Biochimica et Biophysica Acta, 363 (1974) 127–133

BBA 76729

THE pH-DEPENDENCE OF ESR SPECTRA FROM NITROXIDE PROBES IN LECITHIN DISPERSIONS

MARTIN D. BARRATT and PETER LAGGNER*

Biosciences Division, Unilever Research Laboratory Colworth/Welwyn, The Frythe, Welwyn, Herts. (U.K.)

(Received March 27th, 1974)

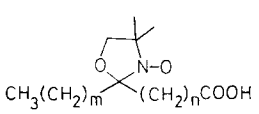
SUMMARY

1. The ESR spectra of spin labelled fatty acids dissolved in dispersions of lecithin and lecithin–cholesterol mixtures show a marked pH dependence. The motion of the spin probes becomes more anisotropic as the pH is increased from 4 to 7. The increase in anisotropy is attributed to a change in location of the spin probes on ionisation of their carboxylic acid groups.

2. It is proposed that the spin probes are anchored in the lecithin head-group regions by hydrogen-bonding to the phosphate group at low pH (below pH 4) and by ion-pairing with the choline group at high pH (above pH 7). The distance between these two locations, estimated from the change in the ESR order parameter is about 6 Å, suggesting that the choline head-group is extended perpendicular to the plane of the bilayer.

INTRODUCTION

Since their introduction by Waggoner et al. [1], the spin-labelled fatty acids have found a wide range of uses in the investigation of lipid, lipoprotein and membrane structure (see reviews by McConnell and McFarland, Jost et al. and Smith [2–4]). In this paper we describe a hitherto unreported pH-dependence of the ESR spectra of the spin-labelled fatty acids (I and II) in lecithin and lecithin–cholesterol mixtures.



I, (m=12, n=3)
II, (m=5, n=10)

* Permanent address: Institut für Röntgenfeinstrukturforschung, Forschungszentrum, Graz, Austria.

MATERIALS AND METHODS

Pure egg lecithin was purchased from Lipid Products, South Nutfield U.K. and cholesterol was from B.D.H. Ltd, Poole.

Spin label I was purchased from Syva Inc., Palo Alto, Calif. Spin label II and its methyl ester were synthesised in this laboratory using previously reported methods [1].

Samples were prepared by dissolving the lipids and spin label in chloroform followed by evaporation of the solvent under reduced pressure and dispersion of the resultant mixture in buffer. Constant ionic strength buffers were prepared using sodium acetate/acetic acid, sodium phosphate and Tris–HCl.

ESR spectra were run at ambient temperature on a Varian E-4 instrument using an aqueous sample cell.

THEORY

When fatty acid spin labels such as I and II are dissolved in phospholipid dispersions, they undergo rapid anisotropic rotation about an axis perpendicular to the surface of the lipid bilayer [5]. If we ascribe a coordinate system to the nitroxide radical such that the N–O bond is along the x-axis and the $2p\pi$ orbital is along the z-axis, then the mean square deviation of the z-axis during rotation about the axis perpendicular to the bilayer surface is $\langle\cos^2\theta\rangle$ where θ is the angle between the two axes. The degree of order of the z-axis with respect to the bilayer is given by

$$S_3 = \tfrac{1}{2}(3\langle\cos^2\theta\rangle - 1)$$

(using the notation of Seelig [6]).

The order parameter S_3 may be determined experimentally from the expression

$$S_3 = \frac{T_{//} - T_{\perp}}{T_{zz} - T_{xx}}$$

where T_{zz} and T_{xx} are the values of the hyperfine coupling tensor in the z- and x-directions (assuming axial symmetry) and $T_{//}$ and $T_{\perp}$ are measured as indicated in

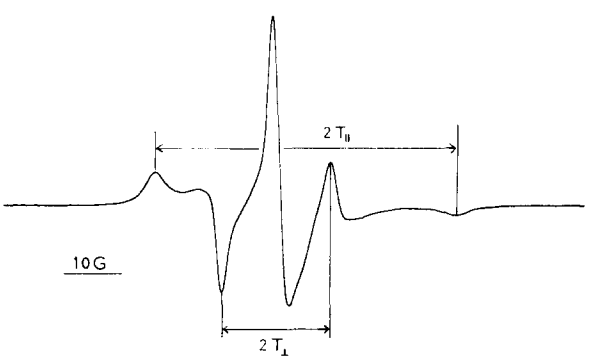


Fig. 1. ESR spectrum of label I (10^{-4} M) in a 2% egg lecithin dispersion, pH 7.5 (ionic strength 0.05).

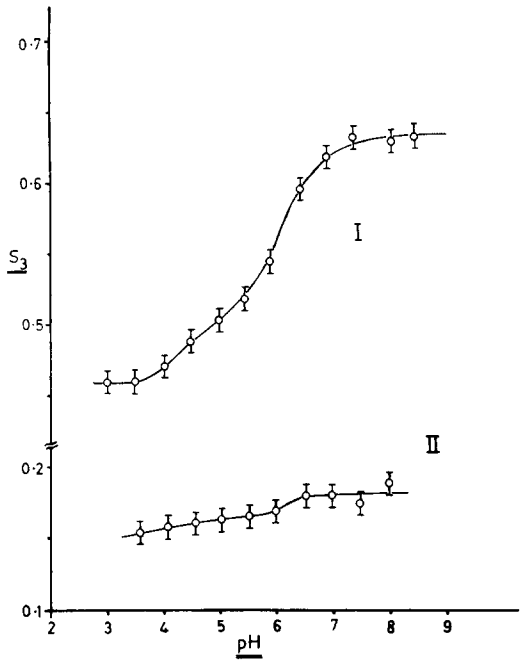


Fig. 2. pH-dependence of S_3 for labels I and II (10^{-4} M) in 2 % egg lecithin (ionic strength 0.05).

Fig. 1. $T_{zz}-T_{xx} = 26.3$ G was used for all calculations of S_3 in this paper. (A detailed derivation of S_3 is to be found in ref. 6). The isotropic hyperfine coupling constant A_N was calculated from the equation

$A_N = \frac{1}{3}(T_{//}+2T_{\perp})$.

RESULTS AND DISCUSSION

The pH-dependences of the order parameter S_3 for the labels I and II in lecithin dispersions are shown in Fig. 2. The experimental values for $T_{//}$, $T_{\perp}$, S_3 and A_N for label I in a 2 % egg lecithin dispersion are listed in Table I. In all cases the observed values of S_3 show a marked increase on raising the pH from 3.5 to 7.5. The methyl ester of label II in lecithin gave S_3 values of 0.150 ± 0.005 which were independent of pH in the range between pH 3.5 and 8.5.

The absence of any significant effect of pH on the methyl ester of label II in lecithin dispersions suggests that there is no intrinsic change in order in the lecithin bilayer with pH. This is in agreement with the results of Hsia and Boggs [7] using an uncharged cholesterol spin probe in lecithin cholesterol mixtures. This finding, together with the approximately sigmoidal shapes of the S_3 versus pH curves observed with labels I and II, strongly suggests that the changes in S_3 are correlated with the ionisation of the carboxylic acid groups of the spin labels. The pH-range, where the changes are observed, is well above the pK values for aliphatic fatty acids (pK = 5) in the bulk phase. Since, as generally accepted, the carboxylic acid groups of labels I

TABLE I

THE EXPERIMENTAL VALUES FOR $T_{//}$, $T_{\perp}$, S_3 AND A_N FOR LABEL I IN 2% EGG LECITHIN DISPERSION

pH	$T_{//}$ (Gauss)	$T_{\perp}$ (Gauss)	S_3	A_N (Gauss)
4.04	22.8	10.3	0.47	14.5
4.50	23.1	10.2	0.49	14.5
5.00	23.4	10.2	0.50	14.6
5.46	23.4	9.8	0.52	14.4
5.92	24.1	9.8	0.55	14.6
6.48	25.2	9.5	0.60	14.7
6.92	25.7	9.4	0.62	14.8
7.40	25.9	9.3	0.63	14.8
8.10	25.9	9.4	0.63	14.9
8.50	25.9	9.3	0.63	14.8

and II are located in the region of the phospholipid polar head-groups, this difference may be explained by a local environment, which is different from the bulk phase in terms of ionic strength, pH, and effective dielectric constant.

Seelig [6] and Hubbell and McConnell [8] have carried out systematic studies on the ESR spectra of lecithin dispersions containing fatty acid spin probes with the nitroxide group attached to different chain carbon atoms. They established the existence of a flexibility gradient along the hydrocarbon chains of the labels, using the relationship between log S_3 and the position of the nitroxide group along the chains.

Fig. 3 shows a plot of log S_3 versus nitroxide group location for a number of spin-labelled fatty acids. Data from Seelig and Hasselbach [9] are combined with those from this work; $S_3 = 0.10$ for 2-ethyl-2-(14-carboxytetradecyl)-4,4-dimethyl-oxazolidine-1-oxyl ($m = 1$, $n = 14$) in 5% egg lecithin dispersion (pH 7.4) (Barratt,

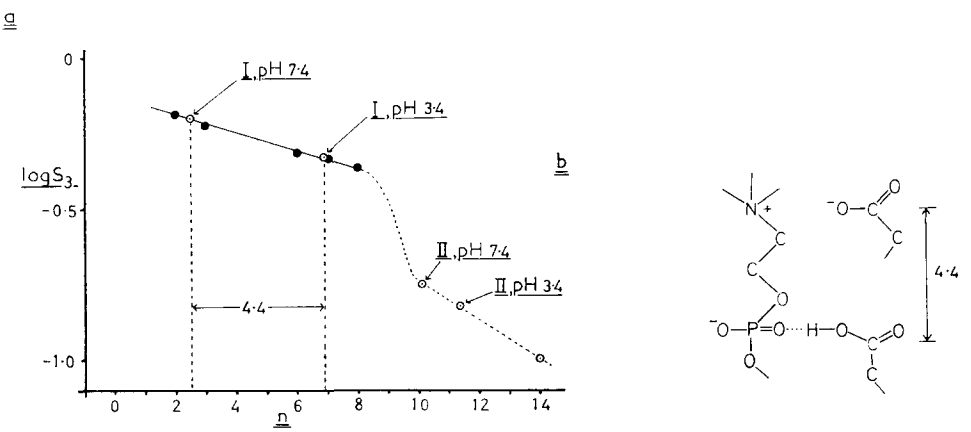


Fig. 3. (a) Graph of log S_3 vs n for spin-labelled fatty acids (●–●, data of Seelig and Hasselbach [9]), and (b) a diagrammatic representation of the two locations of label I with respect to the lecithin head-group. (The distances indicated represent C–C bond lengths).

M. D. and Laggner, P., unpublished). As Fig. 3 demonstrates, our observed order parameter of label I in lecithin dispersions at pH 7.5 is in reasonable agreement with the value found by Seelig and Hasselbach [9] at pH 8.5. At low pH however, label I shows an order parameter which corresponds to that of a nitroxide group attached to carbon 9 at pH 8.5. Furthermore label I displays increased isotropic hyperfine coupling constants (A_N) as the pH is increased (Table I). This shows directly an increasingly polar environment around the nitroxide group on pH increase [10].

These observations may be explained in terms of a change in the position of the probe due to a combination of hydrophobic bonding, hydrogen bonding and electrostatic attraction. At low pH, where the carboxylic acid group of the label is not ionised, hydrogen bonds may be formed between the carboxylic acid group and either the phosphate groups or the ester carbonyls of the phospholipid molecules. The phosphate group by its higher electronegativity seems to be the more likely of the two. On ionisation, the negatively-charged carboxylate ion is repelled by the phosphate group and is attracted by the positively-charged choline nitrogen. This causes a change in the probe position which corresponds to a distance of 4 to 5 chain carbon atoms perpendicular to the plane of the bilayer surface. From this we conclude that in the neighbourhood of the spin label the negative and positive charges of the lecithin polar head-groups are oriented approximately perpendicular to the bilayer surface. This result is in good agreement with the finding of Phillips et al. [11] who extrapolated the X-ray long spacings of a homologous series of lecithins to zero chain length.

The pH dependence of S_3 observed for label II corresponds to a movement of a little over one carbon atom. Whilst the errors involved in estimating the movement of this label are larger than those for label I, it must be recognised that this change is significantly smaller than that observed for label I. The nitroxide group of label II is however separated from its carboxyl group by 10 carbon atoms compared with only three for label I. Because of the elasticity of the chain, any changes due to the ionisation of the carboxyl group might be expected to have only minor effects on the mobility around carbon 12. For the same reason, flexibility gradient data from reporter groups located far from their points of anchorage, are probably subject to quite large errors.

The order parameter of label I in dispersions of bovine brain phosphatidylserine also showed a strong pH dependence. This was more complex than that observed for lecithin, probably due to the combination of effects produced by the ionisation of both the spin label and the phosphatidylserine itself [12].

The effect of adding cholesterol to lecithin containing labels I and II at pH 3.5 and 7.5 is shown in Fig. 4. The S_3 value for methyl ester of label II in lecithin-cholesterol mixture (1 : 1, molar ratio) was independent of pH. The increase in order due to the incorporation of cholesterol into lecithin dispersions is well documented [13–15] and in this respect our results agree with those reported previously. There is however one further point arising out of the data shown in Fig. 4. The addition of cholesterol to lecithin decreases the pH-dependence of label I whilst enhancing it for label II. Evidently when label I moves towards the head-group region of the bilayer as a result of dissociation the nitroxide group gets further away from the ordering influence of the cholesterol molecules. Conversely when label II is moved closer to the head-group region, its motion becomes more strongly inhibited by the closer

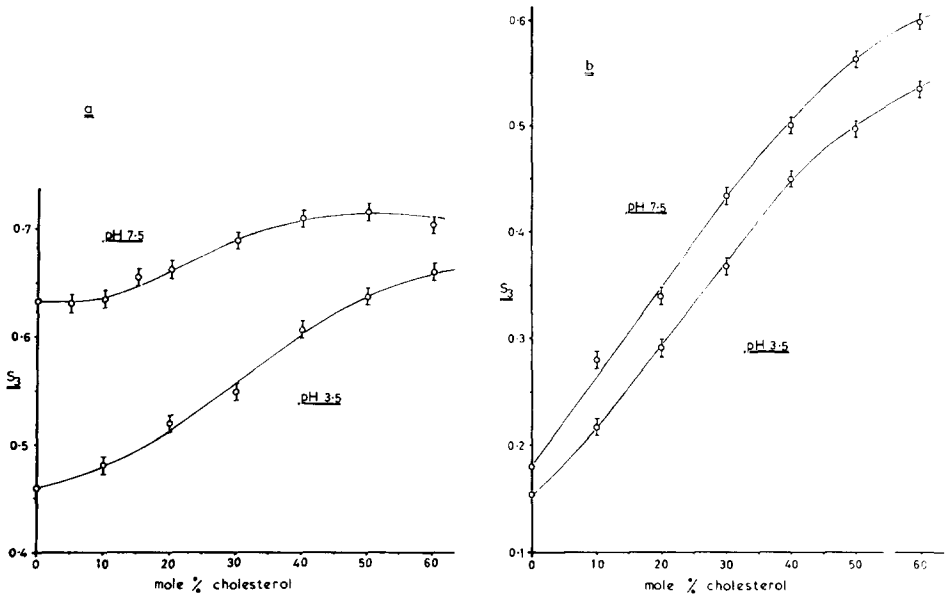


Fig. 4. (a) Variation of S_3 for label I (10^{-4} M) vs cholesterol content of 2% lecithin–cholesterol mixtures at pH 3.5 and 7.5 and (b) variation of S_3 for label II (10^{-4} M) vs cholesterol content of 2% lecithin–cholesterol mixtures at pH 3.5 and 7.5.

neighbourhood of cholesterol. This behaviour agrees well with the observations of Darke et al. [16], who showed that the ordering effect of cholesterol on lecithin is at its greatest between approximately carbons 4 and 8 of the lecithin hydrocarbon chains.

It is also interesting to note that these experiments do not provide any clear-cut evidence for the "clustering" of lecithin–cholesterol complexes [16]. This suggests that the spin labels are distributed throughout the lecithin–cholesterol system and are thus reporting an average environment.

The results of our experiments show clearly that the parameters derived from ESR spectra of ionisable spin-probes in lecithin dispersions are strongly dependent on the pH value. Results obtained from "aqueous" dispersions, as frequently reported, have to be interpreted with care, since different phospholipids, depending on their chemical nature, generate different pH values when dispersed in an unbuffered medium.

In conclusion, the pH-dependence of the order parameters derived from spin-labelled fatty acids in phospholipid systems offers new possibilities. The versatility of the method is increased, i.e. one label may be used in different locations in the bilayer simply by varying the pH of the system. It also leads to a novel way of measuring the distances between the anchoring points for the label in the head-group region of the bilayer.

ACKNOWLEDGEMENTS

The authors are grateful to Dr H. Hauser and Dr M. C. Phillips for helpful discussions. The technical assistance by R. Whitehurst is gratefully acknowledged. One of us (P.L.) performed this work under a Long Term Fellowship of the European Molecular Biology Organisation.

REFERENCES

1 Waggoner, A. S., Kingzett, T. J., Rottschaeffer, S., Griffith, O. H. and Keith, A. D. (1969) Chem. Phys. Lipids 3, 245–253
2 McConnell, H. M. and McFarland, B. G. (1970) Q. Rev. Biophys. 3, 91–136
3 Jost, P., Waggoner, A. S. and Griffith, O. H. (1971) in Structure and Functions of Biological Membranes (Rothfield, L. I., ed.), pp. 83–144, Academic Press, New York
4 Smith, I. C. P. (1972) in Biological Applications of Electron Spin Resonance (Swartz, H. M., Bolton, J. R. and Borg, D. C., eds), pp. 483–539, Wiley-Interscience, New York
5 Hubbell, W. L. and McConnell, H. M. (1969) Proc. Natl. Acad. Sci. U.S. 64, 20–27
6 Seelig, J. (1970) J. Am. Chem. Soc. 92, 3881–3887
7 Hsia, J. C. and Boggs, J. M. (1972) Biochim. Biophys. Acta 266, 18–25
8 Hubbell, W. L. and McConnell, H. M. (1971) J. Am. Chem. Soc. 93, 314–326
9 Seelig, J. and Hasselbach, W. (1971) Eur. J. Biochem. 21, 17–21
10 Dodd, G. H., Barratt, M. D. and Rayner, L. (1970) FEBS Lett. 8, 286–288
11 Phillips, M. C., Finer, E. G. and Hauser, H. (1972) Biochim. Biophys. Acta 290, 397–402
12 Schreier-Muccillo, S., Butler, K. W. and Smith, I. C. P. (1973) Arch. Biochem. Biophys. 159, 297–311
13 Chapman, D. and Penkett, S. A. (1966) Nature 211, 1304–1305
14 Hsia, J. C., Schneider, H. and Smith, I. C. P. (1970) Chem. Phys. Lipids 4, 238–242
15 Phillips, M. C. (1972) in Progress in Surface and Membrane Science (Danielli, J. F., Rosenberg, M. D. and Cadenhead, D. A., eds), Vol. 5, pp. 139–221, Academic Press, New York
16 Darke, A., Finer, E. G., Flook, A. G. and Phillips, M. C. (1972) J. Mol. Biol. 63, 265–279