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Effects of Potassium on Lipid–Protein Interactions in Light Sarcoplasmic Reticulum†

Barry S. Selinsky‡ and Philip L. Yeagle*
Department of Biochemistry, School of Medicine, University at Buffalo (SUNY), Buffalo, New York 14214
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ABSTRACT: This study shows the effect of K^+ on phospholipid–protein interactions in light sarcoplasmic reticulum (LSR) as measured by ^{31}P NMR. In the presence of 110 mM K^+, a substantial effect of the membrane protein on the behavior of the phospholipids was detected. Subtracting the spectrum of the LSR lipid extract from the spectrum of the intact LSR membrane produced a difference spectrum of much greater breadth than the normal phospholipid bilayer powder pattern. This powder pattern is indicative of a phospholipid domain considerably more motionally restricted than the phospholipids in a normal phospholipid bilayer. The apparent axially symmetric powder pattern is consistent with axial diffusion. In a reconstituted membrane containing the calcium pump protein at a lipid/protein ratio much less than in the light sarcoplasmic reticulum, the broad component was more prominent. The relative resonance intensity of the broad component appeared to be proportional to the lipid/protein ratio of the membrane. In 10 mM K^+, no broad powder pattern is observed in the corresponding difference spectrum. Thus, in the absence of potassium, the membrane protein has much less influence on the phospholipid of the membrane, as measured by ^{31}P NMR. In addition to the effects of K^+ on the membrane structure of the sarcoplasmic reticulum, K^+ modulated the function of the calcium pump. The rate of calcium-dependent ATP hydrolysis increased in light sarcoplasmic reticulum when [K^+] increased from 10 to 110 mM. The rate of calcium transport was also stimulated by an increase in K^+.

The effects of phospholipids upon the structure and function of integral membrane proteins have been vigorously explored. Some membrane-bound enzymes have a clearly demonstrated kinetic requirement for specific membrane lipids [reviewed by Sandermann et al. (1978)]. For example, the enzyme β-hydroxybutyrate dehydrogenase has an absolute requirement for phosphatidylcholine (Gazzotti et al., 1974), while phosphatidic acid has been recently shown to activate diacylglycerol kinase isolated from *Escherichia coli* (Russ et al., 1988). Other

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‡Present address: Department of Chemistry, Villanova University, Villanova, PA 19085.

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integral proteins, such as the acetylcholine receptor (Jones et al., 1988), require a lipid annulus to maintain biological function. Cholesterol stimulates the activity of some membrane proteins (Craido et al., 1982; Yeagle et al., 1988; Vemuri & Philipson, 1989).

The (Ca^{2+}, Mg^{2+})-ATPase of sarcoplasmic reticulum has been one of the most extensively studied integral membrane proteins with respect to protein–lipid interactions (Hidalgo, 1987). In native sarcoplasmic reticulum, the ATPase had no apparent functional preference for specific phospholipid headgroups (East & Lee, 1982); in recombined membranes, phosphatidylethanolamine or phosphatidylglycerol was required to generate maximal calcium transport function (Navarro et al., 1984; Cheung et al., 1986). The ATPase did show a preference for phospholipid over cholesterol (Silvius et al., 1984; Selinsky, 1984). Using delipidated sarcoplasmic reticulum, Hidalgo et al. (1986) have shown that 37 phospholipids per (Ca^{2+}, Mg^{2+})-ATPase were required to maintain full ATP hydrolysis activity, but the phosphoenzyme levels were not affected until the lipid:protein ratio was lowered to less than 23:1. These activity data strongly suggested a functional requirement for some phospholipids around the ATPase.

Physical measurements of sarcoplasmic reticulum also demonstrated the presence of phospholipids closely associated with the (Ca^{2+}, Mg^{2+})-ATPase. Fluorescence experiments using the probe diphenylhexatriene (Lentz et al., 1983) or diphenylpropane (Almeida et al., 1982) indicated that one layer of phospholipid which surrounds the (Ca^{2+}, Mg^{2+})-ATPase has different motional properties than phospholipid distant from the protein. Electron spin resonance experiments suggested that 20–34 phospholipids are motionally restricted by each (Ca^{2+}, Mg^{2+})-ATPase polypeptide chain (Hidalgo, 1984).

We previously demonstrated the existence of a broad spectral feature in ^{31}P nuclear magnetic resonance (NMR)[1] spectra of sarcoplasmic reticulum and recombined membranes containing the (Ca^{2+}, Mg^{2+})-ATPase (Selinsky & Yeagle, 1984, 1985). Our results were challenged by Ellena et al. (1986), who did not observe a broad component in their ^{31}P NMR spectra of sarcoplasmic reticulum. In this paper, we demonstrate that the discrepancy between ourselves and Ellena et al. (1986) primarily results from differences in the potassium ion concentrations in our respective sarcoplasmic reticulum preparations. In their report, Ellena et al. (1986) provided results from membranes suspended in low potassium concentrations. As we have previously shown using other methods (Albert et al., 1981; Selinsky & Yeagle, 1983), the addition of physiological concentrations of potassium ions results in a significant increase in the number of phospholipids motionally restricted per (Ca^{2+}, Mg^{2+})-ATPase. In this paper, we present ^{31}P NMR difference spectra which clearly show that changing the potassium ion concentration from 10 to 110 mM introduces a broad spectral component in the ^{31}P NMR spectrum from light sarcoplasmic reticulum characteristic of a motionally restricted phospholipid environment in this membrane.

Function of the calcium pump is also modulated by potassium. The rate of calcium dependent ATP hydrolysis increased in light sarcoplasmic reticulum when K^+ is increased from 10 to 110 mM. The rate of calcium transport was also stimulated by an increase in K^+.

Materials and Methods

Chemicals. Phosphoenolpyruvate, ATP, ADP, pyruvate kinase, lactic acid dehydrogenase, NADH, and Arsenazo III were purchased from Sigma Chemical Co., St. Louis, MO.

Isolation of Sarcoplasmic Reticulum. Sarcoplasmic reticulum was isolated from white hind leg muscles of New Zealand White rabbits as previously described (Eletr & Inesi, 1972). The crude isolate was further purified into light and heavy fractions by discontinuous sucrose density gradient centrifugation (Fernandez et al., 1980). Membrane samples were stored in 20 mM HEPES/1 M sucrose, pH 7.0, under argon at 4 °C. Measurements were made within 2 days of preparation. We have observed that aged material apparently behaves differently than freshly isolated material with regard to interactions with potassium ions, as reported previously (Nakamura & Konishi, 1978).

Total Lipid Extracts from Light Sarcoplasmic Reticulum. Lipid extracts were prepared by using the Folch (1957) method; 1 mM EGTA was included in the aqueous phase to chelate free calcium. The extracted lipids were dried first under nitrogen and then briefly under vacuum and suspended without sonication in 20 mM HEPES, 100 mM KCl, 1 M sucrose, and 1 mM EGTA for NMR studies.

Lipid-Depleted Sarcoplasmic Reticulum Preparation. Lipid-depleted sarcoplasmic reticulum was prepared according to the following procedure. Light sarcoplasmic reticulum was suspended at 2.5 mg/mL in the following buffer: 0.3 M sucrose, 0.5 M KCl, 1 mM EDTA, 1.5 mM $MgCl_2$, 20 mM $CaCl_2$, and 10 mM Tris at pH 8. Dithiothreitol was added to 0.4 mg/mL. Recrystallized deoxycholate was added to 0.5 mg/mL. The solution was vortexed during the addition of deoxycholate. This was then incubated 10 min on ice and subsequently centrifuged at 196000*g* at 4 °C for 1.5 h. After centrifugation, the pellet was resuspended in a small amount of the same solution and vortexed. This was then bath-sonicated alternately for 30 s for 4 cycles. The volume was then brought up to the original volume with the same solution and the mixture centrifuged at 196000*g* at 4 °C for 1.5 h. The pellet was then resuspended in 5 mM HEPES, 0.3 M sucrose, 0.5 M KCl, 0.5 mM $MgCl_2$, and 10 μM $CaCl_2$ at pH 7. The suspension was vortexed and then centrifuged at 196000*g* at 4 °C for 1 h. This material was then applied to a sucrose density gradient, 0–40%, and a single band was harvested. This was pelleted and resuspended in the same buffer used for the NMR experiments. NMR spectra were obtained immediately. The lipid/protein ratio was determined by phosphate and protein assays as described below.

Assays. ATP hydrolysis was measured by the coupled assay method as described previously (Selinsky & Yeagle, 1984).

Calcium transport was measured in the same buffer in the presence of 50 μM Arsenazo III, a calcium-sensitive dye (Herbette et al., 1977), with potassium phosphate buffers. Calcium was omitted from the asssay buffer and then added to a final concentration of 0.1 mM to calibrate the absorbance change. The assay was initiated by the addition of ATP. The change in absorbance with time was measured on an Aminco DW2 dual-wavelength spectrophotometer with a sample wavelength of 660 nm and a reference wavelength of 685 nm. Typical measured ratios of calcium transported to ATP hydrolyzed in 125 mM phosphate were 1.2–1.6, comparable to previous preparations (Meissner, 1975). Calcium transport was reduced in the 10 mM phosphate buffers due to the reduction in calcium precipitation ability.

Flame photometry was performed on a Radiometer/Copenhager FLM 3 flame photometer. The monovalent cation concentrations, when no additional KCl was added, were 9.5 mM K^+ and 12 mM Na^+. Phospholipid concentration was determined by measuring total phosphate (Bartlett, 1959). Protein concentrations were determined by the Lowry method

[1] Abbreviations: LSR, light sarcoplasmic reticulum; NMR, nuclear magnetic resonance.

(Lowry et al., 1951), using bovine albumin as a standard.

Sample Preparation for Nuclear Magnetic Resonance. For NMR measurements, 1 mL of vesicles with a phospholipid concentration of 20–60 mM was pipetted into a flat-bottomed NMR tube. Vesicles were suspended in 20 mM HEPES/0.8 M sucrose, pH 7.0, unless otherwise stated. The potassium concentration of the sample was brought to 100 mM by the addition of solid KCl. After the addition of KCl, the samples were hand-shaken and allowed to incubate at room temperature (22 °C) before measurement. Measurements before and after NMR analysis demonstrated that less than 10% of the ATPase activity was lost during the course of the NMR experiment.

Nuclear Magnetic Resonance. ^{31}P nuclear magnetic resonance (NMR) spectra were obtained with a JEOL FX270 Fourier-transform spectrometer on a broad-band probe in 10-mm tubes at 30 °C. A fully phased cycle (32 pulse) chemical shift anisotropy (CSA) echo was used with a 20-μs echo ($\pi/2$ = 10 μs). The CSA echo sequence eliminates base-line artifacts, removing the need for first-order phase corrections (Rance & Byrd, 1983). Gated proton decoupling (on only during acquisition) at a decoupling field of 9 kHz was employed to eliminate sample heating. A 50-kHz spectral width was used. A delay time of 1 s was used between pulses. Spectral subtractions were performed in the frequency domain, using the JEOL FX data system. The only ^{31}P nuclei in the preparation were in the phospholipid component of these membranes. The protein was not phosphorylated under the conditions of these experiments.

Results

^{31}P NMR Powder Patterns Obtained from Light Sarcoplasmic Reticulum in the Presence of 110 mM K^+. The ^{31}P NMR spectrum derived from the phospholipids in the light sarcoplasmic reticulum in the presence of 110 mM K^+ appears in Figure 1A. Prominent in this spectrum is the axially symmetric powder pattern of the phospholipid bilayer in the light sarcoplasmic reticulum. Additional resonance intensity appears downfield of the normal bilayer powder pattern, indicating there may be a second domain of phospholipid in this membrane characterized by a different ^{31}P powder pattern.

Figure 1B shows the ^{31}P NMR powder pattern of the total lipid extract of the light sarcoplasmic reticulum in the presence of 110 mM K^+. This spectrum is characterized by an axially symmetric powder pattern characteristic of a phospholipid bilayer. No evidence for any other spectral components can be seen.

Subtracting the spectrum in Figure 1B from that in Figure 1A produced the difference spectrum shown in Figure 1C. This difference spectrum resembles an axially symmetric powder pattern, but is more than twice the width of the normal phospholipid bilayer powder pattern observed in the light sarcoplasmic reticulum or in the total lipid extract. This difference spectrum accounts for the resonance intensity downfield of the phospholipid bilayer powder pattern in the ^{31}P NMR spectrum of the light sarcoplasmic reticulum, in the presence of 110 mM K^+.

^{31}P NMR Powder Patterns Obtained from Light Sarcoplasmic Reticulum in the Presence of 10 mM K^+. The ^{31}P NMR spectrum of light sarcoplasmic reticulum in the presence of much lower [K^+] than above, 10 mM, appears in Figure 2A. The spectrum is dominated by an axially symmetric powder pattern, much like one of the powder patterns in the spectrum in Figure 1A. Little evidence can be found in this spectrum for an additional powder pattern, such as that found in the presence of 110 mM K^+.

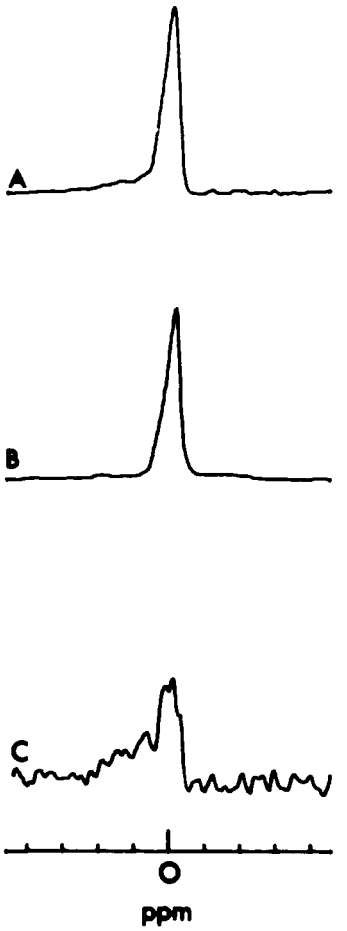

FIGURE 1: 109-MHz ^{31}P NMR spectra of light sarcoplasmic reticulum in 110 mM K^+, obtained with 80000 transients on material 20 mg/mL in protein. The smallest divisions on the chemical shift scale are 50 ppm. The top panel (A) is light sarcoplasmic reticulum. The middle panel (B) is the total lipid extract of light sarcoplasmic reticulum. The bottom panel (C) is the difference spectrum of the top two spectra, using the criteria for spectral subtraction described in the text. The vertical gain has been increased over the top two spectra by a factor of 4.

Figure 2B shows the ^{31}P NMR powder pattern of the total lipid extract of the light sarcoplasmic reticulum in the presence of 10 mM K^+. This spectrum is characterized by an axially symmetric powder pattern characteristic of a phospholipid bilayer. No evidence for any other spectral components can be seen.

Figure 2C shows the difference spectrum obtained by subtracting Figure 2B from Figure 2A. Little evidence was found for the kind of difference spectrum found in Figure 1C. Apparently, in low K^+, only one significant powder pattern can be found in the ^{31}P NMR spectra, and thus ^{31}P NMR is sensitive to only one domain of phospholipids in this membrane under these conditions. This is in good agreement with a previous report which employed a different approach (Selinsky & Yeagle, 1983).

^{31}P NMR Powder Patterns Obtained from Lipid-Depleted Light Sarcoplasmic Reticulum. Using the procedures described under Materials and Methods, preparations of calcium pump protein with a significantly reduced (compared to native light sarcoplasmic reticulum) lipid/protein ratio were harvested from a single band on a sucrose density gradient. If the broad powder pattern observed in Figure 1 was due to the presence of the calcium pump protein in the membrane, then at a lower lipid/protein ratio one might expect to see a more prominent

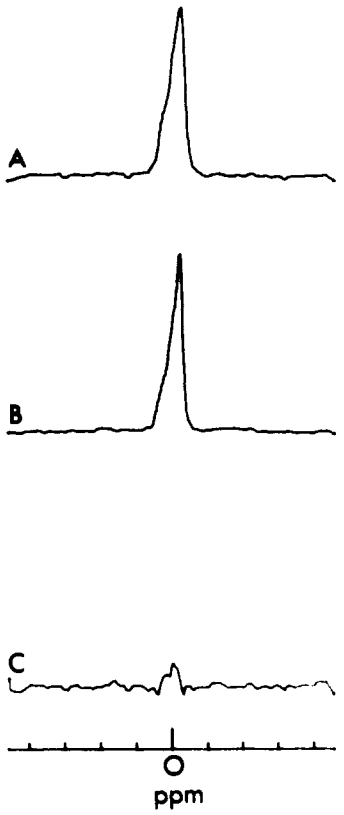

FIGURE 2: 109-MHz ^{31}P NMR spectra of light sarcoplasmic reticulum in 10 mM K^+, obtained with 80000 transients on material 20 mg/mL in protein. The top panel (A) is light sarcoplasmic reticulum. The middle panel (B) is the total extract of light sarcoplasmic reticulum. The bottom panel (C) is the difference spectrum of the top two spectra. The vertical gain has been increased over the top two spectra by a factor of 2.

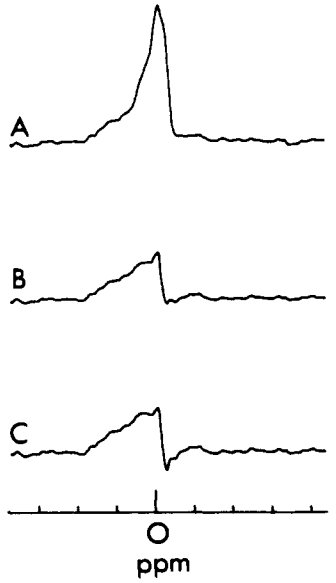

FIGURE 3: 109-MHz ^{31}P NMR spectra of lipid-depleted light sarcoplasmic reticulum in 110 mM K^+ at a 70/1 lipid/protein ratio, obtained with 150000 scans on material 30 mg/mL in protein. The top panel (A) is the lipid-depleted light sarcoplasmic reticulum. The middle panel (B) is the difference spectrum obbtained by subtracting a spectrum of the total lipid extract from the spectrum in (A), using the criteria for subtraction described in the text. The bottom panel (C) represents the subtraction of 10% more of the lipid extract powder pattern than used in (B).

contribution of the broad powder pattern to the spectrum. Figure 3 shows such an experiment. The spectrum in Figure 3 looks much like the spectrum in Figure 1, except that the broad feature is more prominent. Subtraction of the pure lipid spectrum from the observed spectrum produced the difference spectrum in Figure 3B. The degree of sensitivity of this procedure to the magnitude of the resonance subtracted is shown in Figure 3C. In this display, the magnitude of the normal bilayer resonance subtracted from the spectrum in Figure 3A is 10% greater in Figure 3C than in Figure 3B. This produced a difference spectrum with apparent negative resonance intensity in a portion of the spectrum. Subtracting 10% less of the normal bilayer only adds intensity to the broad component in the more shielded region of the powder pattern. The criterion in the procedure we used was to subtract the maximum of the normal bilayer resonance possible without obtaining significant negative deviations in the difference spectrum. Therefore, the procedure used provides a modestly sensitive determination of the minimum contribution of the broad component to the observed spectrum and exhibits less sensitivity to the maximum contribution of the broad component. Information on the shape of the broad ^{31}P powder pattern arising from light sarcoplasmic reticulum is presently available only by the subtraction method used here. However, the powder pattern obtained is similar to that reported previously for phospholipids bound to glycophorin (Yeagle & Kelsey, 1989).

Table I: Quantitative Analysis of the Resonance Intensity of the ^{31}P Powder Patterns of Membranes Containing Calcium Pump Protein from Light Sarcoplasmic Reticulum

sample	no. of samples	lipid/protein ratio (%)	lipids in broad component[a]
light sarcoplasmic reticulum	2	118 ± 10	19 ± 3
lipid-depleted sarcoplasmic reticulum	2	75 ± 10	18 ± 3

[a] Calculated as described previously (Selinsky & Yeagle, 1984), as lipids per calcium pump protein in the membrane giving rise to the broad powder pattern in the ^{31}P NMR spectrum.

Table I summarizes the results in a quantitative manner for the light sarcoplasmic reticulum and the delipidated membranes. It is not possible to obtain precisely the same lipid/protein ratio from two truly independent preparations of lipid-depleted sarcoplasmic reticulum. Therefore, the entry in the table for lipid-depleted sarcoplasmic reticulum reflects an average of two preparations with similar lipid/protein ratios. Table I shows that the proportion of the phospholipids contributing to the broad powder pattern is directly related to the protein content of the membrane. Thus, when the data are normalized for the protein content, similar values for the number of lipids per protein in the broad component are obtained from both the native membrane and the lipid-depleted membrane. This, plus the absence of the broad component in the absence of the membrane protein, suggests that the calcium pump protein plays a role in the formation of the phospholipid domain that gives rise to the broad component in the ^{31}P NMR spectra.

Effect of K^+ on Calcium Pump Activity. Light sarcoplasmic reticulum was isolated from sarcoplasmic reticulum by sucrose density gradient centrifugation. The activity of this membrane fraction was determined. Using phosphate as a calcium precipitating agent, ATP hydrolysis and calcium transport were monitored continuously under the same conditions at the same time in different spectrophotometers. Although the two assays were suboptimal with respect to the activity expressed, the assays were optimized with respect with comparison to each

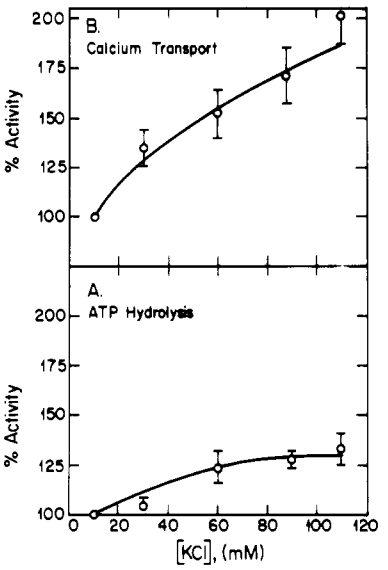

FIGURE 4: Effect of $[K^+]$ on the activity of LSR. The bottom panel (A) shows the stimulation of ATPase activity by K^+ and the top panel (B) shows the stimulation of calcium transport by K^+. Activity is presented as percent of activity at 10 mM K^+. Assays performed as described in the text. The data points represent the average and standard deviation of 14 independent experiments at each K^+ concentration.

other. That is, the main effort here was to measure calcium transport and ATP hydrolysis under conditions similar to those used for the NMR experiments, except for the phosphate in the activity assays.

The tracings for the two assays were similar, with a short "burst" period followed by an extended linear period. Each assay was found to be linear in the range of protein concentrations used (5–20 mg of protein). Typical values obtained for ATP hydrolysis activities ranged from 1 to 2 μmol (mg of Ca-ATPase)$^{-1}$ min^{-1}, depending upon the rabbit preparation and the sarcoplasmic reticulum fraction measured. Calcium transport activities ranged from 0.1 to 2.0 μmol (mg of Ca-ATPase)$^{-1}$ min^{-1}. The apparent low values for calcium transport are the result of the low (10 mM) phosphate concentration in our assay medium. Other investigators (Mitchell, 1983) have demonstrated that 125 mM phosphate is necessary for full transport activity to be measured. We have measured our samples in the presence of 125 mM phosphate or 10 mM oxalate (data not shown) and see activities similar to other preparations (Meissner, 1975). However, oxalate inhibits the coupled assay system used for the ATP hydrolysis measurements, and high phosphate concentrations introduce an unwanted counterion into our buffers (the ^{31}P NMR experiments were performed without phosphate in the buffers).

Measurements of ATP hydrolysis were made in the presence and absence of the calcium ionophore A23187. Under optimal conditions (100 mM KCl, 10 mM A23187), the ATP hydrolysis activity was 14.5 μmol (mg of Ca–ATPase)$^{-1}$ min^{-1}. The relative increases in activity due to potassium were equal in the presence and absence of A23187.

The potassium concentration dependence of the ATP hydrolysis and calcium transport activities of the light sarcoplasmic reticulum are shown in Figure 4. The data shown in this figure represent the average of 14 independent experiments. All measurements were made using freshly isolated material which was never frozen.

The ATP hydrolysis and calcium transport activities both increase as the potassium concentration increases. In all cases, the values for 110 mM potassium are significantly greater than the values for 10 mM potassium, at the 99% confidence level.

Discussion

These data show the effect of K^+ on phospholipid–protein interactions in light sarcoplasmic reticulum as measured by ^{31}P NMR. In the presence of 110 mM K^+, a substantial effect of the membrane protein on the behavior of the phospholipids was detected. Subtracting the spectrum of the lipid extract from the spectrum of the intact membrane would remove from the result the spectral component corresponding to the normal phospholipid bilayer in the sarcoplasmic reticulum spectrum. What was left, if anything, might be expected to correspond to another domain of phospholipid in the membrane with properties different from the normal phospholipid bilayer.

The difference spectrum obtained appeared to be an axially symmetric powder pattern of much greater breadth than the normal phospholipid bilayer powder pattern. This powder pattern is indicative of a phospholipid domain considerably more motionally restricted than the phospholipids in a normal phospholipid bilayer. The axially symmetric powder pattern is consistent with axial diffusion, although the orientation of that axis cannot be determined from the present data.

This broad powder pattern was seen in another case of phospholipid interacting with a membrane protein. Human erythrocyte glycophorin with four phospholipids tightly bound to it was reconstituted into a glycolipid bilayer, so that the only phospholipids contributing to the ^{31}P NMR powder pattern were those tightly bound to glycophorin. The ^{31}P NMR spectrum obtained was an axially symmetric powder pattern about 90 ppm broad (Yeagle & Kelsey, 1989), very much like the broad powder pattern observed in this study.

In the title of their report, Ellena et al. (1986) stated that no immobilized component could be detected in ^{31}P NMR spectra of sarcoplasmic reticulum. When we repeated their experiments using their same salt conditions, we too could not detect a broad component (Figure 2A). Ellena et al. (1986) did observe a small amount of intensity outside of the normal phospholipid pattern and calculated that if all of this intensity arose from phospholipids motionally restricted by the (Ca^{2+}, Mg^{2+})-ATPase, approximately 10% of the phospholipids would be motionally restricted. Using different methodology, we estimated that in 20 mM HEPES buffer with no added potassium that 10% of the phospholipids were motionally restricted (Selinsky & Yeagle, 1983). Thus, if proper attention is given to experimental details as described here, there is no disagreement between the work of Ellena et al. (1986) and Selinsky and Yeagle (1984).

Recent NMR studies have documented lipid behavior at the lipid–protein interface in other systems. In membranes containing human erythrocyte glycophorin (van Zoelen et al., 1978; Romans et al., 1979; Utsumi et al., 1980; Lau & Cowburn, 1981; Romans & Yeagle, 1981; Yeagle, 1984), in sarcoplasmic reticulum mebranes (Robinson et al., 1972; Stoffel et al., 1977; Selinsky & Yeagle, 1984, 1985), in cytochrome oxidase containing membranes (Longmuir et al., 1977; Rajan et al., 1981; Seelig & Seelig, 1985), in retinal rod outer segment disk membranes (Albert & Yeagle, 1983; Albert et al., 1985), in lipid–protein complexes of the ATP–ADP exchange protein from mitochondria (Beyer & Klingenberg, 1985), in Sendai virus envelope membrane (Abidi & Yeagle, 1984), and in serum low-density lipoproteins (Finer et al., 1975; Yeagle et al., 1977; Lund-Katz & Phillips, 1986), NMR studies provided evidence for at least two phospholipid domains in the presence of proteins.

The NMR data reported here suggested a structural change in the light sarcoplasmic reticulum induced by K^+. Some

limited data in the literature also suggest some sort of structural change induced by changes in K^+ concentration. Trypsin digestion of the Ca-ATPase is much more complete when digested in 10 mM histidine buffer in the absence of potassium ions than when digested in the presence of 100 mM KCl (Champeil, 1986; Louis, 1974).

The data reported here suggested that K^+ specifically induced changes in the function of the light sarcoplasmic reticulum. Both the calcium transport and the ATP hydrolysis activities were stimulated by K^+. Examination of Figure 4 suggested that the addition of potassium ions caused an apparent change in the calcium transported to ATP hydrolyzed ratio in the assay system employed. Finally, the effect of the K^+ on ATP hydrolysis persisted when the vesicles were made leaky to calcium (data not shown).

Monovalent cations interact with sarcoplasmic reticulum in two specific ways. First, monovalent cations, of which potassium is the best characterized, affect the activity of the Ca-ATPase. Potassium ions accelerated the decomposition of the phosphoenzyme intermediate in the reaction cycle of the Ca-ATPase. This effect was relatively specific for K^+ (and Na^+) in that LiCl, choline chloride, and Tris-HCl had no effect (Shigekawa, 1976). Potassium also inhibited the conversion of the phosphoenzyme from the ADP-sensitive to the ADP-insensitive form (Shigekawa, 1978), which may be the rate-limiting step in the overall stimulation of the enzyme by K^+. Furthermore, potassium accelerated the dissociation of ATP from the enzyme, but this effect could also be produced by choline chloride, so it was likely not a specific K^+ effect (Shigekawa, 1982).

Second, the longitudinal tubules contain a monovalent cation pore. The pore may allow the flow of monovalent cations opposite to the influx of calcium during muscle relaxation (Meissner, 1983).

It is possible that the structural changes induced by potassium and the alterations in membrane function induced by potassium are connected. However, the physical interpretation which relates these effects is unclear. We have proposed that differing $[K^+]$ and buffer conditions may affect the quaternary structure of (Ca^{2+}, Mg^{2+})-ATPase in the membrane, thereby altering the annular size and the number of phospholipids per protein in that annulus (Selinsky, 1984). At present, we have no data which directly supports this hypothesis. We do note with interest earlier data suggesting a correlation between ATPase activity and protein oligomeric structure [see Squier et al. (1988) and references cited therein].

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Spectroscopic Characterization of the Light-Harvesting Complex of *Rhodospirillum rubrum* and Its Structural Subunit†

Mary C. Chang,‡ Patricia M. Callahan,‡,§ Pamela S. Parkes-Loach,‡ Therese M. Cotton,‖ and Paul A. Loach*,‡
Department of Biochemistry, Molecular Biology, and Cell Biology, Northwestern University, Evanston, Illinois 60208-3500, and Department of Chemistry, University of Nebraska, Lincoln, Nebraska 68588-0304
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ABSTRACT: The spectroscopic properties of the light-harvesting complex of *Rhodospirillum rubrum*, B873, and a detergent-isolated subunit form, B820, are presented. Absorption and circular dichroism spectra suggest excitonically interacting bacteriochlorophyll *a* (BChl *a*) molecules give B820 its unique spectroscopic properties. Resonance Raman results indicate that BChl *a* is 5-coordinate in both B820 and B873 but that the interactions with the BChl C2 acetyl in B820 and B873 are different. The reactivity of BChl *a* in B820 in light and oxygen, or $NaBH_4$, suggests that it is exposed to detergent and the aqueous environment. Excited-state lifetimes of the completely dissociated 777-nm-absorbing form [1.98 ns in 4.5% octyl glucoside (OG)], the intermediate subunit B820 (0.72 ns in 0.8% OG), and the in vivo like reassociated B873 (0.39 ns in 0.3% OG) were measured by single-photon counting. The fluorescence decays were exponential when emission was detected at wavelengths longer than 864 nm. An in vivo like B873 complex, as judged by its spectroscopic properties, can be formed from B820 without the presence of a reaction center.

The pigment-protein complexes of photosynthetic bacteria responsible for the initial light-capture and charge-separation events of photosynthesis, namely, the light-harvesting (LH)[1] complexes and reaction centers (RC), absorb light of longer wavelengths (greater than 700 nm) than that absorbed by oxygenic organisms. This ability to utilize light of longer wavelengths is due partly to their use of bacteriochlorophyll (BChl), instead of chlorophyll (Chl), as the chromophore. BChl *a*, in comparison to Chl *a*, contains an acetyl group rather than a vinyl group at position 2 of ring I and a reduced double bond between the β-carbon atoms of ring II which alters its symmetry properties. As a result of these structural differences, about a 100-nm red shift (2000 cm^{-1}) of the major near-infrared (NIR) absorption band (the Q_y transition) is observed for monomeric BChl *a* relative to monomeric Chl *a* in acetone (770 versus 662 nm, respectively). However, an additional 100-nm red shift (1500 cm^{-1}) of the Q_y band is typically observed in vivo for BChl *a* containing LH complexes (van Grondelle, 1985; Thornber, 1986; Cogdell, 1986) and RC (Feher & Okamura, 1978; Gingras, 1978). The furthest red-absorbing BChl *a* containing bacterium known, *Chromatium tepidum*, has a Q_y band at 920 nm (Garcia et al., 1986).

In case of RC, recent X-ray crystallographic results (Deisenhofer et al., 1984; Allen et al., 1986, 1987a,b; Chang et al., 1986; Michel et al., 1986; Yeates et al., 1988) have confirmed that the primary electron donor consists of two BChl

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* Address correspondence to this author.
‡ Northwestern University.
§ Present address: Department of Chemistry, University of Missouri-Rolla, Rolla, MO 65401.
‖ University of Nebraska.

[1] Abbreviations: BChl, bacteriochlorophyll; B881, light-harvesting complex of wild-type *Rs. rubrum*; B873, light-harvesting complex of G-9 carotenoidless mutant or of benzene-extracted wild-type chromatophores; B820, subunit form of B873; 777(dissoc), 777-nm absorbing material formed by titrating B820 with 4.5-5.0% OG; B873(reassoc), light-harvesting complex formed by reassociation of B820; CD, circular dichroism; Chl, chlorophyll; CTAB, cetyltrimethylammonium bromide; DPG, diphosphatidylglycerol; EDTA, ethylenediaminetetraacetic acid; FWHM, full width at half-maximum; HEPES, *N*-(2-hydroxyethyl)piperazine-*N'*-2-ethanesulfonic acid; HPLC, high-performance liquid chromatography; LH, light harvesting; LHCII, accessory light-harvesting complex in oxygen-evolving organisms; NIR, near-infrared; OG, *n*-octyl β-D-glucopyranoside; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; RC, reaction center(s); RR, resonance Raman; THF, tetrahydrofuran.

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