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PROTON NMR STUDY OF THE BINDING OF CONCANAVALIN A ON MYELOMA PLASMA MEMBRANES

Charles Tellier[1], *Chantal Curtet*[2], *Serge Poignant*[1*], *Anne Godard*[3] *and Jacques Aubry*[2]

[1]*Laboratoire de Chimie Organique Physique, CNRS ERA N° 315, Faculté des Sciences, 44072 Nantes Cedex, France.*
[2]*Laboratoire de Biologie, INSERM U 211, Faculté de Médecine, 44035 Nantes Cedex, France.*
[3]*Laboratoire de Biochimie, Faculté de Médecine, 44035 Nantes Cedex, France.*

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The dynamics of the lipids inside plasma membranes is investigated using proton NMR spectroscopy. Spectra are performed on two different fractions of purified membranes collected on a zonal rotor. A lectin, concanavalin A, provides a restriction of mobility of the lipids on one of these fractions. Enzymatic assays and chemical determinations are investigated in order to discuss the NMR results.

INTRODUCTION

All cells have surface receptors for various ligands. Activation of a cell to respond to a ligand requires the transfer of information across the plasma membrane. Relatively little is known about the various possible mechanisms of transmembrane signalling. Several hypotheses have been suggested, either involving proteins such as membrane bound enzymes interacting with the cytoskeleton (1), or a highly ordered cooperative structure in the membrane (2) or membrane fluidity and lipid environment of cell surface receptors and plasma membrane enzymes (3).

Various early events of the stimulation of lymphocytes by Con A were described at the surface. Until now, contradictory results have been obtained

* *Author to whom correspondence should be addressed.*

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ABBREVIATION : - NMR : Nuclear Magnetic Resonance
- ConA: Concanavalin A

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on the state of the lipids and membrane viscosity after the binding of Con A using fluorescent probes and ESR probes (4, 5).

We report here the use of proton NMR spectroscopy to study the modification of lipid dynamics in isolated plasma membranes of a murine plasmocytoma upon the binding of Con A.

MATERIALS AND METHODS

Preparation of plasma membranes

The plasma membranes were purified from MF_2S cells derived from the murine plasmocytoma MOPC 173 grown as ascites in Balb/c mice. After washing, the cells were placed in a hypotonic medium (5mM Tris HCl pH 8, 2mM $CaCl_2$, 1mM $NaHCO_3$) and the plasma membranes isolated by zonal rotor centrifugation (TI 15) as previously described (6). Two fractions characterized by enzymatic determinations were collected : F I and F II. Enzymatic assays of 5' nucleotidase (EC.3.1.3.5.) and Na^+/K^+ATPase (EC.3.6.1.3.) were determined according to (7). For NMR studies, freshly prepared plasma membranes were extensively washed with a 20 mM phosphate buffer pD 7.4 NaCl 100 mM D_2O. A similar concentration of membrane proteins, 10 mg/ml, was adjusted in the buffer for each sample, for fraction I as for fraction II. Con A dissolved in the same buffer was then added directly to the NMR tube and preincubated for 30 mn at 37°C in each case.

Chemical determinations

Lipid extracts from plasma membranes were prepared according to the method of WAYS and HANAHAN (8). Phospholipids were separated by thin layer chromatography on plastic sheets (5 x 10 cm) with silica gel G (Merk) using chloroform/petroleum ether/acetic acid (65/33/2, v/v/v) as solvent and after drying chloroform/methanol/acetic acid/water (25/12/4/2, v/v/v/v) (8). They were revealed by exposure to iodine vapor and the amount of inorganic phosphate of the different spots was determined according to (7). Cholesterol was estimated with a Biochemica Test Combination Kit (Boehringer, Mannheim). The fatty acid composition was determined by gas chromatography (Carlo Erba) after methylation with a capillary column filled with Carbowax 20 M. The standard fatty acids were obtained from Alltech Ass.

NMR spectra

Proton NMR experiments were performed on a CAMECA 250 Fourier Transform NMR spectrometer operating at 250 MHz. Accumulated free induction decays were obtained from 1000-2000 transients at 37°C corresponding to 20-45 mn for each experiment.

RESULTS

The NMR spectrum analysis of plasma membranes required a large amount of material purified by zonal rotor centrifugation. Two purified plasma membrane fractions termed F I and F II were obtained and displayed several differences in the lipid/protein ratio (Table I) and the distribution of phospholipid classes (Table II). The fatty acid profile was equivalent in both populations (data not shown). Other characteristics of these membranes have been described previously (6).

TABLE I

Chemical composition of plasma membranes from fraction I and fraction II (Results from four preparations).

	F I	F II
Protein	32.1	50.6
Total lipid	67.9	49.4
Lipid/Protein (W/W)	2.12	0.98
Phospholipid/Protein (W/W)	0.74	0.60
Cholesterol/Protein (W/W)	0.13	0.11
Cholesterol/Phospholipid (molar ratio)	0.37	0.38

The proton NMR spectrum of plasma membranes I and II (figures1 and 2A) shows several higher resolution peaks due to terminal CH_3-, lipid chain $-CH_2-$, CH_2-CO-, $-CH_2$-CH=C and $-\overset{+}{N}-(CH_3)_3$ protons. The latter signal was only seen in fraction II spectra, the relative concentration of which was higher than in fraction I (cf. Table II). These data reveal that the most mobile lipid constituents are well resolved.

No modification of the proton NMR spectrumwas obtained upon the binding of increasing amounts of Con A (up to the ratio Con A/membrane protein 1:1) with fraction I. On the contrary, Con A greatly modified the proton NMR spectrum of the plasma membrane fraction II (figure 2B). Both a decrease in intensity and a line broadening of the CH_2 resonance peak were produced.

TABLE II

Phospholipid composition of plasma membrane from fraction I and fraction II (Results from four preparations).

Phospholipids (%)	F I	F II
Phosphatidyl ethanolamine	29.3	24.5
Phosphatidylserine	15.1	15.0
Phosphatidylcholine	27.9	37.6
Sphingomyelin	26.5	19.7
Lysophosphatidylcholine	1.3	3.1

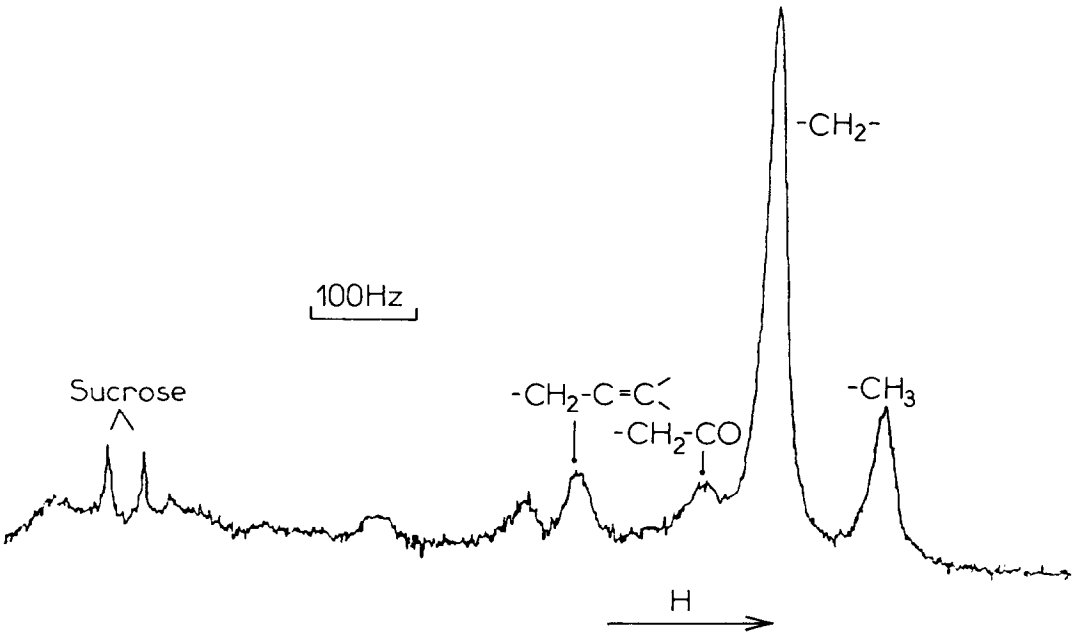

FIG. 1 - The upfield portions of the 250 MHz spectra of Fraction I of plasma membranes from MF_2S cells in a phosphate buffer 20 mM pD 7.40, NaCl 100 mM, D_2O. (1000 scans, 37°C).

The variation with respect to the amount of Con A of the ratio of the $-CH_2-$ and $-CH_3-$ resonance peak intensities is given (figure 3). This ratio was expressed in this way in order to exclude the possibility of any small increase in sample inhomogeneity due to membrane agglutination and to account for differences in the lipid dynamics only. Con A was proved to cause an important decrease in lipid mobility from the value 1/10 - 1/4 mg Con A/mg membrane proteins.

The multiple binding sites of Con A raise the nature of interaction between receptors and lipids. Membrane vesicles were reconstituted with total lipid extract of plasma membrane. The resulting proton NMR spectrum of these vesicles was similar to those obtained in F I and F II. No effect of Con A on the proton NMR spectrum was monitored. The Con A receptors, binding glycoproteins in particular seem necessary to induce a perturbation of lipid dynamics.

We also checked the activity of the membrane bound 5′ nucleotidase upon binding of Con A. Significant inhibition of enzymatic specific activity was

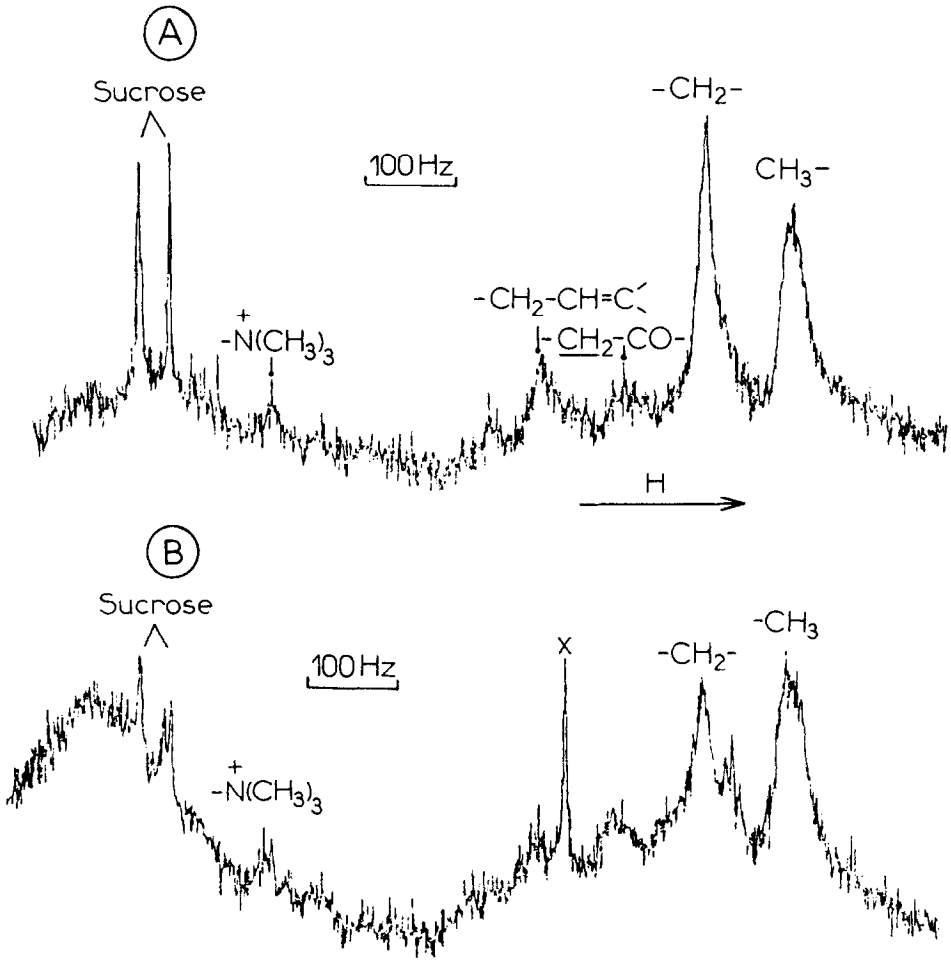

FIG. 2 - The upfield portions of the 250 MHz proton NMR spectra of fraction II of plasma membranes from MF_2S cells in a phosphate buffer 20mM, pD 7.40, NaCl 100 mM, D_2O (2000 scans, 37°C). A : without Con A. B : after incubation with Con A (0.25 mg Con A/mg membrane proteins). X : signal from Con A.

observed with increasing amounts of Con A with the two fractions (figure 4). It reached a plateau at 50-60 %. The absence of a Con A effect on the proton NMR spectrum of plasma membrane fraction I does not seem to depend on the absence of Con A receptors.

DISCUSSION

The effect of Con A binding to the plasma membrane of a murine plasmocytoma was monitored by proton NMR. This technique revealed that treatment of the membranes with Concanavalin A reduced the mobility of the chain $-CH_2$ pro-

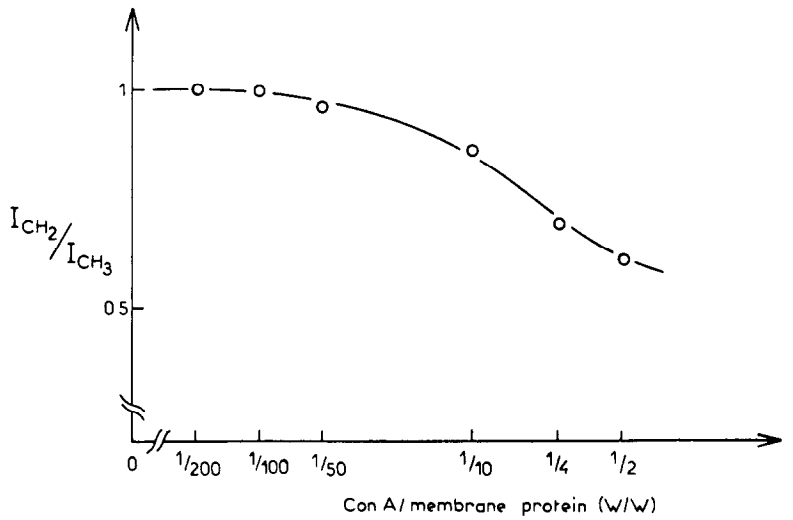

FIG. 3 - Ratio of intensity of methylene peak over methyl peak plotted against the ratio of Con A concentration over membrane proteins concentration (fraction II of plasma membranes).

tons from the lipids. A quantitative interpretation of the $-CH_2-$ resonance linewidth has been attempted by several authors (9). It has been shown that the major contribution to the resonance linewidths of phospholipid bilayers

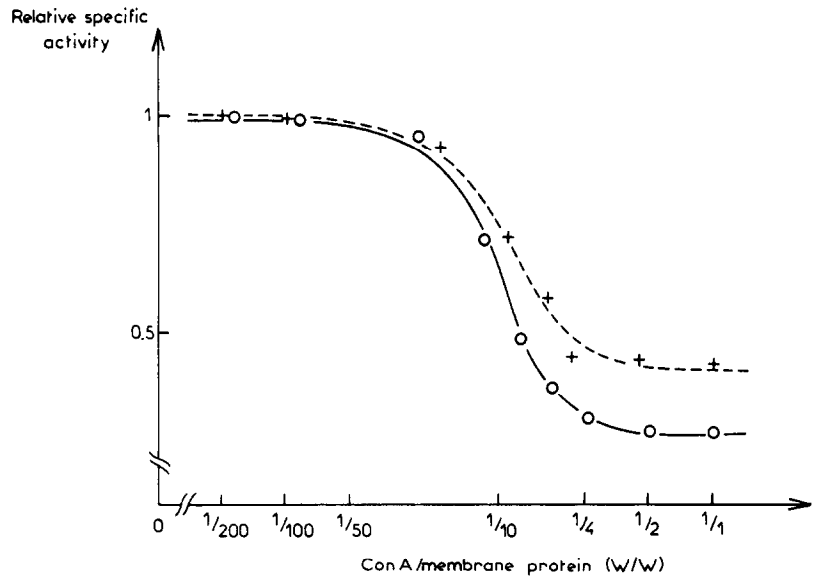

FIG. 4 - Modulation of the 5'-nucleotidase activity by adding various amounts of Con A to fraction I (X.....X) and fraction II (O.....O). The relative specific activity is the ratio between the specific activity in the presence of Con A and the specific activity without the lectin.

is dipolar in origin (10) but interpretation remains difficult because the methylene resonance is a superposition of individual resonances submitted to a gradient of motion proceeding from one end of the fatty acid chain to the other. However, magnetic dipolar interactions are partially averaged by relatively low frequency inter and intra molecular motions such as the rapid angular reorientation of phospholipids by lateral diffusion, large amplitude segmental motion and vesicle rotation (11). The differential broadening of the linewidths in the proton NMR spectrum of F II excludes the vesicle tumbling contribution, it seems that Con A produces zones of restricted mobility in the plasma membrane lipids.

The resonance peak of the methylene groups was diminished but remained relatively sharp. This could take account of the proportion of inside out vesicle lipids which were not affected by Con A.

Until now, several approaches have been tried in order to correlate some properties of plasmic membranes and cells with the fluidity of the ConA receptor by cross linking of the lectin receptor complexes (12, 13). The measurement of the dynamic properties of the lipid bilayer obtained by the use of fluorescent probes and electon spin resonance probes shows discrepancies due to the use of these probes (5, 14). By the use of proton NMR without any additional probe, we have shown that Con A binding produces restricted mobility of the lipid bilayer which correlates the modulation of enzyme activities.

The reason for the difference between responses of fractions I and II upor Con A binding remains to be determined.

An electron microscopic examination of the vesicles (6) showed that they were heterogeneous in size and rather large (0.1 μ to 1μ) but with a mean size smaller in fraction I than in fraction II. The authors deduced that during cell lysis, or when the pellet was suspended in a sucrose solution, plasma membranes of the plasmocytoma cells were broken up into fragments with different properties.

Another interesting data might be the relative proportion of inside out and right side out vesicles in the two fractions, which might change the degree

of Con A binding. It was previously shown (15) that there was regularly about fifty percent of each form in fraction II but more inside-out form in fraction I (about seventy percent). Nethertheless the proportion of the right side out form in fraction I is not negligible and NMR results cannot be explained by a complete absence of Con A receptors as shown before from enzymatic assays.

The low protein-lipid ratio in fraction I is probably related to a low lectin receptor-lipid ratio ; the amount of lipids immobilized by the clustering and the cross linking of Con A receptor is probably too low to be detected by proton NMR.

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