar extent. A smaller effect can be only observed for UQH_2-10. The anisotropy changes caused by $PQ(H_2)$-2 are similar to those induced by the oxidized and reduced UQ-2, whereas both PQ-9 forms show the same, very small ordering effect on DPPC bilayers.

For comparative purposes, the influence of α-tocopherol and α-tocopherol acetate on fluorescence anisotropy of DPH and TMA-DPH in EYL liposomes, in the range of 0–10 mol%, was investigated. The DPH anisotropy changed linearly from 0.090 in pure EYL to 0.130 for α-tocopherol and to 0.100 for α-tocopherol acetate (at 10 mol%), and TMA-DPH anisotropy changed from 0.195 in EYL to 0.220 for α-tocopherol and to 0.200 for α-tocopherol acetate (data not shown).

4. Discussion

The reduced forms of plasto- and ubiquinones which are more polar than the corresponding quinones and localize closer to the membrane surface could be expected to change the membrane order more than the quinones, especially in regions occupied by TMA-DPH. However, it is not the case but rather just the opposite, especially for UQ homologues. There can be a few factors responsible for such an effect. Only slight changes in the anisotropy upon the presence of the quinols might be a result of their poor incorporation into liposomal membranes or aggregation within membranes. Our measurements of incorporation (above 95%) exclude the first possibility. Moreover, we have also found that the DPH and TMA-DPH anisotropy for liposomes with the quinones, after reduction with externally added $NaBH_4$, changed to a value corresponding to that for the quinols obtained for the liposomes with the incorporated quinols. Similar effect was observed in the experiment where the liposomes containing quinols were left to allow their autooxidation to quinone forms. This indicates that both prenylquinone(ol) forms incorporate efficiently into liposomes and can easily exchange their locations in the membranes in accordance with their redox state.

The anisotropy increase of both DPH probes was accompanied by quenching of their fluorescence in our system (data not shown). Quenching of DPH fluorescence by quinone(ol) molecules, accompanied by shortening of its lifetime, could give rise to the increase of DPH apparent anisotropy which is not a result of lipid order change, however, the steady-state anisotropy of DPH in lipid membranes is approximately equal to the limiting anisotropy and is, therefore, a measure of the orientational order in the membrane rather than of the probe dynamics [26,29]. The fluorescence excited state lifetime of DPH is roughly eight times the rotational correlation time [30]. It means that even considerable quenching of DPH fluorescence (shortening of its lifetime) by quinone(ol)s should have no effect on the observed DPH anisotropy and cannot be a source of artifacts. The change in the fluorescence yield of the probes may be caused not only by quinone(ol)–DPH interactions but also partially by the fluorescence intensity change of DPH connected with its anisotropy change in pure lipid bilayers. In the case of DPPC bilayers, DPH fluorescence intensity decreased about 1.5-fold going from 10 to 55°C [25].

Another factor which can influence the observed anisotropies is the possibility of laterally heterogeneous distribution of fluorescent probes between membrane areas of different order induced by quinone(ol) molecules. In the case of DPH, it was found that it shows a tendency to partition equally between lateral domains of different order in a membrane [27]. This has been demonstrated in the case of membranes of different phases [31], different cholesterol content [22], and membranes containing transmembrane proteins [32]. Thus, even if quinone(ol)s induce some heterogeneity in the membrane order, DPH should be evenly distributed between phases of different order and show the average membrane order. In contrast to DPH, TMA-DPH was shown to partition preferentially into the more fluid areas of a membrane [33], however, prenylquinone(ol)s were not found to form any aggregates close to the membrane surface [8] where TMA-DPH resides and therefore they should not also induce significant membrane heterogeneity with TMA-DPH rich and poor regions.

DSC thermograms of liposomes containing UQH_2-10 [20] showed presence of a separate peak between the pretransition and main transition peaks of phospholipids, probably responsible for UQH_2-10 rich phases which undergo cooperative thermal transition. However, this effect should not be attributed to large UQH_2-10 aggregates but rather to UQH_2-10 molecules bound to phospholipids via hydrogen bonds. Interestingly, DSC thermograms of liposomes containing α-tocopherol which was suggested to be hydrogen bound to lipid carbonyl groups, and α-tocopherol acetate which has no hydroxyl group, show the presence of only one peak which corresponds to the shifted main transition peak [21]. This indicates that the physical state of UQH_2-10 in membranes may be different than that of α-tocopherol or α-tocopherol acetate. The tendency of prenylquinols to form hydrogen bonds was shown by infrared spectroscopy [34]. A higher ordering effect of

UQH_2-4 than of α-tocopherol which in turn was higher than for α-tocopherol acetate may indicate that for molecules of aromatic prenyllipids of similar size, the ordering effect increases with the number of hydroxyl groups in their molecules. The considerably higher ordering effect of ubiquinones than of ubiquinols, α-tocopherol and its acetate suggests that the quinone ring of ubiquinones is responsible for this effect. Since in the case of PQ homologues these effects are not as high as for ubiquinones, the presence of methoxyl groups in UQ molecules seems to be most important in this respect. Why these groups are not 'active' in ubiquinols molecules is an open question. The presence of the aromatic ring or the intermolecular hydrogen bonds with the hydroxyl groups might change the interaction of methoxyl groups with the molecular surroundings. It is noteworthy that the changes in calorimetric thermograms were detected at UQ-3/lipid ratios as low as 1:300 [6].

Concluding, even though the reduced forms of ubiquinones and plastoquinones reside closer to the membrane surface, they show much lower ordering effect than the oxidized forms both in the hydrophobic membrane interior as well as close to its surface. These differences are considerably higher for UQ homologues than in the case of plastoquinones. The relatively low perturbation of membrane lipid ordering by prenylquinols might be important for their function, ensuring them high mobility in natural membranes, necessary to function as the mobile hydrogen carriers.

Acknowledgements

This work was supported by Committee for Scientific Research (KBN) grant No. 6 6075 92 03.

References

[1] Gutman, M. (1980) Biochim. Biophys. Acta 594, 53–84.
[2] Rich, P.R. and Moss, D.A. (1987) in The Light Reactions (Barber, J., ed.), pp. 421–445, Elsevier, Amsterdam.
[3] Crane, F.L. (1965) in Biochemistry of Quinones (Morton, R.A., ed.), pp. 183–206, Academic Press, New York.
[4] Lichtenthaler, H.K. (1980) in Biogenesis and Function of Plant Lipids (Mazliak, P., Benveniste, P., Costes, C. and Douce, R., eds.), pp. 299–310, Elsevier/North-Holland, Amsterdam.
[5] Katsikas, H. and Quinn, P.J. (1982) Eur. J. Biochem. 124, 165–169.
[6] Alonso, A., Gomez-Fernandez, J.C., Aranda, F.J., Belda, F.J.F. and Goni, F.M. (1981) FEBS Lett. 132, 19–22.
[7] Katsikas, H. and Quinn, P.J. (1982) Biochim. Biophys. Acta 689, 363–369.
[8] Kingsley, P.B. and Feigenson, G.W. (1981) Biochim. Biophys. Acta 635, 602– 618.
[9] Ulrich, G.L., Girvin, M.E., Cramer, W.A. and Markley, J.L. (1985) Biochemistry 24, 2501–2508.
[10] Aranda, F.J., Villalain, J. and Gomez-Fernandez, J.C. (1986) Biochim. Biophys. Acta 861, 25–32.
[11] Castresana, J., Alonso, A., Arrondo, J.R., Goni, F.M. and Casal, H. (1992) Eur. J. Biochem. 204, 1125–1130.
[12] Fato, R., Battino, M., Degli Esposti, M., Parenti Castelli, G. and Lenaz, G. (1986) Biochemistry 25, 3378–3390.
[13] Katsikas, H. and Quinn, P.J. (1983) Eur. J. Biochem. 131, 607–612.
[14] Spinsi, A., Sartor, G. and Masotti, L. (1981) Membr. Biochem. 4, 149–157.
[15] Quinn, P.J. and Esfahani, M.A. (1980) Biochem. J. 185, 715–722.
[16] Kruk, J., Strzałka, K. and Leblanc, R.M. (1992) Biochim. Biophys. Acta 1112, 19–26.
[17] Jemioła-Rzemińska, M., Kruk, J., Skowronek, M. and Strzałka, K. (1995) Chem. Phys. Lipids, in press.
[18] Rich, P. and Harper, R. (1990) FEBS Lett. 269, 139–144.
[19] Kruk, J. (1988) Biophys. Chem. 32, 51–56.
[20] Aranda, F.J. and Gomez-Fernandez, J.C. (1985) Biochim. Biophys. Acta 820, 19–26.
[21] Villalain, J., Aranda, F.J. and Gomez-Fernandez, J.C. (1986) Eur. J. Biochem. 158, 141–147.
[22] Lentz, B.R., Barrow, D.A. and Hoechli, M. (1980) Biochemistry 19, 1943–1954.
[23] Van Ginkel, G., Korstanje, L.J., Van Langen, H. and Levine, Y.K. (1986) Faraday Discuss. Chem. Soc. 81, 49–61.
[24] Umemura, J., Cameron, D.G. and Mantsch, H.H. (1980) Biochim. Biophys. Acta 602, 32–44.
[25] Borenstain, V. and Barenholz, Y. (1993) Chem. Phys. Lipids 64, 117–127.
[26] Deinum, G., Van Langen, H., Van Ginkel, G. and Levine, Y.K. (1988) Biochemistry 27, 852–860.
[27] Lentz, B.R. (1993) Chem. Phys. Lipids 64, 99–116.
[28] Degli Esposti, M., Bertoli, E., Parenti-Castelli, G., Fato, R., Mascarello, S. and Lenaz, G. (1981) Arch. Biochem. Biophys. 210, 21–32.
[29] Pottel, H., Van der Meer, W. and Herreman, W. (1983) Biochim. Biphys. Acta 730, 181–186.
[30] Jähnig, F. (1979) Proc. Natl. Acad. Sci. USA 76, 6361–6365.
[31] Lentz, B.R., Barenholz, Y. and Thompson, T.E. (1976) Biochemistry 15, 4521–4528.
[32] Moore, B.M., Lentz, B.R. and Meissner, G. (1978) Biochemistry 17, 5248–5255.
[33] Parente, R.A. and Lentz, B.R. (1985) Biochemistry 24, 6178–6185.
[34] Kruk, J, Strzałka, K. and Leblanc, R.M. (1992) Biophys. Chem. 45, 161–169.