Biochimica et Biophysica Acta, 507 (1978) 525—530

BBA Report

BBA 71327

PHYSICO-CHEMICAL STATE AND CALCIUM REACTIVITY OF NORMAL AND QUAKING MOUSE MYELIN

FRANÇOIS LETERRIER[a], JOEL BRETON[a], DENIS DAVELOOSE[a], JACQUES VIRET[a], FRANÇOISE LE SAUX[b] and SERGE POLLET[b]

[a]*Division de Biophysique du Centre de Recherches du Service de Santé des Armées, 1 bis rue du Lieutenant Raoul Batany, F 92141-Clamart and* [b]*Laboratoire de Neurochimie, Inserm U 134, Hôpital de la Salpetrière, 47, Boulevard de l'Hôpital, F 75013-Paris (France)*

(Received October 4th, 1977)

Summary

The ordering and rigidity as well as the effect of calcium have been studied on adult normal, immature normal (12 days) and adult quaking mouse myelins by the spin label method. The rigidity decreases in the order adult normal, quaking and 12-days myelin. Quaking and 12 days myelin are rigidified by Ca^{2+}, whereas normal adult is not. The respective roles of phospholipids and proteins are discussed.

In a recent study of rat brain membranes by the fatty-acid spin label method, we have evidenced structural differences between myelin and synaptic membranes, and have shown that the latter were rigidified by divalent cations, particularly Ca^{2+} [1—3].

The quaking mutation in the mouse is characterized by a decrease in myelin synthesis and by modifications of its lipid and protein composition [4]. We have thus undertaken a comparative study of the physical properties of adult normal and quaking mouse myelin. Furthermore, in order to observe if such modifications could be related to the maturation phenomena we have also studied myelin of immature 12-days old normal mice.

This preliminary report shows the differences concerning the rigidity and its modification under the effect of calcium observed on the three types of myelin.

Three different myelin preparations were made using each time 10 brains of adult (mean weight 400 mg), 20 brains of normal immature (12-days old; mean weight 290 mg) and 20 brains of adult quaking (mean weight 380 mg) C 57 BL mice. These brains were homogenized (Potter apparatus) in 20 times their weight of 0.32 M sucrose. 18 ml of homogenized brains were layered on

15 ml of 0.85 M sucrose and centrifuged 30 min at 21 000 × *g* (SW 27 Spinco rotor).

The bands of crude myelin were washed twice with bidistilled water, and pelleted at 21 000 × *g* (15 min). Pellets were resuspended in 0.32 M sucrose and again layered over 0.85 M sucrose. Bands of purified myelin were again washed with bidistillated water and the pellets were collected in 0.005 M phosphate buffer, pH 7.5. Protein concentrations, measured by the Lowry method [5], were adjusted in each case in order to obtain approximately 2—3 mg/ml.

Spin labelling was performed by adding 5 μl of a 10^{-2} M doxyl fatty acid solution in dimethylsulphoxide to 1 ml of myelin suspension. We have used 5-doxylstearate (5-DS), 16-doxylstearate (16-DS) (SYVA Corp., Palo Alto) and 8-doxylpalmitate (8-DP) (gift of Dr. Devaux). Electron spin resonance (ESR) spectra were recorded with a Varian E 3 ESR spectrometer equipped with a laboratory-built temperature regulator.

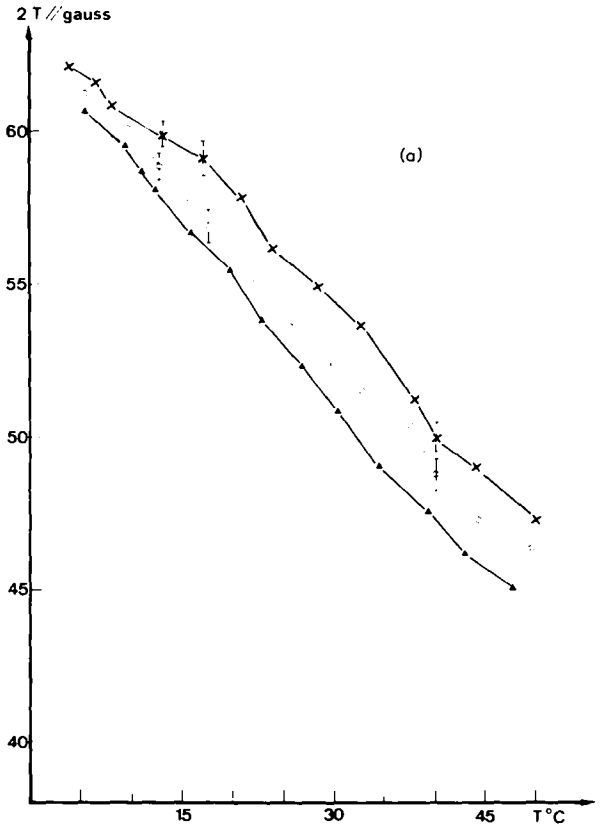
For the phospholipid analysis, myelins were lyophilized and lipids extracted with chloroform/methanol (1:1, v/v) by sonication. Phospholipids were qualitatively and quantitatively determined on two-dimensional thin-layer chromatography with multiple development (Pollet, S., Ermidou, S., Le Saux, F., Mauve, O. and Baumann, N., (1977) unpublished).

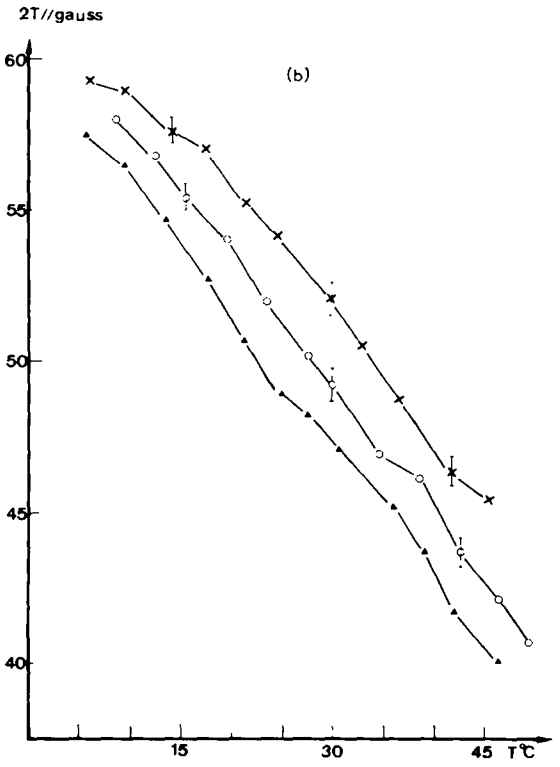
With each spin label, we have recorded the spectra obtained on the three types of myelin as a function of temperature between 5 and 50° C. All the spectra were interpreted by measuring the maximum coupling constant $2T_{//}$. The results are given in Fig. 1 a, b and c. It can be observed with each spin label on the three myelins studied, that the temperature variations are approximatively linear. The coupling constants of normal myelin are always the highest, whereas that of 12-days myelin are the lowest, and that of the quaking myelin have intermediary values. The same results are obtained when the spectra are interpreted by measuring the order parameter (S) [6]. However, this measurement is possible only in the case of 5-DS and 8-DP. With 16-DS, the coupling constant $2T_{\perp}$ is not measurable due to the high fluidity of the phospholipidic region this label explores. Table I gives the values of the order parameter as a function of temperature, in order to allow comparisons with other biological membrane structures [1].

The effect of Ca^{2+} was studied in the concentration range $3 \cdot 10^{-4}$—10^{-2} M, at 35° C on the three types of myelin and with the three labels. No effect was observed with 8-DP or with 16-DS. On the other hand, a noticeable increase of the order parameter measured with 5-DS in quaking or 12-days myelin was observed and only a slight increase at the maximum Ca^{2+} concentration studied in the case of normal myelin (Fig. 2).

The phospholipid composition of normal adult and quaking myelin is given in Table II. These results complete and confirm those obtained previously by Singh et al. [7]. It can be observed that acidic phospholipids are not significantly different in either myelin type.

Fig. 1. Variations of the $2T_{//}$ coupling constant as a function of temperature, measured on: X——X, normal adult myelin; ○——○, quaking myelin; ▲——▲, 12-days myelin. The bars indicate the maximum deviations obtained with the three different preparations experimented in each case. a, 5-Doxylstearate; b, 8-doxylpalmitate; c, 16-doxylstearate.


2 T // gauss
60
55
50
45
40
(a)
15
30
45
T°C


2T//gauss
60
55
50
45
40
(b)
15
30
45 T°C

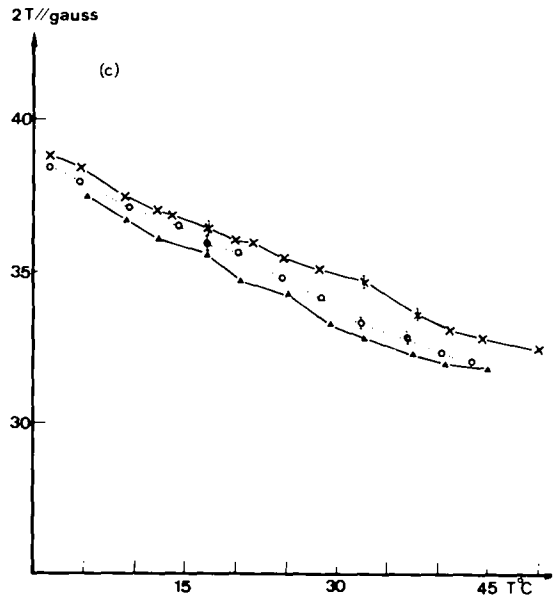

2T//gauss
40
35
30
(c)
15
30
45 T°C

TABLE I

TEMPERATURE VARIATIONS OF THE ORDER PARAMETER (*S*) MEASURED ON MYELINS LABELLED WITH 5-DOXYLSTEARATE AND 8-DOXYLPALMITATE

Temperature (°C)	Normal adult myelin	Normal 12-days myelin	Quaking adult myelin
5-Doxylstearate			
25	0.74	0.68	0.71
35	0.63	0.57	0.62
45	0.56	0.49	0.53
8-Doxylpalmitate			
25	0.71	0.60	0.65
35	0.59	0.51	0.54
45	0.49	0.38	0.43

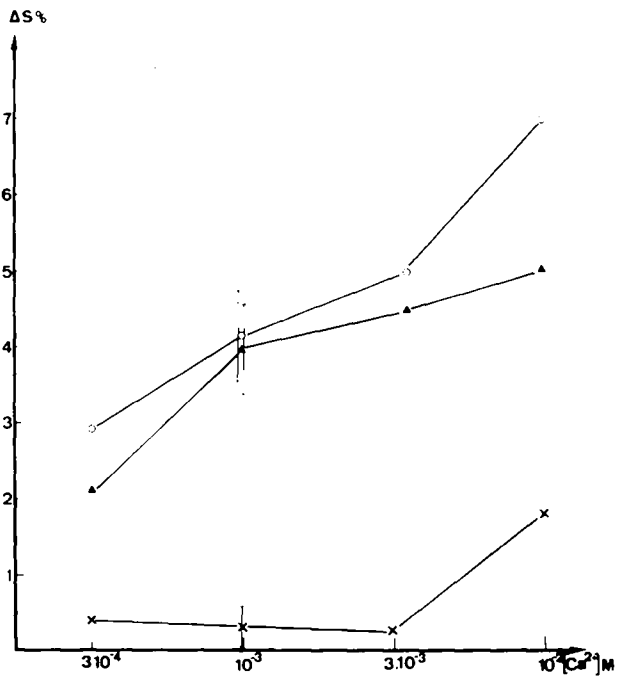


Fig. 2. Relative variations of the order parameter measured at 35°C with 5-doxylstearate on the three types of myelins as a function of Ca^{2+} concentration. (Same symbols as Fig. 1.)

TABLE II

MOUSE MYELIN PHOSPHOLIPIDS (μg/mg dry weight)

	Normal	Quaking
Phosphorus	18.1 ± 0.8	19.4
Phosphatidylethanolamine	4.6 ± 0.2	2.9
Phosphatidylcholine	6.6 ± 0.3	9.1
Phosphatidylserine	4.1 ± 0.5	3.8
Phosphatidylinositol	1.4 ± 0.2	1.0
Sphingomyelin	1.4 ± 0.2	2.6

TABLE III

MAIN CHARACTERISTICS OF THE MOUSE MYELIN COMPOSITION

	Normal	12 Days	Quaking	Ref.
Total protein (%)	21	26	33	4
Basic protein (μg/100 μg)	37.3	17.1*	17.1	9,10
Proteo-lipid protein (μg/100 μg)	24.1	8.0*	7.6	9,10
Cholesterol (mg/100 mg lyophilized myelin)	16	18	17	4
% Total lipid	22.8		19	7
Total phospholipids (mg/100 mg lyophilized myelin)	38	37	37	4
% Total lipid	47.8		56.4	7
Total glycolipids (% total lipid)	29.4		24.5	7
Galactolipids (mg/100 mg lyophilized myelin)	24	19	11	4
Principal fatty acids (% total phosphatides)				
16 : 0	19.5		28.5	7
18 : 0	24.6		27.8	
18 : 1	27.6		17.2	
20 : 1	5.0		1.1	
20 : 4	7.7		7.7	
22 : 6	8.1		12.0	

*10 days.

The variations of the spin label spectroscopic parameters are qualitatively identical with those observed on many other biological membranes. Normal mouse myelin is slightly more rigid than rat myelin, particularly when the intermediate region of the phospholipid layer is studied (8-DP) [2].

It is difficult to correlate the observed variations of order and fluidity of the three myelins studied with the known modifications of their lipid composition. The observed variations of cholesterol, long chain and double bonded fatty acids (see Table III) do not allow their resulting effects on the physical state of the membrane to be predicted. In fact, the cholesterol and the total phospholipid contents are not modified and non-systematic variations of the principal fatty acid levels are observed.

The fact that the 2 $T_{//}$ values measured on adult quaking myelin are intermediate between that of normal and 12-days myelins can be interpreted as indicating a lack of maturation in the structural disposition of the membrane constituents of quaking myelin.

The more interesting observation of this preliminary study seems to be the increase of the order parameter of 12-days and quaking myelin in the presence of Ca^{2+}. This ion is known to bind with high affinity to acid phospholipids of the membrane [8]. However, no important modifications of this type of phospholipids are observed between the different myelins (Table II). On the contrary, previous studies have shown that 12-days and quaking myelin are characterized by a decrease in their Folch proteolipid and basic protein contents (Table III) by comparison with that of the adult myelin. It has been shown that these proteins with basic properties bind on acidic phospholipids [11—13] and that the basic protein could be the organizer molecule

responsible for the myelin compactness [14,15]. It can be suggested that the decrease of these proteins in quaking and 12-days myelin either unmasks acidic phospholipid groups, increasing the affinity for Ca^{2+}, or leads to a less tight structure, making more phospholipids accessible to Ca^{2+}.

This work was done with the financial support of DRME (Contracts No. 76/1148 and 76/34041).

References

1 Viret, J. and Leterrier, F. (1976) Biochim. Biophys. Acta 436, 811—824
2 Viret, J. (1976) Doctoral Thesis, Orsay
3 Breton, J., Viret, J. and Leterrier, F. (1977) Arch. Biochem. Biophys. 179, 625—633
4 Baumann, N., Bourre, J.M., Jacque, C. and Harpin, M.L. (1973) J. Neurochem. 20, 753—759
5 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265—275
6 Hubbel, W.L. and McConnell, H.M. (1971) J. Am. Chem. Soc. 93, 314—326.
7 Singh, H., Spritz, N. and Geyer, B. (1971) J. Lipid, Res. 12, 173—181
8 Jacobson, K. and Papahadjopoulos, D. (1975) Biochemistry 14, 152—161
9 Greenfield, S., Norton, W.T. and Morell, P. (1971) J. Neurochem. 18, 2119—2128
10 Jacque, C.M., Louis, C.F., Guede, C. and Baumann, N.A. (1972) C.R. Acad. Sci. 274, 126—129, Sér. D
11 London, Y. and Vossenberg, F.G.A. (1973) Biochim. Biophys. Acta. 307, 478—490
12 Papahadjopoulos, D., Moscarello, M., Eylar, E.H. and Isac, T. (1975) Biochim. Biophys. Acta. 401, 317—335
13 Crang, J.A. and Rumsby, M.G. (1977) Biochem. Soc. Trans., 5, 110—112
14 Feinstein, M.B. and Felsenfeld, H. (1975) Biochemistry 14, 3041—3048
15 London, Y., Demel, R.A., Geurts van Kessel, W.S.M., Vossenberg, F.G.A. and van Deenen, L.L.M. (1973) Biochim. Biophys. Acta 311, 520—530