^{31}P NMR as a tool for monitoring detergent solubilization of sarcoplasmic reticulum membranes

Michel Roux and Philippe Champeil

Département de Biologie, Service de Biophysique, CEN Saclay, F-91191, Gif-sur-Yvette Cédex, France

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Sarcoplasmic reticulum vesicles were solubilized stepwise by the nonionic detergent octaethyleneglycol monododecyl ether; ^{31}P NMR enabled the extent of phospholipid solubilization to be monitored by following the conversion of the broad resonance peak characterizing the phospholipids inserted in the bilayer to the narrow resonance signal characterizing phospholipids inserted into a mixed micelle. Up to 0.25 g detergent/g protein could be incorporated into the membrane without solubilization. Higher detergent concentrations of up to 1.5–2 g detergent/g protein led to gradual solubilization. Although the method allows us to monitor the extent of solubilization of individual phospholipid classes, there was no evidence of either preferential solubilization or retention of a specific class of phospholipids.

^{31}P NMR *Phospholipid* *Detergent* *Sarcoplasmic reticulum ATPase*

1. INTRODUCTION

Solubilization of membranes by detergents has aroused widespread interest [1]. We describe here the use of ^{31}P NMR to document this process.

In biological membranes, phospholipid headgroups give rise to relatively broad ^{31}P NMR resonances, due to the slow tumbling rate of the membrane fragments and to the constraints imposed on motional freedom by the bilayer structure. On the other hand, if detergent is added in order to obtain partial membrane solubilization, the phospholipid molecules incorporated into small detergent micelles experience much faster motion and therefore give rise to a narrow NMR signal. If chemical exchange between the micellar and bilayer states is slow on the ^{31}P NMR time scale, a two-component spectrum is observed, allowing quantification of the fraction of phospholipid solubilized.

Solubilization of pure lipid phosphatidylcholine bilayers by the nonionic detergent octyl glucoside has already been studied by this method [2]. Such experiments may benefit from the fact that the major classes of solubilized phospholipids can be detected as well resolved resonances [3]. We show here that this technique can be helpful in studying the solubilization of biological membranes derived from sarcoplasmic reticulum. The detergent used was octaethyleneglycol monododecyl ether ($C_{12}E_8$). Our experiments confirmed that up to a certain level, small amounts of detergent can be incorporated into the bilayer, without giving rise to phospholipid solubilization [4]. The threshold measured was consistent with the one determined independently with a Sephacryl S-300 column by measuring the maximal amount of detergent eluting at the void volume together with non-solubilized lipid and protein [4]. Our results were also consistent with the idea that no particular class of phospholipid is extracted preferentially upon SR membrane solubilization.

2. EXPERIMENTAL

Sarcoplasmic reticulum vesicles (SR) were prepared and tested as already described [4,5]. Their phospholipid content, measured after mineralization, was about 0.5 g phospholipid/g protein. Lipids were extracted from SR as in [6].

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Pure lipids were obtained from Sigma. Liposomes were prepared from dry lipid by resuspension in buffer, with or without sonication. The buffer contained 100 mM KCl, 10 mM *N*-tris[hydroxymethyl]methyl-2-aminomethanesulfonic acid (Tes), 0.15 mM $CaCl_2$, and 0.05 mM EDTA in order to chelate possible metallic line-broadening agents (pH 7.5). $C_{12}E_8$ was obtained from Nikko Chemicals, Tokyo.

NMR measurements were performed with a Bruker WH 90 Fourier-transform spectrometer operating at 36.4 MHz for ^{31}P, with continuous broadband ^{1}H decoupling and a probe temperature set at 20°C. A small quartz tube (5 mm in diameter) containing D_2O was inserted into the larger sample-containing tube (10 mm in diameter), in order to lock the spectrometer on the D_2O signal. To quantitate the amount of phospholipid solubilized, we used 90° pulses, 2 s acquisition time, and a 20 s interval between acquisitions (because of the long relaxation time of the ^{31}P nucleus); 100–200 scans and 1 Hz line broadening were used. For measurement of the longitudinal (T_1) relaxation time, a conventional inversion-recovery sequence was used.

3. RESULTS AND DISCUSSION

Fig.1 shows some of the spectra obtained when detergent was added stepwise to a concentrated suspension of SR vesicles (50 mg/ml protein). Inorganic phosphate (P_i) was also included together with the vesicles in order to provide an internal reference. In the absence of detergent, the phospholipid headgroups gave rise to such a broad ^{31}P resonance that it did not appear under the conditions of this scan, and only the narrow resonance signal of P_i was visible (note that the SR fragments used have diameters ranging from 400 to 2000 Å; we measured a bandwidth of several hundreds of Hz (not shown) for the resonance of membrane phospholipids). When 0.25 g $C_{12}E_8$/g protein was added, no peak characteristic of solubilized phospholipid appeared; we did not attempt to quantify precisely the effect of these subsolubilizing concentrations of detergent on the spectrum of the phospholipids in the bilayer, but fluidification and slight narrowing of the overall ^{31}P envelope was to be expected [2,4]. Only for higher detergent concentrations did the spectrum show the superposition of new peaks characteristic of phospholipids in mixed micelles.

A similar spectrum with 3 main peaks was obtained with high concentrations of another detergent, *n*-octyl glucoside. When detergent was added to liposomes made from SR lipids, peaks with identical chemical shifts were found. Comparison with standard lipids solubilized under the same conditions allowed assignment of the peaks to phosphatidylcholine, phosphatidylethanolamine and phosphatidylinositol. High levels of $C_{12}E_8$ (see below) gave spectra with enough resolution (omitting the 1 Hz line broadening) to allow quantification of the percentage of each individual phospholipid. The values measured were in good agreement with previous analyses of SR phospholipids [3,7,8].

Table 1 gives the resonance characteristics of some of these phospholipids; our data completely agree with those obtained with Triton X-100 [3].

Fig.2 shows the fraction of phospholipid solubilized as a function of the concentration of the detergent added (under our conditions, the concentration of monomeric detergent in the medium was negligible compared to the concentrations of both the micellar detergent and the detergent incorporated into the bilayer). Solubilized phospholipid was estimated by comparing the integral of the micellar phospholipid ^{31}P signal (the area of the peak) to the integral of the P_i signal. Results of 3 different experiments are plotted in fig.2. Solubilization started slightly above 0.25 g detergent/g protein, in perfect agreement with measurements by a column technique of the max-

Table 1

^{31}P chemical shift and longitudinal relaxation time (T_1) of phospholipids and inorganic phosphate

Species	Chemical shift (ppm) from external 85% H_3PO_4	T_1 (s) (measured at 27°C)
PC	+0.95	3.12
PE	+0.28	2.88
PI	+0.70	
PS	+0.43	
P_i (pH 7.5)	−2.10	5.40

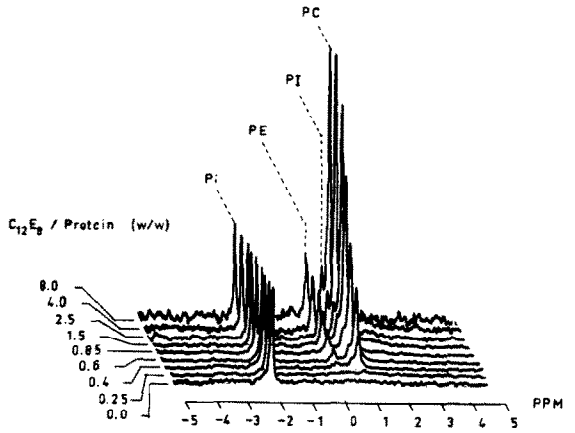

Fig.1. ^{31}P spectra of sarcoplasmic reticulum membranes in the presence of different amounts of detergent. P_i was included as a reference in a concentrated suspension of SR vesicles. Various amounts of $C_{12}E_8$ from a stock solution (100 mg/ml) were added, in order to achieve the indicated final detergent/protein ratios (w/w). Spectra were filtered with a line broadening of 1 Hz and standardized in relation to the inorganic phosphate peak. Pi, inorganic phosphate; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol.

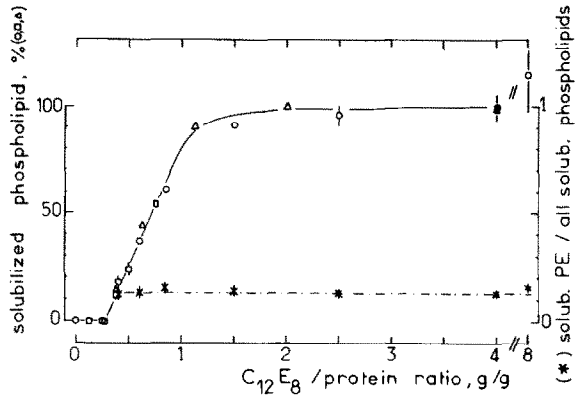

Fig.2. Phospholipid solubilization as a function of the final detergent/protein ratio (w/w). (○, □, △) Total solubilized phospholipids (sum of integrated phospholipid peak areas), in relation to the P_i peak, expressed as % of maximal value. Different symbols refer to different experiments. (*) Ratio of solubilized PE over total solubilized phospholipids.

imal amount of detergent incorporated into an SR membrane [4]. Solubilization was virtually complete for 1.5–2 g detergent/g protein, a proportion commonly used for ATPase solubilization [9].

It is apparent from fig.1,2 that, although the solubilized phospholipid peaks have a constant integral above 1.5–2 g $C_{12}E_8$/g protein, they become still narrower and therefore better separated when the detergent concentration is further raised. This pattern was also present when pure lipid liposomes were solubilized stepwise by detergent (not shown); this enhanced resolution might be due to the general reduction in size of the phospholipid-containing mixed micelles.

This varying bandwidth for the extracted phospholipids made it more difficult to quantitate precisely the relative solubilization of the individual classes of phospholipids, since it is not possible to use the relative height of the peak as a reasonable index. For instance, the phosphatidylinositol peak is more severely contaminated by the 'foot' of the phosphatidylcholine peak at intermediate detergent concentrations than at high concentrations. We could, however, reasonaby quantify the fraction of solubilized phosphatidylethanolamine *vs* total solubilized phospholipids from the corresponding integrals, as shown in fig.2 (*); no preferential solubilization or retention of this phospholipid could be detected within the present experimental limits. The significance of this finding in terms of absence of specific protein–lipid interaction was ascertained by performing a similar solubilization experiment in the absence of protein; i.e., with pure lipid liposomes (not shown). Again, after a critical amount of detergent had been incorporated into the liposomes, phosphatidylethanolamine solubilization occurred concomitantly with that of all the phospholipids.

We here confirm that ^{31}P NMR offers a convenient, if sample-consuming, means of detecting phospholipid solubilization without any preliminary separation of solubilized material. Results are consistent with previous experiments [8,10,11], showing that there is no preferential binding to the protein of a large number of phospholipids of a particular chemical class. However, it has been suggested that there might be a few discrete, non-annular binding sites for amphiphiles around the protein [12]; for instance, fluorescent derivatives of phosphatidylethanolamine have been suggested to remain closely linked to the ATPase, even after solubilization [13]. Our experiments are not con-

clusive enough to either support or contradict these interesting suggestions.

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