ELSEVIER

Biochimica et Biophysica Acta 1194 (1994) 143–148

BBA Biochimica et Biophysica Acta

Effect of β-carotene on structural and dynamic properties of model phosphatidylcholine membranes. II. A ^{31}P-NMR and ^{13}C-NMR study

Iwona Jeżowska [a], Adam Wolak [a], Wiesław I. Gruszecki [b], Kazimierz Strzałka [c,*]

[a] *Institute of Physics, Jagiellonian University, Kraków, Poland*
[b] *Institute of Physics, Maria Curie-Skłodowska University, Lublin, Poland*
[c] *Department of Plant Physiology and Biochemistry, The Jan Zurzycki Institute of Molecular Biology, Jagiellonian University, Al. Mickiewicza 3, 31–120 Kraków, Poland*

Received 3 February 1994

Abstract

Spin label EPR studies (Strzałka and Gruszecki (1994) Biochim. Biophys. Acta 1194, 138–142) revealed that β-carotene affects structural and dynamic properties of model dipalmitoylphosphatidylcholine (DPPC) membranes (multilamellar liposomes) more than polar carotenoid lutein. NMR measurements presented in this paper demonstrate that β-carotene exerts different effect on various groups of the DPPC molecule. It was found that β-carotene: (1) increases motional freedom of lipid headgroups as revealed by means of ^{31}P-NMR; (2) increases motional freedom of alkyl chains forming the hydrophobic core of the membrane greater than that of a choline moiety as revealed by means of ^{13}C-NMR. In all cases the effect of β-carotene with respect to the dynamics of DPPC molecules is found to be more pronounced below the main phase transition temperature than in the membrane's fluid state. The influence of β-carotene on the molecular dynamics of DPPC molecules is discussed in terms of localization and orientation of this pigment within lipid bilayer.

Key words: β-Carotene; Lecithin; Model membrane; Molecular dynamics; NMR, ^{31}P-; NMR, ^{13}C-

1. Introduction

There is increasing evidence that carotenoids besides their generally recognized and estabilished functions as antenna pigments [1–5] and components of photodamage and oxidation defence mechanisms [6–10], play a role as modulators of membrane molecular dynamics [11–20]. Carotenoids broaden phase transition temperature of model lipid membranes and in consequence, they rigidify the membrane in its fluid state, but they make it more fluid in a liquid-crystalline state [15,17]. Thus, they ensure optimal fluidity for many membrane-related processes to occur. As recently demonstrated carotenoid-induced changes in membrane molecular dynamics may modulate the oxygen diffusion-concentration product in such membrane [21]. Therefore, understanding the nature of interactions between carotenoids and membranes is of great significance for the elucidation of various functions, actions and associations of carotenoids [22], including those connected with their anti-disease and health promoting role in such vitally important areas as, e.g., cancer and coronary heart disease prevention (recent review see [23]).

In the accompanying paper [17] we compared the effect on the membrane molecular dynamics of β-carotene, the most aboundant hydrocarbon carotenoid in photosynthetic membranes, with a xanthophyll species – lutein, in a broad range of temperatures. β-Carotene is more effective than lutein in fluidization of DPPC model membranes in their crystalline state. It was also more active in increasing the rotational correlation times τ_B and τ_C and in enhancing the partition of 5-DD spin probe into DPPC membranes in the low temperature range.

Abbreviations: 5-DD, 5-doxyldecane; DPPC, DL-α-dipalmitoylphosphatidylcholine; EPR, electron paramagnetic resonance; NMR, nuclear magnetic resonance.

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

SSDI 0005-2736(94)00136-D

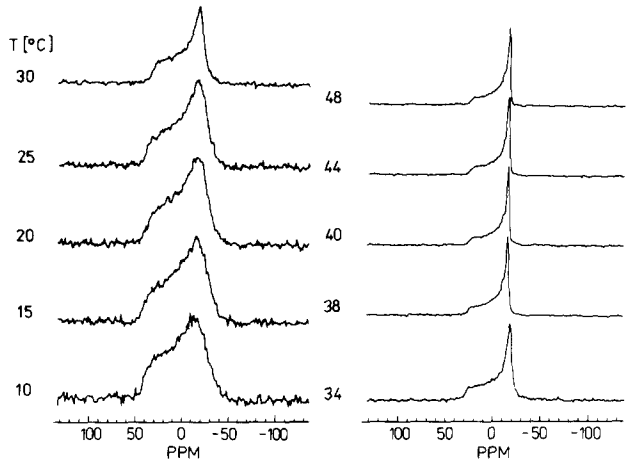

Fig. 1. ^{31}P-NMR spectra of multilamellar vesicles of DPPC at different temperatures, as indicated.

The greater effect of β-carotene than lutein on these measured parameters can be attributed to the lack of oxygen atoms in its ionone rings. Instead of exerting a stabilizing interaction with polar lipid headgroups as in the case of xanthophylls (polar interactions, hydrogen bond formation) repulsive forces dominate and in consequence, β-carotene introduces more disorder in regular membrane lipid structure than polar lutein. To obtain additional information concerning the effect β-carotene exerts on different parts of DPPC molecule, we performed ^{31}P-NMR and ^{13}C-NMR measurements. The advantage of the applied method is that it is non-invasive and does not require an introduction into the membrane of a probe which reports about the molecular dynamics of its environment. Also it gains informations which are difficult to obtain by traditional methods (calorimetry, spin labels). This approach shows that the effect of β-carotene varies with respect to different groups in the DPPC molecule and in addition it depends also on the temperature range studied.

2. Materials and methods

β-Carotene was a gift from Hoffmann-La Roche (Basle, Switzerland). DL-α-Dipalmitoylphosphatidylcholine (DPPC) was purchased from Sigma. Dispersion of multilamellar liposomes of DPPC or DPPC containing β-carotene was prepared as described in the accompanying paper [17]. Double distilled D_2O instead of a buffer was used in preparation of samples for NMR measurements (DPPC/D_2O = 40:60, by weight). ^{31}P-NMR measurements were made with Bruker AM-400 Fourier transform spectrometer operating at a resonance frequency 162 MHz. A phase-cycled Hahn echo sequence [24] with high power proton decoupling was used. The 90° pulse lenght was 37 μs and the pulse spacing was 60 μs. 85% phosphoric acid was used as

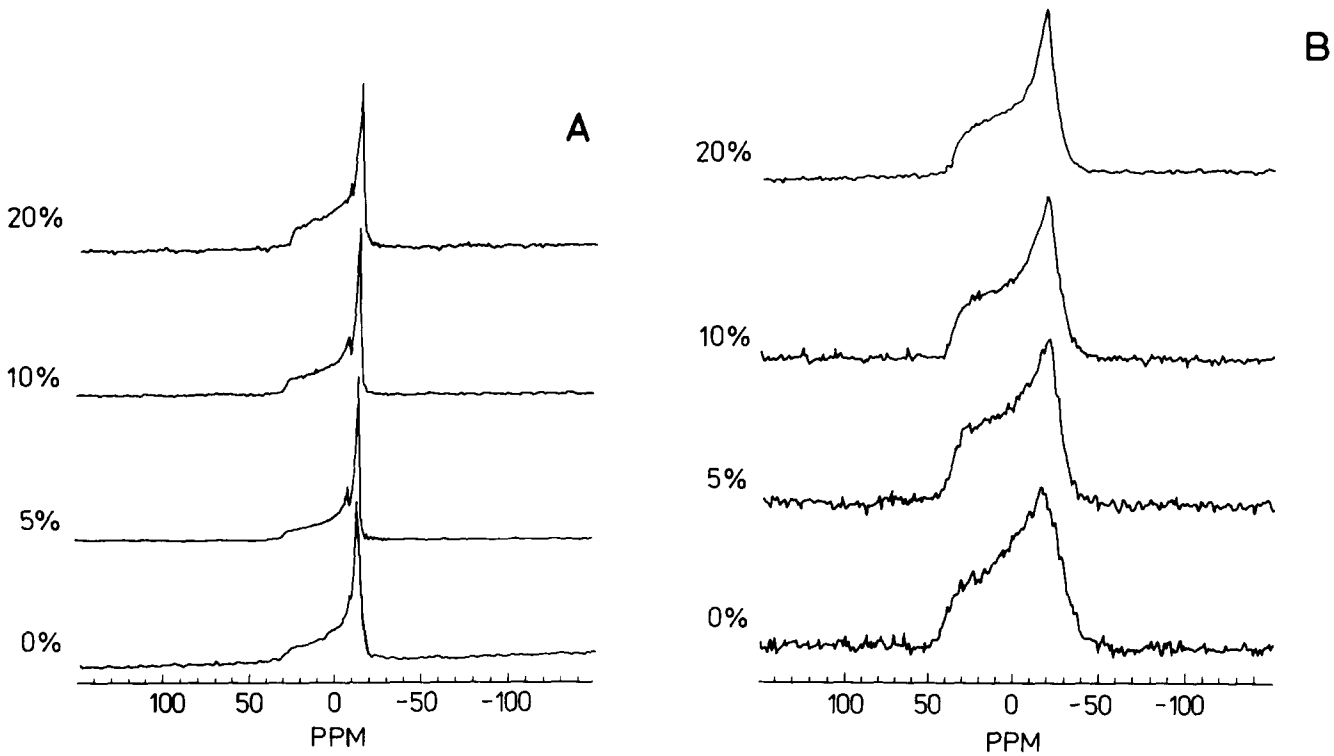

Fig. 2. ^{31}P-NMR spectra recorded at 48°C (A) and at 15°C (B) of multilamellar vesicles of DPPC containing different molecular ratio of β-carotene, as indicated.

the external standard. ^{13}C-NMR spectra were obtained at 100.6 MHz by applying one pulse with the length 4 μs and broad-band proton decoupling (WALTZ-16). The 90° pulse length was 12.5 μs. All presented data are the results of heating experiments. Prior to measurements, the multilamellar vesicle suspension was adapted to a temperature of 0°C.

3. Results

Nuclear magnetic resonance is a powerful experimental technique able to shine a new light on many aspects of lipid bilayer modification. Fig. 1 presents ^{31}P-NMR spectra of DPPC multilamellar vesicles at different temperatures. Temperature increase results in a narrowing of the spectrum. This effect may be interpreted as directly related to an increase of motional freedom of polar headgroups in a bilayer, where a phosphorus atom is located. Similarly to the temperature effect on EPR spectra [17], the most pronounced changes in the shape of NMR spectrum are noticed below the main phase transition temperature, i.e., between 25°C and 38°C.

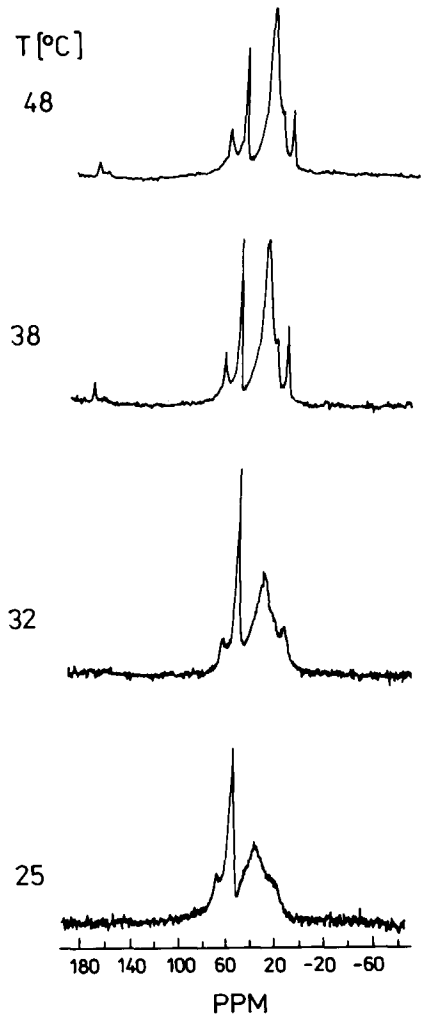

Fig. 3. ^{13}C-NMR spectra of multilamellar vesicles of DPPC at different temperatures, as indicated.

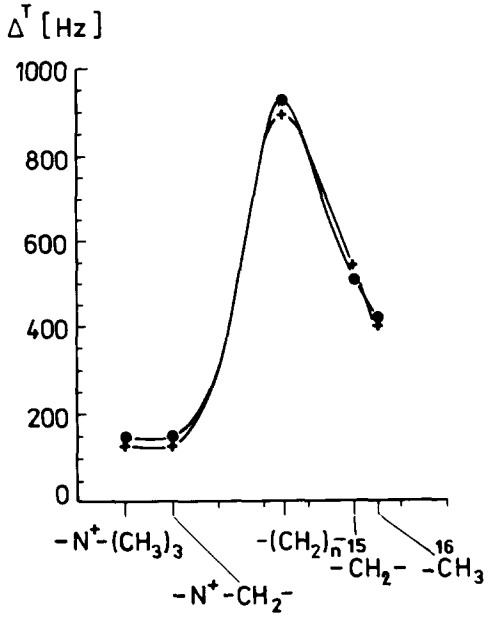

Fig. 4. Values of a parameter Δ^T (see Eq. (1)) calculated for different groups on the basis of ^{13}C-NMR spectra of DPPC vesicles at 38°C (+) and 48°C (•).

Incorporation of β-carotene into DPPC membranes produces an effect similar to a rise of temperature, increasing motional freedom of polar headgroups. This is seen in the ^{31}P-NMR spectra presented in Fig. 2. Again, the dynamic effect of β-carotene is much more pronounced at low temperatures (15°C, Fig. 2B) than at higher temperatures (48°C, Fig. 2A).

The ^{13}C-NMR technique was applied in the present study to examine the dynamic effect of β-carotene on different groups in the DPPC molecule in liposome membranes. Fig. 3 presents ^{13}C-NMR spectra of DPPC multilamellar vesicles at different temperatures. The appearance of new spectral lines accompanying the narrowing of other spectral lines, following a rise of temperature, indicates an increase of motional freedom of acyl chains. The effect of temperature on motion of different groups may be analyzed as follows:

$$\Delta^T = \Delta\nu^{\circ}_{1/2} - \Delta\nu^T_{1/2} \quad (1)$$

where: $\Delta\nu^{\circ}_{1/2}$ and $\Delta\nu^T_{1/2}$ are half-widths of a selected spectral line at 32°C as a reference and another spectral line at a higher temperature, respectively.

Fig. 4 presents values of Δ^T calculated for temperatures of 38°C and 48°C for different groups in the DPPC molecule (for assignment see Fig. 6). One may conclude from the presented results in Fig. 4 that the temperature rise predominantly increases motional freedom of fatty acid CH_2 and terminal CH_3 groups

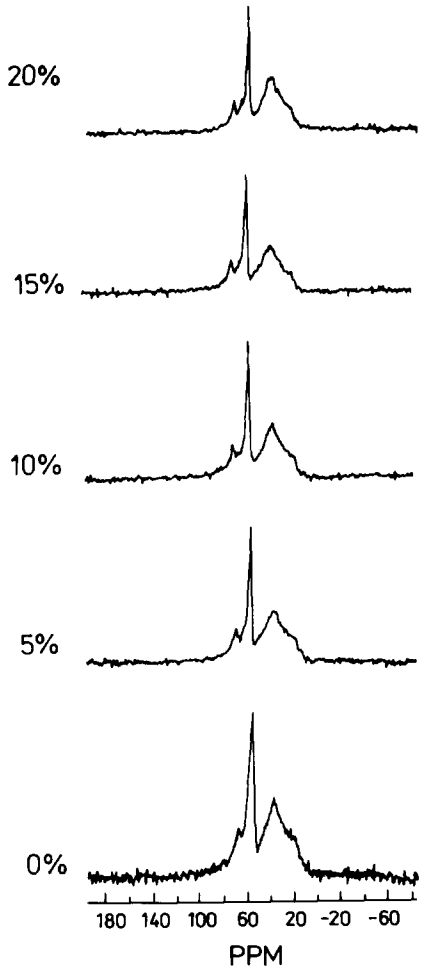

Fig. 5. Typical ^{13}C-NMR spectra recorded at 25°C from the suspensions of DPPC vesicles containing different molecular ratio of β-carotene, as indicated.

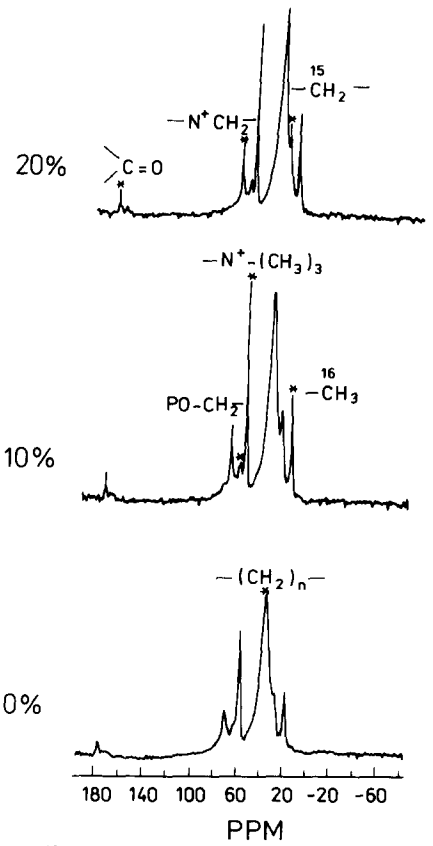

Fig. 6. Typical ^{13}C-NMR spectra recorded at 48°C from the suspensions of DPPC vesicles containing different molecular ratio of β-carotene, as indicated.

forming lipid membrane core and to a lesser degree the choline moiety motional freedom. Another conclusion is that the increase of temperature from 38°C to 48°C (crossing the main phase transition) has not as much effect as temperature increase from 32°C to 38°C.

Effect of β-carotene on ^{13}C-NMR spectra was measured for several temperatures ranging from 25°C to 48°C. Although there are distinct changes in the shape of the 25°C spectrum under the influence of increasing concentration of β-carotene (Fig. 5), it is difficult to ascribe these changes to specific groups of the DPPC molecule because of poor spectral resolution at this temperature. On the other hand at 48°C the spectrum is well resolved but the effect of β-carotene is not so strong (Fig. 6). Therefore, for analysis of concentration effects of β-carotene, spectra measured at 32°C were chosen. At this temperature the effect of β-carotene was pronounced and good resolution of the spectrum, allowed the peaks corresponding to different groups in

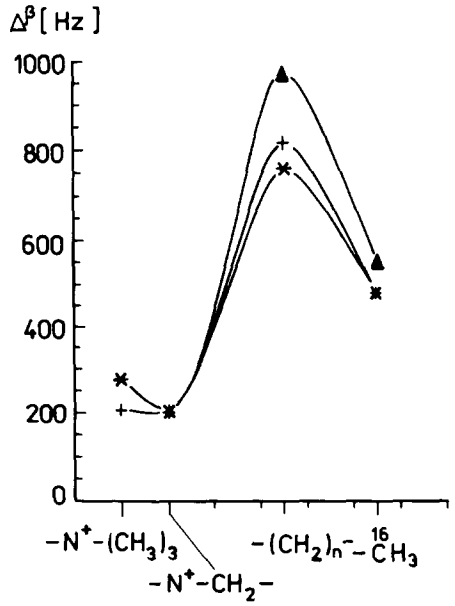

Fig. 7. Values of a parameter Δ^β (see Eq. (2)) calculated for different groups on the basis of ^{13}C-NMR spectra recorded at 32°C from multilamellar suspension of DPPC containing different molecular ratio of β-carotene: *, 10 mol%; +, 15 mol%; △, 20 mol%.

DPPC molecule to be identified. The β-carotene effect at a given temperature is analyzed similarly to that presented above:

$$\Delta^{\beta} = \Delta\nu_{1/2}^{DPPC} - \Delta\nu_{1/2}^{\beta} \quad (2)$$

where: $\Delta\nu_{1/2}^{DPPC}$ and $\Delta\nu_{1/2}^{\beta}$ are the half-width of a given spectral line of the spectrum of DPPC membrane and of DPPC membrane modified with β-carotene, respectively.

Fig. 7 presents calculated values of Δ^{β} at 32°C for different groups at different concentrations of β-carotene. As it is evident from comparison of the graphs presented in Fig. 7 and Fig. 4, the effect of β-carotene incorporation into DPPC membranes is the same as the effect of increased temperature and results in a pronounced increase of motional freedom of acyl chains. It may be also noticed that motional freedom of acyl chains is greater in DPPC membranes modified with larger amounts of β-carotene.

4. Discussion

Physical properties of model DPPC membranes containing β-carotene are the result of both lipid–lipid interaction (as in an one-component system) and a lipid-additive interaction. As presented in the accompanying paper using spin probe technique [17], β-carotene strongly influences structural properties of a membrane by increasing its fluidity in liquid-crystalline state and exerting some rigidifying effects when the membrane becomes fluid. ^{31}P-NMR and ^{13}C-NMR permit detailed insight into the nature of these interactons at the molecular level. The rigid, rod-like β-carotene molecule is long enough (about 30 Å) to span the hydrophobic core of DPPC membrane [25]. By contrast to xanthophylls [26,27], β-carotene has no polar groups at opposite sides of the molecule which could interact with both polar regions of the bilayer and orient the long axis of the pigment perpendicularly to the membrane plane. This is probably the reason why β-carotene orientation in the lipid bilayer is not as well defined as it is in the case of polar carotenoids [26–30].

NMR measurements of β-carotene enriched DPPC membranes indicate an increased motional freedom within the lipid core of the membrane (^{13}C-NMR) and also within the head-group region (^{31}P-NMR). Since β-carotene apolar molecules are not likely to be present in the polar region of the membrane due to repulsive forces between the lipid headgroups and the pigment, such a result indicates a large-scale mismatch in the well ordered lipid bilayer structure, directly related to the presence of this hydrocarbon carotenoid. This may explain the different behaviour of various groups in the DPPC molecule when influenced by β-carotene. The effect of this pigment is more prominent in the hydrophobic core where all CH_2 groups are located (Fig. 7) and is analogous to the effect of temperature increase, when comparing the profiles presented in Figs. 4 and 7. High mobility of fatty acid methylene groups indicates on creation of some free space in the network of well ordered lipid molecules due to a *gauche-trans* isomerisation (temperature effect) or mismatch (β-carotene effect). This is in agreement with the results obtained for the partition coefficient change of 5-DD by the influence of β-carotene [17].

The reported here effect of β-carotene on dynamics of DPPC molecule in membranes aids to understand a possible mechanism of its health-promoting action. Although not definitely proven, the anti-disease activity of carotenoids appears to be directly or indirectly related to their antioxidant properties [8,31–35]. As it was found [36] antioxidant properties of several membrane-occurring antioxidative agents differ in the media of various polarity and their free radical scavenging efficiency also depends on protic or aprotic character of the immediate environment [37]. Biological as well as natural membranes differ in both these respects depending which part, polar head group area or the hydrophobic core, is concerned and there exists a gradient of polarity across these two zones. Thus, localization and orientation of β-carotene as well as other carotenoid species in a membrane should be an important factor affecting their antioxidant activity and efficiency.

Acknowledgements

This study was financially supported by the Committee of Scientific Research of Poland under project Nos. 6 6075 92 03 and 2 2411 91 02. Thanks are due to Dr. W. Dietrich from Ruhr Universität in Bochum for the possibility of the use of a Bruker AM-400 spectrometer.

References

[1] Davidson, E. and Cogdell, R.J. (1981) Biochim. Biophys. Acta 635, 295–303.
[2] Larkum, A.W.D. and Barrett, J. (1983) Adv. Bot. Res. 10, 1–219.
[3] Siefermann-Harms, D. (1985) Biochim. Biophys. Acta 811, 325–355.
[4] Gillbro, T., Cogdell, R.J. and Sundström, V. (1988) FEBS Lett. 235, 169–172.
[5] Koyama Y. (1991) J. Photochem. Photobiol. B. Biol. 9, 265–280.
[6] Demmig, B., Winter, K., Krüger, A. and Czygan, F.-C. (1987) Plant Physiol. 84, 218–224.
[7] Krinsky, N.I. (1989) Free Radical Biol. Med. 7, 617–635.
[8] Terao, J. (1989) Lipids 24, 659–661.

[9] Bilger, W. and Björkman, O. (1990) Photosynth. Res. 25, 173–185.
[10] Conn, P.F., Schalch, W. and Truscott, T.G. (1991) J. Photochem. Photobiol. B. Biol. 11, 41–47.
[11] Huang, L. and Haug, A. (1974) Biochim. Biophys. Acta 352, 361–370.
[12] Rohmer, M., Bouvier, P. and Ourisson, G. (1979) Proc. Natl. Acad. Sci. USA 76, 847–851.
[13] Rottem, S. and Markowitz, O. (1979) J. Bacteriol. 140, 944–948.
[14] Milon, A., Lazrak, T., Albrecht, A.-M., Wolff, G., Weill, G., Ourisson, G. and Nakatani, Y. (1986) Biochim. Biophys. Acta 859, 1–9.
[15] Subczyński, W.K., Markowska, E., Gruszecki, W.I. and Sielewiesiuk, J. (1992) Biochim. Biophys. Acta 1105, 97–108.
[16] Gruszecki, W.I. and Strzałka, K. (1991) Biochim. Biophys. Acta 1060, 310–314.
[17] Strzałka, K. and Gruszecki W.I. (1994) Biochim. Biophys. Acta 1194, 138–142.
[18] Milon, A., Wolff, G., Ourisson, G. and Nakatani, Y. (1986) Helv. Chim. Acta 69, 12–24.
[19] Chaturvedi, V.K. and Kurup, C.K.R. (1986) Biochim. Biophys. Acta 860, 286–292.
[20] Lazrak, T., Milon, A., Wolff, G., Albrecht, A.-M., Mieh, M., Ourisson, G. and Nakatani, Y. (1987) Biochim. Biophys. Acta 903, 132–141.
[21] Subczyński, W.K., Markowska, E. and Sielewiesiuk, J. (1991) Biochim. Biophys. Acta 1068, 68–72.
[22] Olson, J.A. (1989) J. Nutr. 119, 94–95.
[23] Krinsky, N.J. (1993) Annu. Rev. Nutr. 13, 561–587.
[24] Rance, H. and Byrd, R.A. (1983) J. Magn. Reson. 52, 221–240.
[25] Subczyński, W.K., Markowska, E. and Sielewiesiuk, J. (1993) Biochim. Biophys. Acta 1150, 173–181.
[26] Gruszecki, W.I. and Sielewiesiuk J. (1990) Biochim. Biophys. Acta 1023, 405–412.
[27] Gruszecki, W.I. and Sielewiesiuk, J. (1991) Biochim. Biophys. Acta 1069, 21–26.
[28] Yamamoto, H.Y. and Bangham, A.D. (1978) Biochim. Biophys. Acta 507, 119–127.
[29] Johansson, L.B.-A., Lindblom, G., Wieslander, A. and Arvidson, G. (1981) FEBS Lett. 128, 97–99.
[30] Van de Ven, M., Kattenberg, M., Van Ginkel, G. and Levine, Y.K. (1984) Biophys. J. 45, 1203–1210.
[31] Garewal, H.S. (1992) Ann. N.Y. Acad. Sci. 669, 260–269.
[32] Canfield, L.M., Forage, J.W. and Valenzuela, J.G. (1992) Proc. Soc. Exp. Biol. Med. 200, 260–265.
[33] Di Mascio, P., Murphy, M.E. and Sies, H. (1991) Am. Chem. Clin. Nutr. 53, 194S–200S.
[34] Olson, J.A. (1993) J. Nutr. Sci. Vitaminol. 39, S57–S65.
[35] Ames, B.N., Shigenaga M.K. and Hagen, T.M. (1993) Proc. Natl. Acad. Sci. USA 90, 7915–7922.
[36] Mukai, K., Morimoto, H., Kikuchi, S. and Nagaoka, S. (1993) Biochim. Biophys. Acta 1157, 313–317.
[37] Fragata, M. and Bellemare, F. (1985). J. Colloid Int. Sci. 107, 553–559.