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Phosphorus Nuclear Magnetic Resonance Studies of Lipid–Protein Interactions: Human Erythrocyte Glycophorin and Phospholipids†

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ABSTRACT: Human erythrocyte glycophorin containing four molecules of phospholipid tightly bound to the protein was isolated from human red cell ghosts. This protein preparation was reconstituted into a digalactosyl diglyceride bilayer. The ^{31}P NMR spectrum of this reconstituted membrane produced an axially symmetric powder pattern arising exclusively from the phospholipids bound to glycophorin. The width of the powder pattern, about 90 ppm, is about twice as broad as that normally exhibited by a phospholipid bilayer. The chemical shift tensor is perturbed relative to phospholipids in a bilayer. The spin–lattice relaxation rate of these protein-bound phospholipids is found to be nearly an order of magnitude faster than phospholipids in a bilayer. The results are consistent with phospholipids tightly bound to the membrane protein and undergoing rotational diffusion, perhaps as a complex of phospholipid and protein.

Lipid–protein interactions in membranes have been the subject of intense interest for a number of years because interactions between these two major components of biological membranes have been expected to be important to membrane function. However, before an adequate understanding of the potential roles of such interactions in cell membrane function can be obtained, a clear picture of the possible modes of interaction between lipids and proteins must be drawn.

It is reasonably well agreed that lipids and proteins in membranes must have some influence upon each other. For example, lipids (or their detergent replacements) are often required to activate membrane enzymes (Mitchell et al., 1983).

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Proteins in turn affect the behavior of membrane lipids, as evidenced by calorimetry data (van Zoelen et al., 1978), by electron spin resonance (Griffith & Jost, 1976), and in the morphological behavior of membrane lipids (Albert et al., 1984). The lipids immediately adjacent to the membrane protein were suggested to inhabit a distinctly different environment, motionally restricted relative to normal bilayer lipid (with respect to motional rates and/or motional freedom or orientational order). The number of lipids in that environment corresponded to the surface area of the protein in contact with the lipid bilayer (Griffith & Jost, 1976).

Subsequently, a distinctly different suggestion was advanced that lipids immediately adjacent to much larger membrane proteins experienced greater motional freedom than lipids in pure phospholipid bilayers (Deese et al., 1981). This hypothesis was used to explain ^2H NMR data of deuterated lipids in the presence of membrane proteins. Furthermore, the lipids were said to be virtually unrestricted in their exchange between sites in the lipid–protein interface and in the lipid bilayer. These suggestions were based on negative results, that is, the inability to detect separate spectroscopic components using ^2H NMR of deuterated lipids in the presence of membrane proteins. It was later shown why those ^2H NMR experiments did not reveal properties of the phospholipids that were interacting strongly with membrane proteins (Spiess & Sillescu, 1981; Lewis et al., 1984; Albert et al., 1985). Therefore, it was necessary to look elsewhere for information about that special lipid domain.

Recent NMR studies provided evidence concerning lipid behavior at the lipid–protein interface. 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 membranes (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 membrane proteins. One of the domains resembled normal phospholipid bilayers while the other domain was motionally restricted. The population of the motionally restricted domain was directly proportional to the protein content of the membrane. Ellena et al. (1986) suggested an alternative view, presenting ^{31}P NMR spectra of sarcoplasmic reticulum with little evidence for a broad spectral component. However, Ellena et al. (1986) did not accurately repeat the experiments of Selinsky and Yeagle (1984), having left potassium out of their system. Selinsky and Yeagle (1983) showed that in the absence of potassium the broad spectral component is difficult to see, as confirmed by Ellena et al. (1986).

The published studies have not provided a clear picture of the motional behavior of the phospholipids in the domain that is motionally restricted. The present work fills this gap in knowledge. In a novel experiment, a ^{31}P NMR spectrum of phospholipid bound to glycophorin was obtained for the protein–phospholipid complex in a lipid bilayer. The experiment was designed such that no contributions from a normal bilayer powder pattern obscured the powder pattern for the protein-bound phospholipids. The line shape was simulated by a broad axially symmetric powder pattern.

Materials and Methods

Chemicals. Egg phosphatidylcholine was purchased from Avanti Biochemicals, Inc., Birmingham, AL. Phosphoenolpyruvate, ATP, ADP, pyruvate kinase, lactic acid dehydrogenase, NADH, and Arsenazo III were purchased from Sigma Chemical Co., St. Louis, MO. Electrophoresis standards were purchased from Bio-Rad, Richmond, CA. Digalactosyl diglyceride was obtained from Sigma. Some of the digalactosyl diglyceride used was the generous gift of Dr. Raison, Macquarrie University, Sydney, Australia. Octyl β-glycoside was obtained from Calbiochem.

Phospholipid concentration was determined by measuring total phosphate (Bartlett, 1959). Protein concentrations were determined by the Lowry method (Lowry et al., 1951), using bovine albumin as a standard. Polyacrylamide gel electrophoresis was performed as described by Laemmli (1970) and modified by Smith et al. (1975).

Freshly out-of-date human erythrocytes were obtained from the local Red Cross blood bank. White or slightly pink ghosts were obtained by the procedure of Dodge et al. (1963). Glycophorin was isolated from human red cell ghosts by the lithium diiodosalicylate (LIS) extraction procedure of Marchesi and Andrews (1971). LIS was prepared from recrystallized diiodosalicylic acid by reaction with 1.1 equiv of LiOH, followed by recrystallization of the LIS. All glycophorin preparations were checked by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis on 12% gels. The gels were stained with Coomassie blue or periodic acid Schiff stain and the same patterns obtained. Representative gels of these glycophorin preparations were presented previously (Romans & Yeagle, 1981). Glycophorin concentrations were determined by using the procedure of Lowry et al. (1951) in 3% sodium dodecyl sulfate (SDS) in triplicate. An 18% correction was applied to this value (Grefath & Reynolds, 1974). A molecular weight for the protein part of glycophorin of 14 000 was used to calculate the mole ratio of phospholipid/protein. Phospholipid and protein analysis showed that approximately four phospholipids remained bound to glycophorin in our preparation after isolation.

Reconstitution of Glycophorin. Human erythrocyte glycophorin was recombined with digalactosyl diglyceride using the detergent octyl glucoside. Digalactosyl diglyceride was dispersed in 10 mM histidine–1 mM EDTA, pH 7, buffer from a dry powder. The dispersion was then solubilized with octyl glucoside. Glycophorin was added at a weight ratio of 100 mg of glycophorin to 20 mg of glycolipid. No effort was made to extensively delipidate the glycophorin, such as by extraction with organic solvents. After it was determined that all the protein and lipid components were solubilized, the mixture was dialyzed against a 1000-fold excess of the same buffer as above. Two changes of buffer were made over a period of 2 days of dialysis. The buffer was purged with nitrogen extensively prior to dialysis and the dialysis vessel sealed under nitrogen. Dialysis was performed at 21 °C. Following dialysis, the material was centrifuged in a 50 rotor in a Beckman L5-50 ultracentrifuge for 1 h at 49 000 rpm. A pellet was formed that was harvested, separated from the supernatant. Both the supernatant, which was clear, and the pellet, which was firm, contained glycophorin. The pellet was analyzed on a 0–50% continuous, linear sucrose density gradient, by centrifugation in an SW27 rotor for 18 h at 14 000 rpm.

Nuclear Magnetic Resonance. ^{31}P nuclear magnetic resonance (NMR) spectra were obtained with a JEOL FX270

Fourier-transform spectrometer in a broad-band probe in 10-mm tubes at 30 °C. A fully phase-cycled (32 pulse) Hahn echo sequence was used with either a 20-μs or a 40-μs echo. The echo sequence eliminates base-line artifacts, removing the need for first-order phase corrections (Rance & Byrd, 1983). Data were collected prior to the refocusing of the echo, and the FID was transformed from the top of the echo. The procedure avoids artifacts that arise when the receiver is turned on only at the expected refocusing of the echo. Because of the short echo time that must be employed to capture all the resonance intensity (due to very short T_2* of the broad component of the spectra), the finite length of the transmitter pulses (10 μs, 20 μs) must be taken into account in determining the refocusing point of the echo. Because of the very rapid decay in the FID of the broad component in these spectra, care must be taken to transform from the top of the echo because the broad features are readily lost in the Fourier transform if the refocusing point of the echo is missed. The ^{1}H decoupler was gated on during the acquisition and off the remainder of the time to prevent sample heating. The effectiveness of the decoupling was improved by using single-frequency decoupling rather than noise-modulated decoupling, with the frequency set at the resonance frequency of the phospholipid headgroup protons. This concentrates all the power of the decoupler in the small region of the ^{1}H NMR spectrum important to fully decoupling the phosphorus; 9 kHz of decoupling power was used. Exponential line broadening of 50–200 Hz was used. Recalculations showed no introduction of artifacts occurred in this range. Spin–lattice relaxation measurements were made by using the inversion–recovery method. The partially relaxed spectra were obtained by using a modification of the pulse sequence of Rance and Byrd (1983) which employed that pulse sequence for the observe sequence after inverting the magnetization and allowing partial relaxation.

Results

Human erythrocyte glycophorin, isolated as described under Materials and Methods, retained four tightly bound phospholipids. Three of the four phospholipids bound to glycophorin could be removed by a chloroform–methanol extraction, in good agreement with Armitage et al. (1977). Phospholipid analysis showed the presence of phosphatidylinositol among these extracted lipids as a major component, in agreement with Armitage et al. (1977). Analysis by thin-layer chromatography showed no phosphatidylinositol–phosphate. Analysis of the remaining one nonextractable phospholipid bound to glycophorin by ^{31}P NMR showed no phosphatidylinositol–phosphate in our preparations, in contrast to the results of Armitage et al. (1977) but in agreement with Shukla et al. (1979). Therefore, the powder patterns reported in this paper are not complicated by the presence of a phosphomonoester.

The purpose of the first experiment was to obtain the ^{31}P NMR spectrum of phospholipids bound to a membrane protein in a bilayer without interference from a normal phospholipid bilayer ^{31}P powder pattern. Glycophorin with four phospholipids bound was reconstituted into a glycolipid rather than a phospholipid bilayer [digalactosyl diglyceride forms a bilayer (Shipley et al., 1973; Sen et al., 1981)]. Thus, the only phospholipids contributing to the ^{31}P NMR spectrum would be the phospholipids originally bound to glycophorin. By so doing, the motional properties of a phospholipid bound to a membrane protein in a membrane could, in part, be determined from the ^{31}P powder pattern obtained.

The product resulting from reconstitution of glycophorin into membranes consisting of digalactosyl diglyceride was analyzed in several ways. The reconstituted material was placed on a 0–50% (w/w) continuous sucrose gradient. The material formed one predominant band, with one minor second band (10–15% of the material was in the minor band). The upper major band was at about 28% sucrose, and the lower minor band was at about 37% sucrose. The major band was harvested and analyzed further. The phospholipid:protein mole ratio was determined to be 3:1 in the reconstituted membrane. The total lipid:protein mole ratio was much higher due to the presence of considerable glycolipid in the reconstituted membrane. We calculated the lipid:protein ratio for this reconstituted membrane using a density for the protein of 1.47 (Grefrath & Reynolds, 1974). A lipid:protein mole ratio of 61 was obtained for the upper band.

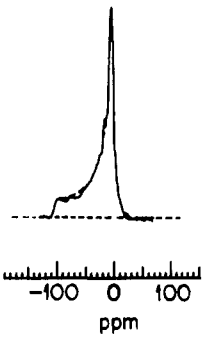

FIGURE 1: 109-MHz ^{31}P NMR spectrum for glycophorin, with three phospholipids bound, reconstituted into digalactosyl diglyceride bilayers. A total of 200 000 scans were obtained with 2K data points in the time domain at 20 °C with a 1-s repetition rate. The spectral simulation is represented by the dashed line. Where the dashed line is not visible, the simulation is identical with the data.

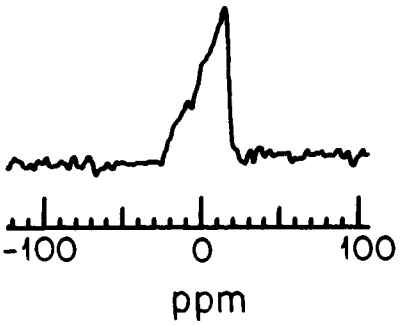

FIGURE 2: 109-MHz ^{31}P NMR spectrum for phosphatidylinositol 10 mol % in digalactosyl diglyceride bilayers. A total of 150 000 scans were obtained with 2K data points in the time domain at 20 °C.

From these analyses, it appears that a reconstituted glycophorin–glycolipid membrane has been formed that still contains three of the four phospholipids that were bound to the glycophorin after isolation and before the reconstitution (one phospholipid per glycophorin was apparently removed during reconstitution). The ^{31}P NMR spectrum of this reconstituted membrane was obtained. The result appears in Figure 1.

Figure 1 shows an axially symmetric powder pattern, indicative of rotational diffusion of the phospholipid. Two features distinguished this ^{31}P powder pattern from that observed in a pure phospholipid bilayer. One feature was the width of the powder pattern. $\sigma_\parallel - \sigma_\perp$ was about 90 ppm. For pure phospholipid bilayers, $\sigma_\parallel - \sigma_\perp$ was normally about 45 ppm (Yeagle, 1982), while for pure phosphatidylinositol $\sigma_\parallel - \sigma_\perp$ was about 57 ppm (spectrum not shown). The principle elements of the chemical shift tensor for phosphatidylinositol, as represented in the powder pattern of the dry powder obtained by cross-polarization ^{31}P NMR (spectrum not shown), were very similar to other phospholipids (Seelig, 1978). Phosphatidylinositol incorporated in the glycolipid did not produce a

Table I: Values for $\sigma_\parallel$ and $\sigma_\perp$ from the ^{31}P NMR Powder Patterns of Phosphatidylinositol in Different Membrane Environments

system	$\sigma_\parallel$ (ppm)	$\sigma_\perp$ (ppm)	$\Delta\sigma$ (ppm)
pure PI, hydrated lamellar	−38	19	57
PI in DGDG bilayers, hydrated lamellar	−24	12	36
PI bound to glycophorin in DGDG hydrated lamellar	−95	−4	91

broad powder pattern as shown in Figure 2. $\sigma_\parallel - \sigma_\perp$ is 36 ppm for phosphatidylinositol in digalactosyl diglyceride (see Table I).

The second feature was the position of the powder pattern on the chemical shift scale. The values associated with $\sigma_\parallel$ and $\sigma_\perp$ for this powder pattern were shifted from those expected from simple motional restriction. For example, $\sigma_\perp$ was more than 10 ppm downfield from the $\sigma_\perp$ of the normal phospholipid bilayer powder pattern. Given the overall width, one might expect an upfield shift of the high-field maximum of the powder pattern (from the normal bilayer powder pattern) to maintain the isotropic chemical shift constant. The downfield shift of $\sigma_\perp$ suggested a change in the chemical shift tensor, resulting perhaps from a change in the environment of the phosphate of the phospholipid when bound to glycophorin.

No resonance appeared characteristic of phospholipids in normal bilayers. An axially symmetric powder pattern of less than half the width would be expected if the phospholipids originally bound to glycophorin left the protein surface and mixed with the glycolipids. These data suggested that the phospholipids remained bound to the protein after reconstitution into the glycolipid bilayer.

Spin–lattice relaxation measurements were made for the broad powder pattern from the phospholipids bound to glycophorin in the glycolipid bilayer. The measurement was obtained from $\sigma_\perp$ because of the considerable challenge in obtaining adequate signal to noise ratio. A value of 0.2 s was obtained which is nearly an order of magnitude shorter than normally observed for phospholipid (Yeagle et al., 1984). Such a short T_1 might be due to paramagnetic contributions to the spin–lattice relaxation from transition-metal ions in the solution. However, the presence of the EDTA makes this unlikely. Furthermore, using the same materials to make buffers for ^{31}P spin–lattice relaxation measurements on pure phospholipids produces values for T_1 of an order of magnitude longer. Therefore, this short T_1 is unlikely the result of paramagnetic relaxation enhancement.

These data appear to be the first report of a ^{31}P NMR spectrum consisting only of a powder pattern of phospholipid bound to a membrane protein in a bilayer. One possible exception is a recent report of a ^{31}P NMR spectrum from lipid-depleted cytochrome oxidase (Seelig & Seelig, 1985) which also reports a very broad powder pattern. However, in that preparation, the protein content is sufficiently high that the ^{31}P NMR spectrum may have been distorted from that characterizing phospholipid next to protein at more moderate protein:lipid ratios, because of close packing of the protein.

In order to understand these spectra, spectral simulations were necessary. The equations of Seelig were used (1978):

$$p(\nu) \propto (1/3)[1 - (\nu - \nu_i)/(\nu_\perp - \nu_i)]^{-1/2}$$

where $p(\nu)$ is the transition probability as a function of the chemical shift and ν_i is the position of the isotropic resonance. This expression describes a single axially symmetric powder pattern of an idealized line shape and a singularity at $\nu_\perp$. Multiplication of this transition probability by a Lorentzian function then produces an appropriate line shape that simulates the real data.

Use of the Lorentzian function permitted the inclusion in the simulation of experimentally derived T_2 values and correction for filtering. In principle, each value of ν had a T_2 associated with it, and T_2 can be anisotropic across the powder pattern (Rajan et al., 1981); Yeagle et al., 1984). However, the use of average T_2 values produced satisfactory simulations and did not change in any substantive way the conclusions in this work. Simulations were calculated by using 500 points across the spectrum. The results were graphed as an analogue version of the digital data. The method for determining the quality of fit was described previously (Albert & Yeagle, 1983).

Simulation of the normal ^{31}P NMR bilayer powder pattern for these membranes containing glycophorin was carried out by using the upfield local maximum (rightmost shoulder) for $\sigma_\perp$ and the downfield shoulder as the value for $\sigma_\parallel$. The line shape used is an axially symmetric powder pattern, and the best fit was obtained with a value for $\sigma_\parallel - \sigma_\perp$ of about 90 ppm. The simulation is also presented in Figure 1.

Discussion

In recent years, a number of NMR studies identified the presence of at least two domains of phospholipids in reconstituted membranes containing human erythrocyte glycophorin. One domain exhibited resonances similar to that observed from pure phospholipid bilayers. The other was much broader (van Zoelen et al., 1978; Romans et al., 1979; Utsumi et al., 1980; Lau & Cowburn, 1981; Romans & Yeagle, 1981; Yeagle, 1984). Interpretation of these results was hampered due to lack of a clear representation of the powder pattern arising from phospholipids bound to a membrane protein in a lipid bilayer.

What was required was a ^{31}P NMR spectrum consisting only of resonances arising from phospholipids bound to a membrane protein in a lipid bilayer. For the success of this experiment, it was essential that the exchange of phospholipids between the motionally restricted domain and the normal phospholipid bilayer occurred very slowly. Otherwise, the ^{31}P NMR spectrum obtained would be a superposition of two powder patterns arising from each of the (at least) two phospholipid domains. Human erythrocyte glycophorin provided an appropriate system. Four of the phospholipids interacting with glycophorin were apparently in very slow exchange with other membrane lipids. They were isolated with glycophorin, and only rigorous treatment was capable of removing them. This protein–phospholipid complex can be incorporated into a glycolipid bilayer, and the phospholipids can be expected to remain bound to the protein. Therefore, glycophorin with these phospholipids tightly bound to it was a good candidate for the experiment to obtain a pure powder pattern from phospholipids bound to a membrane protein in a lipid bilayer.

The results shown in Figure 1 confirmed this expectation. The ^{31}P powder pattern obtained was distinctly different from the ^{31}P powder pattern obtained from pure phospholipid bilayers. The width of the powder pattern is approximately twice that seen from pure phospholipid bilayers. Furthermore, the ^{31}P powder pattern from the protein-bound phospholipids was shifted downfield from pure phospholipids.

Several new pieces of information about phospholipids in protein-induced motionally restricted domains can be extracted from the data in this report. One is that, in a bilayer, the phospholipids bound to the protein are experiencing rotational diffusion. Rotational diffusion is indicated by the axially

symmetric powder pattern. However, the motion of these protein-bound phospholipids is considerably restricted from that experienced by a phospholipid in a pure lipid bilayer. Spectral simulations have previously been published describing the effects of motional restriction on the ^{31}P NMR powder patterns from phospholipids experiencing rotational diffusion (Campbell et al., 1979). Changes in line shape are observed as a function of rotational correlation time. Comparison of those simulations with the broad powder pattern observed in the presence of some membrane proteins suggests a considerable reduction in rotational correlation time from pure phospholipids induced by the protein. The rotational correlation time for the phospholipid headgroup in the absence of protein is about 10^{-9} s in phospholipid bilayers (Yeagle et al., 1975). The rotational correlation time for phospholipids bound to glycoprotein must be much longer than that. It is interesting to compare this to the rotational diffusion correlation time obtained for glycophorin itself. From phosphorescence decays, a rotational correlation time for glycophorin in dimyristoylphosphatidylcholine vesicles of about 10^{-6} s was obtained (Jovin et al., 1981).

In addition to the fact that motion of the protein-bound phospholipids is restricted compared to normal bilayer phospholipids, information about the environment of the phospholipid phosphates when bound to the protein is also available. The chemical shift tensor is altered, as shown under Results. The axially symmetric powder pattern is shifted downfield from that observed for normal bilayer phospholipids. Thus, the environment of the phospholipid phosphate must be different when bound to a membrane protein than when it only has phospholipids as nearest-neighbors. This is a reasonable expectation for phospholipids bound to a membrane protein encountering amino acid side chains rather than phospholipid headgroups as nearest-neighbors.

The short T_1 also provides evidence for an altered environment when the phospholipids are bound to the protein. As shown in previous published work, the shortest T_1 that can be expected for phospholipids in bilayers without protein is about 1 s (Tamm & Seelig, 1983). This is also the shortest value obtained for phospholipids in biological membranes that were in a phospholipid bilayer (Yeagle et al., 1984). To obtain a significantly shorter T_1, an alteration in the nature of the dipolar interactions leading to the T_1 must occur. That is, the dipolar interactions resulting from ^1H–^{31}P internuclear interactions are proportional to the number of spins involved, are a function of the motions and motional rates characterizing the system, and are inversely proportional to the internuclear distance to the sixth power:

$$\frac{1}{T_1} \propto \frac{n}{r^6}\{f(\tau)\}$$

where r is the internuclear distance and $f(\tau)$ is a correlation function describing the anisotropic motions undergone by the headgroup. The short T_1 value obtained therefore is best explained by a change in the internuclear geometry characterizing the ^1H–^{31}P dipolar interactions; as mentioned above, changes only in the correlation function are not expected to cause such a short T_1. Therefore, the short T_1 is best understood in terms of a change in r and/or n, which must result from a change in the phosphate environment when the phospholipids are bound to the membrane protein.

All the evidence in this report is consistent with the concept that a few phospholipids, most likely phosphatidylinositol (Armitage et al., 1977), are tightly bound to glycophorin in membranes. The phospholipids did not exchange with the glycolipids as evidenced by the lack of a normal phospholipid bilayer powder pattern. Cholesterol cannot displace those tightly bound phospholipids (Yeagle, 1984). The state of phosphorylation of the phosphatidylinositol bound to glycophorin regulates the affinity of glycophorin for a linking protein, band 4.1, that links the membrane skeleton of the erythrocyte with the membrane (Anderson & Lovrien, 1984).

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Interaction of Smooth Muscle Tropomyosin and Smooth Muscle Myosin. Effect on the Properties of Myosin†

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ABSTRACT: Several techniques were used to investigate the possibility that smooth muscle tropomyosin interacts with smooth muscle myosin. These experiments were carried out in the absence of actin. The Mg^{2+}-ATPase activity of myosin was activated by tropomyosin. This was most marked at low ionic strength but also occurred at higher ionic strength with monomeric myosin. For myosin and HMM, the activation of Mg^{2+}-ATPase by tropomyosin was greater at low levels of phosphorylation. There was no detectable effect of tropomyosin on the Mg^{2+}-ATPase activity of S1. The KCl dependence of myosin viscosity was influenced by tropomyosin, and in the presence of tropomyosin, the 6S to 10S transition occurred at lower KCl concentrations. From the viscosity change, an approximate stoichiometry of 1:1 tropomyosin to myosin was estimated. The phosphorylation dependence of viscosity, which reflects the 10S–6S transition, also was altered in the presence of tropomyosin. An interaction between myosin and tropomyosin was detected by fluorescence measurements using tropomyosin labeled with dansyl chloride. These results indicate that an interaction occurs between myosin and tropomyosin. In general, the interaction is favored at low ionic strength and at low levels of phosphorylation. This interaction is not expected to be competitive with the formation of the actin–tropomyosin complex, but the possibility is raised that a direct interaction between myosin and tropomyosin bound to the thin filament could modify contractile properties in smooth muscle.

The dominant regulatory mechanism in smooth muscle involves phosphorylation and dephosphorylation of the 20 000-dalton light chains of myosin, catalyzed by myosin light chain kinase (MLCK)[1] and myosin light chain phosphatase, respectively. Phosphorylation is thought to alter the conformation of myosin and facilitate cross-bridge cycling, and this event is essential for initiation of the contractile response (Hartshorne, 1987). Regulation of skeletal muscle activity is different and utilizes the thin filament based regulatory proteins troponin and tropomyosin. Although the exact function of tropomyosin is not established, it is clearly an essential component of the regulatory system in striated muscle. Troponin is not found in smooth muscle (Hartshorne, 1987), although tropomyosin is present in approximately the same stoichiometry with actin as in skeletal muscle. Thus, there is no obvious role for tropomyosin in smooth muscle

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[1] Abbreviations: MLCK, myosin light chain kinase; HMM, heavy meromyosin; S1, heavy meromyosin subfragment 1; S2, heavy meromyosin subfragment 2; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; $NaDodSO_4$, sodium dodecyl sulfate; Tris-HCl, tris(hydroxymethyl)aminomethane hydrochloride; dans-tropomyosin, tropomyosin labeled with dansyl chloride.

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