Biochimica et Biophysica Acta, 465 (1977) 400—407
© Elsevier/North-Holland Biomedical Press

BBA 77627

ON CELL MEMBRANE LIPID FLUIDITY AND PLANT LECTIN AGGLUTINABILITY

A SPIN LABEL STUDY OF MOUSE ASCITES TUMOR CELLS

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(Received July 19th, 1976)

Summary

The fluidity of the plasma membrane of Sarcoma 180 mouse ascites tumor cells has been studied in viable cells using fatty acid spin labels. The order parameter was found to vary from 0.61, approximately four carbon bond lengths removed from the membrane surface, to 0.47 approximately eleven bond lengths removed at 22°C and from 0.55 to 0.33 at 37°C. Thus these cells show similar membrane fluidity to that found in other mammalian cells with the exception of human erythrocytes which are less fluid. The concanavalin A mediated agglutinability of Sarcoma 180 cells was altered by the addition of cytochalasin B and the fluidity was found to be the same as in unaltered cells.

Introduction

The "fluidity" of cell membranes has become a focal point of discussion in recent years because membrane components are known to move laterally rather rapidly and this motion is thought to serve a biological function (see e.g. refs. 1—9). Cell membranes have fruitfully been viewed, for some purposes, as two dimensional fluids in which cell components diffuse [2]. The "fluidity" of this fluid, then provides a vaguely defined variable in which to understand cellular phenomena such as sugar transport [7], clustering viewed in electron microscopy studies [3], patch and cap formation [5], appearance of particles [6], action of cholera toxin [8] etc. In some very simple membranes, this two dimensional fluid view gives rather satisfying correlations between physiological observations and "direct" physical measurements. An example of this is the correlation between sugar uptake by bacteria, the fatty acid composition of the bacteria membrane and the electron spin resonance (ESR) measurements of

lateral phase separation of the lipids [7]. In most cases there is as yet no direct physcial verification of a difference in fluidity which is postulated to lead to a physiological difference. One outstanding example of this is differential cell agglutinability by plant lectins of normal and transformed cells [10—12]. That this is a problem that merits experimental attention is emphasized by the fact that agglutinability differences observed in different cell types are frequently discussed almost interchangeably with the term "difference in mobility of lectin receptors". When the membrane is pictured as a two dimensional fluid, this latter term suggests, as a working hypothesis, turning to differences in membrane fluidity to explain differences in agglutinability [13]. The present work addresses this hypothesis.

Gaffney has recently studied the electron spin resonance of nitroxide labeled fatty acids incorporated into normal and several varieties of transformed mouse 3T3 fibroblasts addressing herself to the same question [9]. She showed that the differences in glycoprotein mobility which are thought to exist in these normal and transformed cells are not accompanied by measurable differences in the lipid chain flexibility as measured with the spin label method. A large body of work [14—29] shows that the method itself, in spite of often discussed shortcomings, is rather sensitive to changes in organization and dynamics of the environments in which the spin label resides. Thus Gaffney's work [9] implies that normal and transformed cells do not differ in "fluidity" if this term is taken to mean lipid flexibility. Caution is, of course, indicated since there is so little data. We decide to carry out a similar experiment in a different cell system and using a different method of inducing a difference in agglutinability to test the generality of Gaffney's result. Also, there is a scarcity of spin label data in intact mammalian cells and these data might very well lead to some useful generalizations about the lipid fluidity in mammalian cells.

We have chosen the Sarcoma 180 mouse ascites tumor cell for study. The concanavalin A mediated agglutinability of these cells has recently been shown [30] to be dramatically enhanced after addition of a low concentration of cytochalasin B. This cell system presents a number of advantages. First, the measurements are carried out on two cell populations that have the same history, the only difference being the presence of the drug. Second, electron microscopy, scanning electron microscopy, and fluorescence microscopy results are available on these cells under identical conditions as those used here so the cell surfaces in both treated and untreated cells are rather well characterized [30]. Finally, these cells have the advantage that, unlike cultured cells, they do not have to be removed from a growing surface.

The results are similar to those found in the case of 3T3 fibroblasts: there is no measurable correlation between lipid fluidity and agglutinability. We discuss implications of this negative result and compare the lipid fluidity of Sarcoma 180 cells and other mammalian cells that have been reported in the literature.

Methods

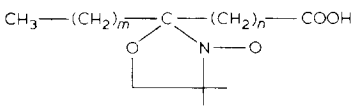
Tumor cell line

Ascites Sarcoma 180 cells were obtained from Dr. Melvin Cohen of the Salk Institute, Mr. Samuel M. Poiley of the National Institute of Health and Dr.

Geoge Tarnowsky of Sloan Kettering (1963, SKI Line). The tumor was maintained by intraperitoneal passage in male Swiss Webster mice as previously described [31]. Mice were sacrificed by cervical dislocation and the peritoneal contents were transferred to Hepes-buffered (20 mM) Hanks' balanced salts solution (Grand Island) (pH 7.4, 37°C). The cells (10^6/ml) were washed several times in the Hanks' solution and then were incubated with and without 50 μg/ml Cytochalasin B (Aldrich) with 1% Me_2SO in Hanks' solution pH 7.4, 37°C, for 30 min. Some control experiments showed that none of the results presented here or previously [30] are affected by the presence of the 1% Me_2SO.

Spin labeled cells

The fatty acid spin labels,



Spin label I (*m*, *n*)

where (*m*,*n*) = (5,10) and (12,3) and were purchased from Syva Assoc., Palo Alto, Calif. and were used as received. A 0.1 M stock solution in 100% ethanol was prepared of each label and was stored in a refrigerator when not in use. The spin labels were incorporated into the cells by the method of Kaplan et al. [32] using 100 μl of stock solution in 100 ml of Hanks' solution containing 2 ml of cells. In most experiments, the cells were incubated with the spin labels 30 min at 37°C during slow rotation, washed three times, sedimented at 250 × *g* and sealed into capillary tubes for the ESR measurements. In a few experiments the cells were incubated with the labels for only 5 min allowing the resonance measurements to be made within 10 min of the application of the labels. The cells treated with cytochalasin B were kept in contact with this drug at all stages of the experiments.

The cell viability was virtually unaffected by the spin labeling procedure. Trypan blue dye exclusion tests carried out after the measurements were completed showed that the viability was 90—95%.

Electron spin resonance measurements

Electron spin resonance derivative spectra of the labels in the cells were measured at 22°C and 38°C in Varian X-band spectrometer using a nitrogen flow temperature control system. The temperature was monitored with a thermocouple placed near the sample in the nitrogen flow tube. Hyperfine parameters were measured by calibrating the field with di-*tert*-butyl nitroxide in water (10^{-4} M) as a standard (a_N = 17.2 G assumed) [33]. In some measurements, the spectra were taken within 10—15 min after application of the spin label. These measurements showed that although the signal level diminished with time, the order parameter (see below) as was independent of time. Thus, most of the data presented here were taken between 30 min and 3 h after the label was added. Care was taken to reduce the microwave power and modulation ampli-

tude sufficiently so as not to influence the results [9]. In some samples, measured soon after addition of the label, line broadening was observed due to interactions between labels. As the signal diminished, the lines narrowed giving spectra identical to those observed in other samples to which less label had been added and which had been measured immediately. Only spectra not displaying broadening effects were included in the data presented here.

Results

Most of the measurements reported here were made using 50 μl/ml of cytochalasin B but one measurement was carried out using 10 μl/ml and one using 20 μl/ml. The addition of this drug to S180 cells increases the Concanavalin A agglutinability. For example, the agglutinability of these cells with 10 μg/ml Concanavalin increases by 60% after 20 min when treated with 20 μl/ml of cytochalasin B [30]. Also, according to electron microscopy studies [30], the surface topography of S180 changes from one characterized by relatively even distribution of surface villi to one showing large ruffles. Fluorescence microscopy shows [30] that in cells treated with cytochalasin B, the receptor sites localize on the ruffles where as in untreated cells, the receptors are dispersed.

ESR spectra of I(12,3) and I(5,10) incorporated into S180 cells are shown in Fig. 1. The spectra are similar to those due to nitroxide spin labels inserted into lipid bilayers [9,15–18,34]. The interpretation of these types of rather sharp spectra is now well documented [17]. The data are extracted from the line

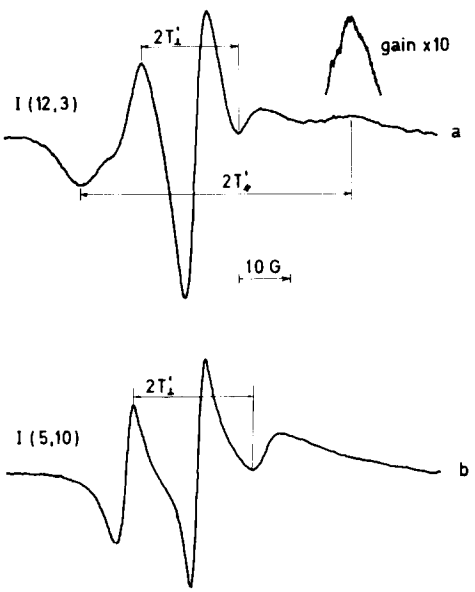


Fig. 1. EPR first derivative, X-band spectra of I(12,3) and I(15,10) incorporated into Sarcoma-180 mouse tumor cells, defines $T'_{\perp}$ and $T'_{/\!/}$ in the case of I(12,3) and defines $T'_{\perp}$ in the case of I(5,10). Spectrum (a) was taken at 22°C and spectrum (b) at 38°C. The horizontal arrow is 10 G long and points toward increasing field.

positions in the ESR spectra. The method of measuring $T'_{\perp}$ and $T'_{/\!/}$ in the case of I(12,3) and $T'_{\perp}$ in the case of I(5,10) is indicated in Fig. 1. These quentities are approximately related to the parallel and perpendicular components of an assumed motionally-averaged, axially-symmetric hyperfine coupling tensor [16]. These quantities in turn lead to an order parameter [9]

$$S = \frac{T'_{\parallel} - T'_{\perp} - C}{T'_{\parallel} + 2T'_{\perp} + 2C} \times 1.723 \tag{1}$$

where the correction factor $C = 1.4\ \text{G} - 0.053\ (T'_{/\!/} - T'_{\perp})$.

The deduction of an order parameter in the case of I(5,10) is less reliable because the outer features of $T'_{/\!/}$ are nor resolved (Fig. 1b). Nevertheless, one may estimate an order parameter by fixing $T'_{/\!/} + 2T'_{\perp}$ and C. Following Gaffney [9] we set $T'_{/\!/} + 2T'_{\perp} = 44.5$ G and $C = 0.8$ G. Thus, for I(5,10)

$$S = \frac{43.7\ \text{G} - 3T'_{\perp}}{46.1\ \text{G}} \times 1.723 \tag{2}$$

We note that the numberical value of S depends on the choice of molecular fixed parameters and the method of calculating C but that relative values of S, which we seek, are valid independent of the choice.

The reader is referred to the literature for details [16,19], but briefly, the order parameter is a measure of the average angular deviation of the nitroxide labeled portion of the fatty acid chain from the average orientation of the fatty acid. The order parameter then is related to the fluidity of the lipid bilayer portion of the membrane in which it resides. A numerically accurate value for the "fluidity" of the bilayer is difficult to measure for a number of reasons, two of which we mention. First, the perturbation of the nitroxide group, may appreciably affect the results [35]. Second, slow motion affects are similar to order-

TABLE I

ESR SPECTRAL PARAMETERS OF FATTY ACID SPIN LABELS IN CELLS

Hyperfine coupling parameters given in Gauss. Order parameter calculated from Eqn. 1. The errors quoted are standard deviations in the number of measurements given in the parentheses.

Cell	Measurement temperature	Label	$2T'_{\parallel}$	$2T'_{\perp}$	Order parameter
Sarcoma 180	22	I(12,3)	52.3 ± 1.2	19.0 ± 0.6	0.61 ± 0.1 (7)
Sarcoma 180 + Cytochalasin B	22	I(12,3)	53.7 ± 0.7	19.0 ± 0.8	0.62 ± 0.02 (3)
Mouse L-cells [a]	23	I(12,3)	54.7 ± 0.6	17.7 ± 0.3	0.68
Human Lymphocyte [a]	23	I(12,3)	53.7 ± 0.9	18.1 ± 0.2	0.65
Human Erythrocyte [a]	23	I(12,3)	59.8 ± 0.5	17.3 ± 0.4	0.76
3T3 Mouse Fibroblasts [c]	25	I(10,3)			0.64
Sarcoma 180	38	I(12,3)	49.8 ± 1.7	19.5 ± 0.7	0.55 ± 0.02 (3)
Sarcoma + 180 Cytochalasin B	38	I(12,3)	49.7 ± 0.6	19.6 ± 0.5	0.54 ± 0.01 (3)
Erythrocyte [d]	37	I(10,3)			0.65
Chick Embryo Fibroblasts [c]	37	I(10,3)			0.58
3T3 Mouse Fibroblasts [c]	37	I(10,3)			0.59

[a] Data taken from reference 16. The calculated order parameters are different than those given in reference 16 because Eqn. 1 is slightly different than that used in reference 16.

[b] Reference 9.

TABLE II

ESR SPECTRAL PARAMETERS OF I(5,10) IN CELLS

Hyperfine coupling parameters given in Gauss. Order parameter computed from Eqn. 2.

Cell	Measurement temperature	Order parameter
Sarcoma 180	22	0.47
Sarcoma 180	38	0.33
Erythrocytes [a]	37	0.45
Chick Embryo fibroblasts [a]	37	0.21

[a] Reference 9.

ing effects in spectra derived from samples that are not macroscopically oriented (such as cells) [36]. Nevertheless, relative values of S should be valid indicators of fluidity differences. The lower the value of S, the higher is the fluidity.

The results are given in Tables I and II. The fluidity is greater in all cases at $T = 38°C$ than at 22°C. A whole body of evidence shows that spin labels of the type I(m,n) are incorporated into lipid bilayers such that the polar head group residues near the water-lipid interface and the nitroxide bearing fatty acid chain extends into the hydrophobic region of the bilayer [9,14—29]. It has been invariably found that these labels report a higher fluidity toward the center of the bilayer than near the polar head group and that is what the data of Tables I and II indicate.

Tables I and II also include data taken from the literature which pertains to spin label measurements of fluidity in mammalian cells to date.

Discussion

It was mentioned in Results that it is generally accepted that fatty acid spin labels reside in the lipid bilayer portion of the cell membrane. It is not obvious that the cellular location of these labels is the plasma membrane although the experiments of Kaplan et al. [32] show that a relatively large portion of these labels are, in fact, found in the surface membrane of other mammalian cells. Gaffney [9] has further argued that (a) the fact that rapid biodegradation of the labels take place and (b) the fact that the order parameters measured within minutes after label introduction are the same as those measured later, suggest that a substantial portion of the label is in the plasma membrane. We also ran a few experiments in which $K_3Fe(CN)_6$ was added to cell samples in which the EPR signal had declined and observed a revival of an EPR signal which was identical to the original signal. Kaplan et al. [32] have proposed this as a method for preferentially observing spin labels in the surface membrane. We also have found no evidence for spin labels located in more than one type of environment including a few experiments in which the temperature was lowered to −20°C. In short, even though cellular location of the spin labels is difficult to prove, there is a body of evidence that supports the hypothesis that either a substantial portion of the label resides in the plasma membrane or the EPR spectra are very similar due to fatty acid spin labels in the various membranes of the cell. In the present work, in which we base our discussion on a lack of

differences in order parameters due to differences in agglutinability we only require that a fraction of the label be in the surface membrane.

The lack of correlation between agglutinability and lipid fluidity has now been demonstrated in two cell types with rather different means of altering the former variable. We do not consider this as a trivial result because the mechanism of differences in lectin agglutinability in some cells has often been suggested to involve differences in mobility of lectin-binding sites. Nicolson [10] outlines three types of mechanisms which could produce such mobility difference: (a) intrinsic lipid bilayer fluidity differences, (b) structural modification of the lectin binding determinant and (c) differences in the interaction of the receptor with some type of peripheral membrane structure. These results and those of Gaffney [9] argue against (a) in the case of 3T3 and Sarcoma 180 cells. Put another way, the satisfying correlation between the rotational (local fluidity) and the translational motion (mobility) of rhodopsin in the disc membrane of retinal rods [37,38] which naturally would follow if the membrane is viewed simply as a two dimensional fluid may not exist between the mobility and fluidity of receptor sites. Certainly the view of the membrane as a two dimensional liquid has been applied to some problems with reasonable predictions [2,7] but in view of the evidence, the agglutinability of a cell does not seem to fit this simple picture. In fact, measurements of "microviscosity" using fluorescence polarization of fluorescent hydrocarbons introduced into normal and transformed 3T3 cells [39] seem to imply an inverse correlation i.e. the higher the agglutinability the higher the microviscosity. The problem with these latter measurements seems to be that one can not be sure what viscosity one is measuring and if the probe is not in fact reporting an average of several environments. In this sense, spin label measurements are probably more reliable at the moment in spite of several shortcomings in the method.

We now turn to a short discussion of the membrane fluidity of the few mammalian cells that have been reported. The striking feature of the results of Table I is that with the exception of erythrocytes which seem to be less fluid, there is remarkable similarity in the various cells near the polar head group. We note that in this comparison, we have grouped the data extracted from the fatty acid I(10,3) with that derived from I(12.3). In fact, the order parameter would not be expected to be very different in these two labels [40]. We also note that the numerical values in Table I are different than originally reported [30,32] because different authors have used different assumptions to calculate S. We have recalculated the order parameter in all cases from the original data using Eqn. 1.

Table II shows that a larger difference in fluidity exists in interior of the lipid bilayer from cell to cell although these data should be viewed cautionsly because of the lack of resolved $T'_{//}$ features in the ESR spectra. It may prove necessary to treat these spectra with more rigorous methods [41].

Obviously, much more work must be done on the agglutinability-fluidity question. Cell agglutinability is a very complicated phenomenon which more than likely involves both microtubules and microfilaments [42].

Acknowledgement

We wish to thank Mr. Jorge Serra for preparing the figure.

References

1 Frye, L.D. and Edidin, M. (1970) J. Cell. Sci. 7, 319—333
2 Shimshick, E.J. and McConnell, H.M. (1973) Biochemistry 12, 2351—2360
3 Nicolson, G.L. (1972) Nat. New Biol. 239, 193—196
4 Taylor, R.B., Duffus, W.P.H., Raff, M.C. and de Petris, S. (1971) Nat. New Biol. 233, 225—229
5 Edidin, M. and Weiss, A. (1972) Proc. Natl. Acad. Sci. U.S. 69, 2456—2459
6 James, R. and Branton, D. (1973) Biochim. Biophys. Acta 323, 378—390
7 Linden, C.D., Wright, K.L., McConnell, H.M. and Fox, C.F. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2271—2275
8 Bennett, V., O'Keefe, E. and Cuatrecasas, P. (1975) Proc. Natl. Acad. Sci. U.S. 72, 33—37
9 Gaffney, B.J. (1975) Proc. Natl. Acad. Sci. U.S. 72, 664—668
10 Nicolson, G.L. (1974) Int. Rev. Cytol. 39, 89—100
11 Roblin, R., Chou, L. and Black, P.H. (1975) Adv. Cancer Res. 22, 203—260
12 Nicolson, G.L. (1976) Biochem. Biophys. Acta 458, 1—72
13 Horwitz, A.F., Hatten, M.E. and Burger, M.M. (1974) Proc. Natl. Acad. Sci. U.S. 71, 3115—3119
14 Caron, F., Mateu, L., Rigny, P. and Azerad, R. (1974) J. Mol. Biol. 85, 279—300
15 Libertini, L.J., Waggoner, A.S., Jost, P.C. and Griffith, O.H. (1969) Proc. Natl. Acad. Sci. U.S. 64, 13—19
16 Hubbell, W.L. and McConnell, H.M. (1969) Proc. Natl. Acad. Sci. U.S. 69, 16—22
17 Hubbell, W.L. and McConnell, H.M. (1971) J. Am. Chem. Soc. 93, 314—326
18 Hubbell, W.L. and McConnell, H.M. (1969) Proc. Natl. Acad. Sci. U.S. 64, 20—27
19 Gaffney, B.J. and McConnell, H.M. (1974) J. Magn. Res. 16, 1—28
20 Seelig, J. (1970) J. Amer. Chem. Soc. 92, 3881—3887
21 Jost, P., Libertini, L.J., Herbert, V.C. and Griffith, O.H. (1971) J. Mol. Biol. 59, 77—98
22 Smith, I.C.P. (1972) in Biological Applications of Electron Spin Resonance (Bolton, J.R., Borg, D. and Swartz, H., eds.), pp. 483—539, John Wiley-Interscience, New York
23 Smith, I.C.P. (1971) Chimia 25, 349—360
24 Lapper, R.D., Paterson, S.J. and Smith, I.C.P. (1972) Can. J. Biochem. 50, 969—981
25 Schreier-Muccillo, S., Marsh, D., Dugas, H., Schneider, H. and Smith, I.C.P. (1973) Chem. Phys. Lipids 10, 11—27
26 Sanioto, D.L. and Schreier, S. (1975) Biochem. Biophys. Res. Commun. 67, 530—537
27 Butler, K.W., Dugas, H., Schneider, H. and Smith, I.C.P. (1970) Biochem. Biophys. Res. Commun. 40, 770—776
28 Schreier-Muccillo, S., Butler, K.W. and Smith, I.C.P. (1973) Arch. Biochem. Biophys. 159, 297—311
29 Hsia, J.C., Schneider, H. and Smith, I.C.P. (1970) Chem. Phys. Lipids 4, 238—242
30 Oppenheimer, S.B., Bales, B.L., Brenneman, G., Knapp, L., Lesin, E.S., Neri, A. and Pollock, E.G. (1976) Exp. Cell. Res., in the press
31 Oppenheimer, S.B. (1973) Exp. Cell. Res. 92, 122—126
32 Kaplan, J., Canonico, P.G. and Caspary, W.J. (1973) Proc. Natl. Acad. Sci. U.S. 70, 66—70
33 Bales, B.L. and Baur, M.E. (1970) Chem. Phys. Lett. 7, 341—348
34 Sackmann, E., Trauble, H., Galla, H. and Overath, P. (1973) Biochemistry 12, 5360—5369
35 Stockton, G.W., Polnaszek, C.F., Tulloch, A.P., Hasan, F. and Smith, I.C.P. (1976) Biochemistry 15, 954—966
36 Mason, R.P., Polnaszek, C.F. and Freed, J.H. (1974) J. Phys. Chem. 78, 1324—1329
37 Cone, R.A. (1972) Nat. New Biol. 236, 39—43
38 Cherry, R.J. (1975) FEBS Lett. 55, 1—7
39 Fuchs, P., Parola, A., Robbins, P.W. and Blout, E.R. (1975) Proc. Natl. Acad. Sci. U.S. 72, 3351—3354
40 Seelig, J. (1972) Biomembranes 3, 267—279
41 Cannon, B., Polnaszek, C.F., Butler, K.W., Eriksson, L.E.G. and Smith, I.C.P. (1975) Arch. Biochem. Biophys. 167, 505—518
42 Shields, R. (1975) Nature 256, 257—258