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19 ABSTRACT (Continue on reverse if necessary and identify by block number) This project is aimed at investigating if a lipid elastic property, known as the spontaneous radius of curvature R_0 , is a regulated membrane property and if its value modulates membrane protein activity. Specific aims reported on here include: 1) Correlation of ion pump/channel activity with R_0 by reconstitution of membrane proteins in vesicles with different R_0 values obtained by different lipid compositions; and 2) The development of new techniques for measuring R_0 which are more suitable for native membranes. Toward these ends we have reconstituted the $\text{Ca}^{++}\text{ATPase}$ of sarcoplasmic reticulum into vesicles and detected a positive correlation of the pump coupling efficiency with R_0 . Combined x-ray diffraction and NMR studies on deuterated lipids are underway to correlate the quadrupole splitting with the x-ray determined R_0 value.					
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INTRODUCTION:

-Despite the enormous progress which has been made in understanding the structure and function of biomembranes, there still is no satisfactory understanding of the factors which determine the polar lipid composition of cell membranes. In particular, the functional interactions between the lipids and intrinsic membrane proteins of cell membranes are unknown. A typical membrane contains a spectrum of lipid species of roughly 10 types of polar headgroups linked to hydrocarbon chains of various lengths and degrees of saturation (Quinn & Chapman, 1980). The lipid spectrum varies considerably from one membrane type to another and according to environmental conditions, leading to two related questions: (1) What determines the types and amounts of lipids which occur in a given membrane? and (2) How does the functioning of the intrinsic membrane proteins depend on the lipid composition?

A number of materials membrane properties are known to modulate membrane protein behavior. By "materials properties", as opposed to chemical properties for which there exists a chemically specific recognition site, we refer to properties which result from the colligative interactions of many lipid molecules. Important materials properties include

the bilayer thickness, surface charge, and ionic permeability. Lipid membranes are known to also have elastic materials properties which have not been extensively explored with respect to protein function. In particular, we have shown that the lipid monolayers of which bilayers are made are endowed with a spontaneous tendency to curl to a specific radius, R_0 , which depends sensitively on the overall lipid composition. Extensive theoretical and experimental studies (Gruner et al, 1988; Gruner, 1989) have shown that non-bilayer mesomorphism in lipid systems may be understood in terms of the spontaneous curvature and that a mesomorphically prone lipid system in the bilayer state is under an anisotropic elastic strain. The hypothesis explored by this project is that this strain is a carefully regulated membrane property and that it modulates the activity of certain membrane proteins (Gruner, 1985). In particular, Navarro et al (1984) showed that the Ca^{++} ATPase coupling ratio is sensitive to the mesomorphic tendencies of the imbedding lipid bilayer.

Methods

Ca^{++} ATPase from rabbit skeletal muscle was isolated and purified. The protein was then reconstituted into various lipid mixtures by deoxycholate mediated exchange into single lamellar bilayer vesicles of lipid mixtures of dioleoylphosphatidylethanolamine (DOPE), N-methyl-DOPE (DOPE-Me), and dioleoylphosphatidylcholine (DOPC). These lipids have been characterized with respect to their spontaneous curvature properties (Gruner, et al, 1988). Because the lipids are not incorporated into the vesicles in the same ratio as in the feed mixture, all lipid compositions were determined by TLC analysis. The protein coupling ratio (number of Ca^{++} pumped/ATP hydrolyzed) was determined by monitoring the ATP hydrolysis via the disappearance of NADH in conjunction with the sequestration of radio- Ca^{++} in the vesicle suspension buffer (Navarro et al, 1984).

No method is known whereby the spontaneous curvature of protein containing lipid bilayers may be measured (see below), so the actual correlation tested was between the pump activity and the spontaneous curvature of the pure lipid system of an identical lipid composition. In so far as the protein is dilute in the bilayer, it is expected to have negligible effect on R_0 . The lipid R_0 values for the specific assayed compositions of the vesicles were measured via the dodecane method of Gruner et al (1988). Here, dodecane is added to induce the H_{II} phase and the size of the H_{II} water core is taken as an approximate measure of the spontaneous radius of curvature according to the formula $R_0 = 0.5d - (16\text{\AA})$, where d is the H_{II} phase basis length and $16\text{\AA}$ is the approximate thickness of the lipid monolayer.

With respect to the distinct goal of developing new methods for determining the spontaneous curvature of lipid bilayers, the experimental protocol has been to compare the quadrupole splitting of perdeuterated lipid chains in lipid bilayers and H_{II} phases (Cullis et al, 1986) with the spontaneous curvature determined by the dodecane method. Mixtures of palmitoyl-oleoyl-phosphatidylcholine (POPC) and -ethanolamine (POPE) are being used as a test system. The choice of this lipid was dictated by the difficulty of fully perdeuterating unsaturated chains. The synthesis route consisted of perparing perdeuterated palmitic acid which is then incorporated into the phospholipid. Since the POPC-POPE system has not previously been used for curvature elasticity studies, the initial experiments consisted of a detailed mapping of the phase behavior as a function of temperature and composition and demonstration that the NMR spectra can be depaked to yield the chain order parameter as a function of chain carbon number.

Results

In collaboration with Drs. M Jaworski and A. Janoff or the Liposome Co., the Ca^{++} ATPase was successfully reconstituted into 11 DOPE-DOPC mixtures. For each

of these vesicle systems, the protein content, lipid composition, ATP hydrolysis rate and Ca^{++} pump rate was determined. Each lipid composition was then mixed in excess water with free dodecane and the size of the water core was determined. The resultant compositions and the coupling ratios are shown in Table 1 and Figure 1, resp. The coupling ratio (Ca^{++} pumped/ATP hydrolyzed) is shown vs. $(1/R_0)^2$, which is the extrapolated upper bound on the strain energy density (Gruner et al, 1988; Gruner, 1989). As can be seen, the points conform well to a smooth curve, irrespective of the specific compositions used to achieve a given R_0 value. Also shown for reference are the coupling ratio vs. $(1/R_0)^2$ values estimated from Navarro et al (1984), where the abscissa values were determined from measurements of the DOPE-DOPC compositions given in Navarro et al (1984). The agreement is good. For a given preparation, the lipid composition varied slightly with the size of the vesicles. As a check, complex H of table one was chromatographically sized. The vesicle size distribution peaked at two different values, each corresponding to slightly different compositions. The two distinct compositions are shown as filled circles on Fig. 1. Note the excellent agreement with the curve.

A number of cautions must be mentioned in interpreting these results: First, the coupling ratio can be biased by the presence of fully open membrane fragments, because such fragments consume ATP without resulting in net Ca^{++} trapping. In order to explain the results, the fraction of such open fragments, which would be unusual in any case, would have to increase systematically with R_0 ie, with the bilayer stabilizing fraction of the lipid. This is counter-intuitive. Even so, control experiments are now being performed to assay the degree of occurrence of open membrane fragments. Second, there was a large rabbit-to-rabbit variation in the ATPase activity of different preparations, presumably due to inactive protein. This variation does not show up in the coupling ratios because dead protein contributes to neither ATP hydrolysis nor Ca^{++} pumping. However, we would

TABLE 1

Biochemical Characterization of Ca^{2+} ATPase Complexes ^{a/}

Complex	Prot. Conc. (mg/ml.)	Lip./Prot. (mol/mol)	mole percent		
			Lipid 1	Lipid 2	Other ^{b/}
A DOPE/DOPC	9.73	34.8	51.5	46.0	2.5
B DOPE/DOPC	8.33	25.6	32.5	58.9	8.6
C DOPE/DOPC	0.90	86.1	27.1	69.6	3.4
D DOPE/DOPC	1.87	20.8	0.0	98.3	1.7
E DOPE/DOPHPE	8.05	36.1	69.0	29.6	1.4
F DOPE/DOPHPE	5.11	30.5	65.3	32.2	2.5
G DOPE/DOPHPE	7.11	17.9	39.0	58.9	2.1
H DOPE/DOPHPE	9.57	27.8	36.5	59.6	3.8
I DOPE/DOPHPE	7.26	34.3	0.0	98.8	1.2
J DOPC/DOPHPE	8.68	30.1	21.0	78.0	1.0
K DOPC/DOPHPE	4.97	26.5	38.7	60.1	1.2

^{a/} Final protein and lipid compositions were determined by assay.

^{b/} Total residual native lipids remaining after reconstitution protocol.

still like to understand the source of the variation. Finally, the coupling ratios should be measured over the full time course of the pumping event to see if the coupling ratios change as the sequestered Ca^{++} increases and the ADP level rises. These experiments are in progress.

The experiments on correlating the spontaneous curvature of POPE-POPC mixtures with the deuterium NMR quadrupole splitting are being done with Drs. M. Laffleur, P. Cullis, and M. Bloom of the University of British Columbia. So far, extensive x-ray and NMR data have been acquired. The behavior of POPE-POPC mixtures with high POPC fractions has turned out to be quite hysteretic, a phenomenon also observed with other large R_0 systems, such as DOPE-Me. This has required careful attention to specimen history protocols. Mapping of the phase diagram of the POPE-POPC system is nearing completion. The depaking of the NMR spectra has been successfully accomplished and order parameter profiles of selected systems have been obtained in both the lamellar and

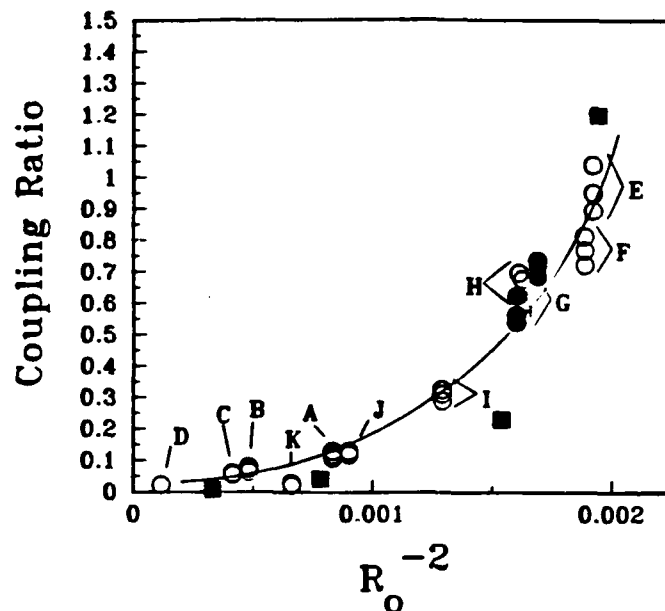


Figure 1. The Ca^{2+} coupling ratio mean for all of the various binary lipid/ Ca^{2+} ATPase complexes are presented as moles Ca^{2+} pumped/moles ATP hydrolyzed vs. R_0^{-2} . The complex labels are indicated in Table 1. Included for comparison are the DOPE/DOPC/ Ca^{2+} ATPase complexes (■) with coupling ratios taken from Navarro et al 91984) and radii which we measured from their compositions as described in the text. Also included are the positions of separated components from complex H(●) (see text). Curve shown was drawn by eye.

H_{II} phases (Lafleur et al, 1989). As soon as the x-ray phase diagram and x-ray determined R_0 values are in hand, selected pope=popc ratios will be chosen for NMR analysis for the R_0 and quadrupole splitting correlation.

SUMMARY

Initial data on the correlation of the pumping efficiency of a Ca^{++} ATPase with the curvature strain energy in bilayer vesicles with different values of the spontaneous curvature indicates a good correlation. Various control measurements are underway to rule out artefacts due to factors such as membrane fragments and denatured protein. Barring

unforeseen results of the control studies, it appears that the spontaneous curvature of lipid mixtures does modulate the protein pump activity as postulated. The implications of this are quite important for understanding the mechanism of membrane pumps and for experiments which utilize reconstituted proteins.

Work on developing methods to measure R_0 in lipids in the bilayer form in the POPE-POPC system is proceeding on schedule. However, it is too early to report definitive results as to whether the hydrocarbon chain order parameter profile can be interpreted in terms of a spontaneous curvature.

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