Interaction of a Type II Myosin with Biological Membranes Studied by ^{2}H Solid State NMR†

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ABSTRACT: Deuterium nuclear magnetic resonance spectroscopy (^{2}H NMR) has been employed to investigate the interaction of lung type II myosin protein with neutral bilayers containing dimyristoylphosphatidylcholine (DMPC) as the only constituent and mixed bilayers containing the negatively charged lipid dimyristoylphosphatidylglycerol (DMPG). DMPC was deuterated at its headgroup by substituting the four protons at the α- and β-positions (DMPC-d_4) and the nine protons at the γ-position (DMPC-d_9). DMPG was perdeuterated at its headgroup (DMPG-d_5). No changes were observed in the quadrupole splittings or spin−lattice relaxation times for the deuterated DMPC headgroup segments when increasing amounts of myosin were added to liposomes, made exclusively of DMPC-d_9 or of DMPC-d_4. However, upon the insertion of the negatively charged lipid DMPG at 1:1 molar ratio into the DMPC bilayers, myosin was found to interact electrostatically with the liposomes, thereby affecting significantly both the quadrupole splittings and spin−lattice relaxation rates of the α-, β-, and γ-deuterons in labeled DMPC. Monitoring DMPG-d_5 in mixed DMPC/DMPG bilayers revealed a direct electrostatic interaction of DMPG with the protein, where positively charged lysine residues located at the tail domain of myosin provide the necessary sites for the interaction to occur. When ATP and Mg^{2+} were complexed to the head domain of myosin, a reduced interaction with the negatively charged bilayers was observed. The results clearly indicate that a type II myosin can interact with membranes without the need for a specific hydrophobic domain or an anchor in the protein molecule, provided that negatively charged lipids are present in the bilayer.

Myosin is an important biological motor responsible for motion in several systems (*1*, *2*). Muscle contraction and relaxation, for example, are performed by myosin, powered by the hydrolysis of ATP, moving along actin filaments on a complex supramolecular arrangement of several distinctive protein molecules. Muscle myosins have similar structures and form dimers, with each unit having a globular head and a long rod (*3*). While the head stays free for interactions with actin (*3*) and responds to the enzymatic ATPase activity, the long rod makes a super-coiled coil with a unit rod from another molecule. More recently, structural models have been described for various myosins, which were initially found in unicellular organisms, but later also in mammalian cells (*4*). This so-called type I myosin (for its single molecular arrangement) or "unconventional" myosin has a feature in common with the type II myosins (or "conventional"), namely the ∼80 kD "head" domain, although they also found it to be attached to a variety of "tail" domains.

Recently, association of single-headed (type I) myosin from *Acanthamoeba* directly with membranes has been reported (*4*−*7*), a fact which could explain several aspects of cellular movements, especially trafficking of organelles. However, the possibility of conventional type II myosins to behave in a similar way has not yet been reported. Due to the importance of the association with membranes as a process essential for the function of both types of myosin from tissues where cellular movement is important, further investigations were carried out in this study.

Deuterium NMR[1] has been used extensively to study membrane surface properties in various systems by using headgroup deuterated lipids as nonperturbing probes in the bilayer (*8*−*13*). Since the conformation of the lipid headgroups responds very sensitively to molecular reorientations which occur upon changes in the electrostatic membrane potential, modulation of these properties can be monitored directly in the observed ^{2}H NMR spectra. Changes in the electrostatic surface potential by adding charges to the bilayer surface is thought to result in a change of the tilt of the phosphatidylcholine electric dipole (P^--N^+) in the headgroup with respect to the membrane. The origin of this change may be external, as in the case of binding of surface ligands such as divalent cations or peripheral proteins or peptides (*10*, *14*−*16*), or internal, within the membrane as in the case of mixing charged phospholipids, amphiphiles, or intrinsic peptides (*9*, *12*, *17*−*20*).

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[1] Abbreviations: NMR, nuclear magnetic resonance; DMPC, 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine; DMPG, 1,2-dimyristoyl-*sn*-glycero-3-phospho-*rac*-glycerol; $\Delta\nu_Q$, deuterium quadrupole splitting; T_{1Z}, spin−lattice relaxation time.

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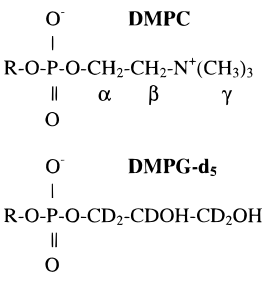
Based on earlier results (*21*), the work presented here studies the association of smooth muscle myosin (type II myosin) from lung with neutral and charged membrane surfaces by employing solid-state ^{2}H NMR techniques. By using deuterons specifically placed in the headgroups of DMPC and DMPG, changes in the observed quadrupole splittings provide information on the structural perturbation in the lipid headgroups upon protein binding (*12*, *22*). The analysis of deuterium NMR line shapes of pure DMPC and DMPC/DMPG bilayers upon binding of protein revealed an interaction of myosin with the bilayer when charged lipids such as DMPG were present. The results are discussed in terms of a specific electrostatic protein−lipid interaction between myosin and charged lipid, as seen in the NMR spectra of labeled DMPG in mixed membranes.

MATERIALS AND METHODS

Materials. Lung myosin was prepared essentially as previously described by Frederiksen et al. (*23*). Myosin was isolated from bovine lung alveoli and purified by precipitation of an actin−myosin complex. The complex was dissociated by ATP/Mg^{2+} treatment and fractionated with $(NH_4)_2SO_4$ to an 11.3 and 2.2-fold enrichment (measured by Ca^{2+} and K^+, EDTA−ATPase activities). The purity of lung myosin preparation was assessed by PAGE showing a single band for the pure myosin with a molecular mass of ca. 200 kDa. This band presented three types of polypeptide chains when treated with β-mercaptoethanol and SDS, with molecular masses of 176, 19, and 16 kDa, a peptide chain assembly typical for type II myosins. Maximum values of enzyme activity were observed at pH 8 and pH 9 for Ca^{2+}, K^+, and EDTA−ATPase activities, respectively.

Deuterated phospholipids were specifically labeled in the headgroup as indicated below, and were prepared as follows. 1,2-Dimyristoyl-*sn*-glycerol-3-phosphocholine was specifically deuterated at the γ-methyls (DMPC-d_9) by methylation of DMPE with CD_3I, as described by Eibl (*24*). DMPC deuterated at the α- and β-methylenes (DMPC-d_4) was prepared from the reaction of CH_3I and DMPE-d_4 which was synthesized from dimyristoylglycerol with perdeuterated ethanolamine (*24*), which in turn was produced by catalytic exchange of protonated ethanolamine against D_2O (*25*). DMPG-d_5 was synthesized by the method of Harlos et al. (*26*) and Sixl et al. (*27*).



Sample Preparation. Samples for NMR experiments were prepared from 50 mg/mL stock solutions of each lipid in $CHCl_3/CH_3OH$ (2:1; vol:vol) by mixing suitable amounts of each solution to give the desired composition. Solvent was removed in a N_2 stream at ambient temperature and dried under high vacuum overnight. The dried lipid or lipid mixture was then fully rehydrated by vortexing in an excess of a buffer containing 20 mM Tris, 20 mM glycine, 1 mM EDTA, 0.6 M KCl, at pH 8.2 (which was the optimum pH and ionic strength for myosin Ca^{2+} ATPase activity) followed by three cycles of freezing under liquid N_2 and thawing at 37 °C. In all cases, deuterium-depleted water (Aldrich, Gillingham, UK) was used.

NMR Measurements. Deuterium NMR spectra were recorded on a Bruker MSL 400, Nicolet 360, or a Bruker DPX 300. For all spectrometers typical pulse lengths were 4−6 μs for 90° pulses. Solid-state spectra were obtained using a quadrupolar echo pulse sequence (90°-τ-90°), and for τ a typical value of 30 μs was set (*28*). Measurements of the spin−lattice relaxation time (T_{1Z}) were carried out applying an inversion−recovery sequence (180°-τ_2-90°-τ-90°) (*29*). The recycle time was 500 ms. All NMR experiments were recorded with quadrature detection and appropriate phase cycling schemes at 303 K (regulated to within ± 0.5 degrees by an N_2 temperature control unit). The number of acquisitions varied between 1000 and 10 000 scans.

RESULTS

The electrostatic interaction of conventional type II myosin with neutral and negatively charged membranes was monitored by ^{2}H NMR of headgroup-deuterated DMPC and DMPG lipids. DMPC was labeled either at the choline moiety as DMPC-d_9 or at the choline backbone as DMPC-d_4, thereby allowing a characterization of electrostatic interactions along the whole headgroup moiety by exploiting characteristic changes in the quadrupole splittings as a function of the membrane surface charge (*9*, *10*, *18*). In the experiments, either neutral bilayers containing DMPC as the only constituent or negatively charged membranes containing DMPC and DMPG in a 1:1 molar ratio were used. The applied lipid-to-protein ratio was mainly varied from 8000:1 to 30000:1 (molar ratio).

Interaction with Neutral Membranes. Deuterium NMR spectra of DMPC-d_4 liposomes containing myosin at various ratios are presented in Figure 1. For DMPC bilayers without any protein, the spectrum obtained (top) exhibits a narrow axially symmetric powder pattern characteristic of randomly oriented lamellar bilayers in the liquid-crystalline (L_α) phase (*17*, *30*). The spectral line shape is dominated by an axially symmetric electric field gradient tensor (EFG), where the intense resonances originate from lipid molecules oriented at 90° relative to the applied magnetic field. The measured quadrupole splitting is 5.6 kHz at 303 K and corresponds to the difference between the maximal spectral intensities in the spectrum. The splitting can be assigned to the β-deuterons, more remote from the bilayer, which overlaps the α-deuteron resonances at this temperature (*31*, *32*). Adding increasing amounts of myosin shows essentially the same broad averaged powder spectra as already obtained for DMPC liposomes without any protein. The quadrupole splittings and the general feature of the line shapes remain unchanged for all protein concentrations used. This result was expected since deuterium resonance spectra of DMPC-d_9 for the same ratios of lipid and myosin did not reveal any spectral changes (*21*). T_{1Z} measurements performed on all the DMPC-d_4/myosin liposomes also revealed no change in the spin−lattice relaxation of the lipid for any of the protein

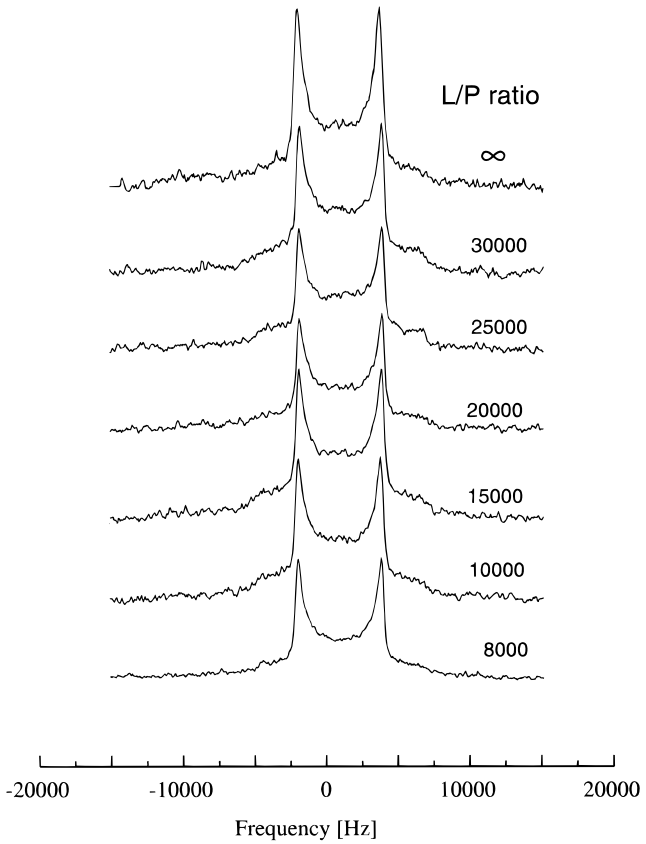


FIGURE 1: ^{2}H NMR spectra of DMPC-d_4 and myosin-DMPC-d_4 complexes (20 mg of lipid) at different lipid to protein molar ratios (as indicated) at 303 K. Experimental conditions are described in the text.

concentrations employed when compared to DMPC liposomes without protein. The NMR spectra and T_{1Z} values obtained for DMPC-d_4 bilayers clearly indicate no specific interaction between myosin and the bilayer surface of neutral DMPC liposomes.

Interaction with Charged Membranes. Binding of myosin to charged membrane surfaces was studied by adding protein at various concentrations to mixed liposomes containing DMPC-d_4 and DMPG in 1:1 molar ratio. The ^{2}H NMR spectra for vesicles with and without protein are shown in Figure 2. Compared to spectra obtained for pure DMPC bilayers, the spectrum of DMPC-d_4 is already substantially altered in mixed lipid vesicles before any addition of myosin. Clearly a reorientation of the DMPC-d_4 headgroup occurred, reflecting the existence of a charged membrane surface due to DMPG, which has a permanent negative charge in the headgroup at physiological pH. This conformational reorientation leads to the observation of two separate quadrupole splittings as described before by Sixl and Watts (*27*). The β-CD_2 quadrupole splitting decreases to 1.4 kHz, while the outer doublet, arising from the α-CD_2 segment, increases to 11.3 kHz. Addition of myosin to the charged liposomes introduces further changes to the conformation and orientation of the choline backbone as seen in the NMR spectra (Figure 2). Upon addition of increasing amounts of protein the splitting for the outer doublet (α-deuterons) decreases steadily, while for β-CD_2 segment an increase of the quadrupolar splitting can be observed. The way in which the increasing concentration of protein modulates the surface charge density of the membrane can more clearly be seen in

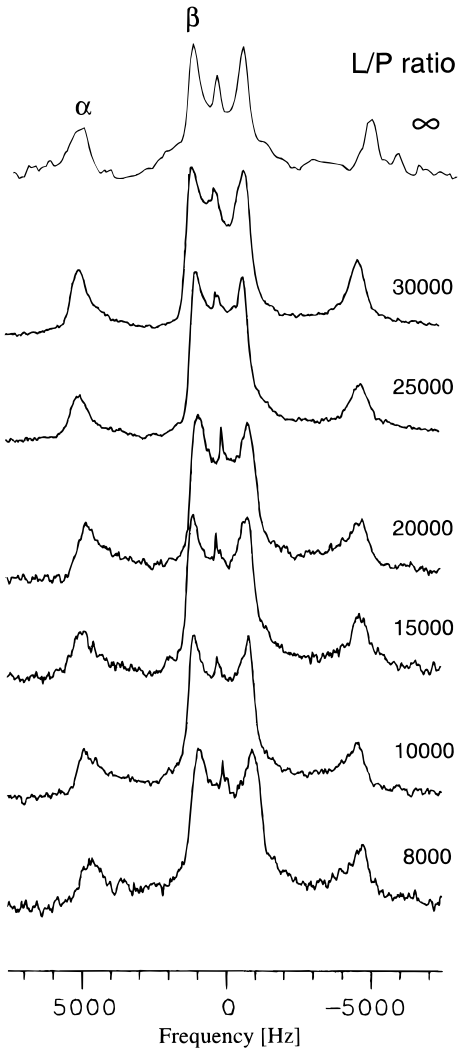


FIGURE 2: ^{2}H NMR spectra of DMPC-d_4/DMPG liposomes (40 mg of lipid) at 1:1 molar ratio, after binding of myosin at various lipid to protein molar ratios (as indicated) at 303 K. Experimental conditions are described in the text.

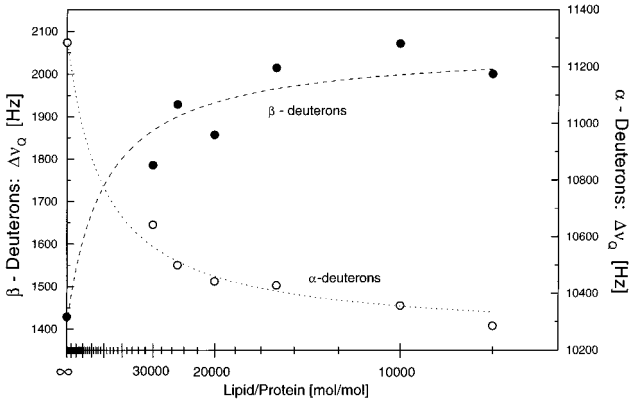


FIGURE 3: Counter-directional charge effect produced by myosin on the quadrupole splittings of DMPC-d_4/DMPG bilayers (1:1 molar ratio) as a function of the concentration of myosin bound to the charged vesicles at 303 K for the α-CD_2 segment (○) of DMPC and for the β-CD_2 segment (●).

Figure 3. Here the measured quadrupolar splittings for both α- and β-segments are plotted against the myosin concentration. The diagram reveals a significant change in the conformational state of the headgroup after addition of myosin in a concentration as low as 30000:1 lipid:protein

molar ratio. Upon further addition of protein the changes are less dramatic but can still be observed. Finally the splitting for the α-deuterons decreases slightly from 11.3 to 10.3 kHz, while the β-CD_2 quadrupole splitting exhibits a pronounced increase from 1.4 to about 2.0 kHz when compared to the measured absolute values.

To monitor the interaction of myosin with the most remote part of the membrane, studies were also performed using DMPC-d_9 in mixed DMPC/DMPG (1:1 molar ratio) vesicles. The results obtained for different myosin concentrations are seen in Figure 4, where the quadrupole splitting is plotted with the protein concentration. A behavior similar to the one observed for the α-deuterons in mixed bilayers of DMPG and DMPC-d_4 at 1:1 mole ratio was found for the γ-deuterons, in agreement with earlier results (*21*). The observed quadrupole splitting of around 1000 Hz decreases after addition of protein, dropping finally to a value below 900 Hz after further addition of myosin (final concentration of 8000:1 lipid:protein molar ratio). It has been shown that by analyzing the decrease in the quadrupole splitting, information about the exchange rate of lipids between the bulk lipid phase and the complexes with a given protein can be derived (*15*, *33*). For a fast, two-site exchange of lipid between the protein-free part of the bilayer and the protein-associated phase the observed quadrupole splitting $\Delta\nu_{Qt}$ can be expressed by a weighted average of the quadrupole splittings in each of the two environments such that:

$$n_t = n_c + n_f$$
$$\Delta\nu_{Qt} = n_c(\Delta\nu_{Qc} - \Delta\nu_{Qf})/n_t + \Delta\nu_{Qf} \qquad (1)$$

where n_t, n_c, and n_f are the numbers of total, complexed, and free lipid molecules per protein molecule; $\Delta\nu_{Qt}$ is the total quadrupole splitting (observed spectra), $\Delta\nu_{Qc}$ is the quadrupole splitting of the complexed lipid, and $\Delta\nu_{Qf}$ is the quadrupole splitting of the free lipid. Therefore, if the assumptions about the exchange mechanism are valid, plotting of $\Delta\nu_{Qt}$ against $1/n_t$ will give a straight line with a slope $= n_c(\Delta\nu_{Qc} - \Delta\nu_{Qf})$ and an intercept $= \Delta\nu_{Qf}$, as shown for some protein systems (*15*). In the case of myosin, an analysis of the observed quadrupole splittings of γ-deuterons of DMPC-d_9 in the mixed bilayers done in the same way, results in a plot displayed in the inset of Figure 4. The curve clearly reveals no simple two-site exchange of a fixed number of lipids associated with the myosin protein, but rather a more complex interaction. However, the observation of a single, axially symmetric Pake doublet indicates that any possible exchange of lipids between different environments must happen at least fast on the ^{2}H NMR line shape time scale.

To study a possible involvement of the head domain of myosin where the ATPase active site and actin binding site are located, ATP and Mg^{2+} were used to complex myosin prior to the interaction with the lipid bilayers. Previous addition of 100 mM ATP and 1mM $MgCl_2$ to the protein induced an observable change in the spin−lattice relaxation T_{1Z} (Figure 5a) and quadrupole splitting (Figure 5b) after adding the protein to DMPC-d_9/DMPG bilayers at various concentrations. The measurements clearly show that the decrease in quadrupole splitting is attenuated when myosin interacted previously with ATP and Mg^{2+}. This effect is also reflected in the T_{1Z} experiments, where the concentration

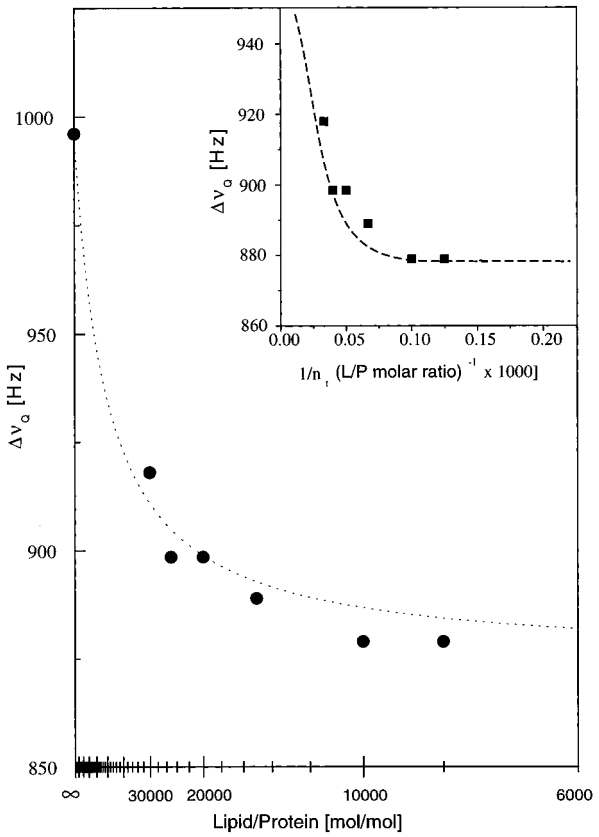


FIGURE 4: Quadrupole splitting for the γ-$(CD_3)_3$ segment of DMPC-d_9 for various myosin concentrations in binary mixtures of DMPC/DMPG (1:1 molar ratio) membranes at 303 K. Inset diagram: Variation of the quadrupole splitting as a function of the reciprocal of the lipid to protein molar ratio ($1/n_t$) according to eq 1.

dependence of the T_{1Z} values for pretreated myosin is very weak (Figure 5a), while for untreated myosin a pronounced increase up to a T_{1Z} maximum could be observed which decreases to 42 ms upon further addition of protein. Our observation suggest that the myosin head domain, where ATP is located, also participates either directly or indirectly in the association with charged membranes.

The above experiments were designed to monitor, by ^{2}H solid-state NMR, the outermost region of the uncharged lipid (deuterated phosphatidylcholine) moiety in neutral and negatively charged membranes. To explore whether phosphatidylglycerol was the component of the bilayer interacting with myosin, mixed liposomes were prepared containing nonlabeled DMPC and phosphatidylglycerol perdeuterated at the headgroup (DMPG-d_5) in a 1:1 molar ratio. The sensitivity of this approach is clearly shown in Figure 6 (bottom), where a ^{2}H NMR spectrum is observed for pure DMPC/DMPG membranes in the fluid phase, revealing well-resolved quadrupolar splittings for the various deuterons incorporated into the headgroup of DMPG. Upon addition of myosin at a 8000:1 (lipid:protein molar ratio) these resonances collapse into a single resonance (Figure 6; top), indicating a direct electrostatic interaction between the charged residues of the myosin molecule with the bilayer. The results of the spin−lattice relaxation experiments performed on these vesicles are summarized in Table 1, together with the other ^{2}H NMR spectral parameter obtained. It can be seen from the reduced splitting for all positions that the presence of myosin in the system reduces signifi-

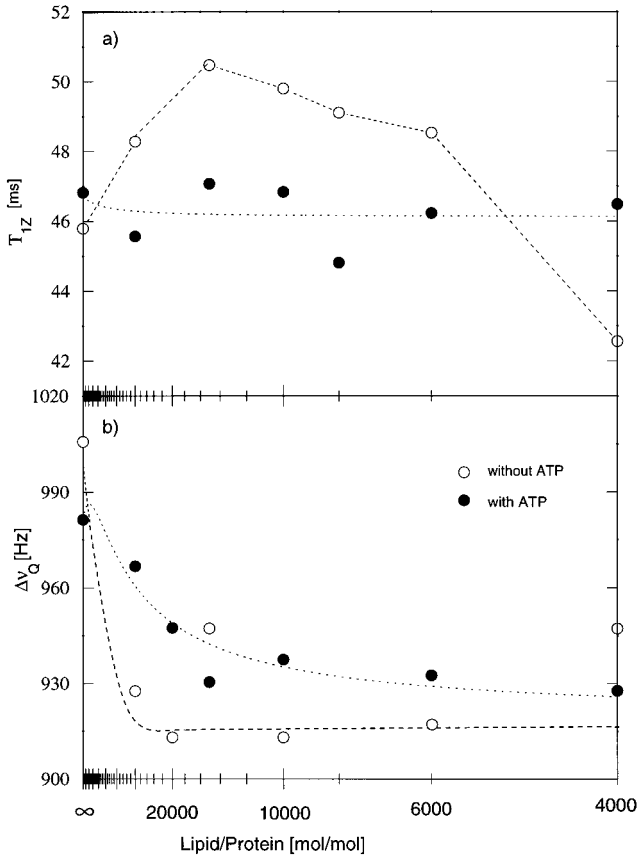


FIGURE 5: Effect of myosin added at various concentrations on spin−lattice relaxation T_{1Z} and quadrupole splitting $\Delta\nu_Q$ for DMPC-d_9 in mixed DMPC/DMPG bilayers (1:1 molar ratio) at 303 K. Closed circles: previous treatment of myosin with 100 mM ATP and 1 mM $MgCl_2$. Open circles: untreated myosin added to vesicles.

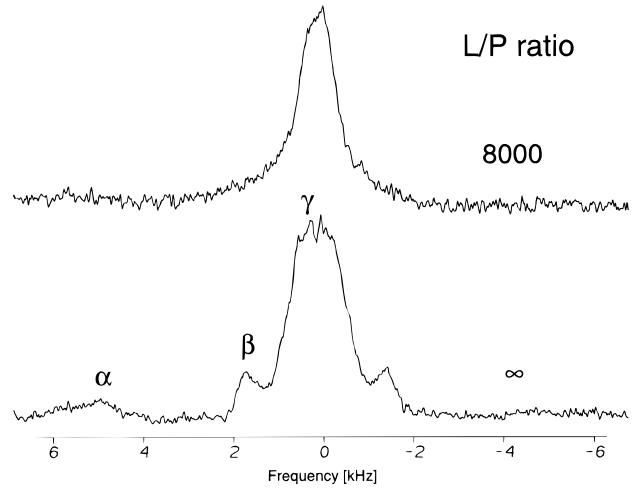


FIGURE 6: ^{2}H NMR spectra for DMPG-d_5 of DMPC/DMPG (1:1 molar ratio) liposomes at 1:1 mole ratio at 303 K without myosin (bottom) and in the presence (top) of myosin at 8000:1 lipid to protein molar ratio.

cantly the order of the lipid headgroup. Interestingly, the fast molecular motions assessed by the T_{1Z} values are only reduced for the γ-^{2}H position.

DISCUSSION

As monitored by ^{2}H NMR, myosin does not interact with neutral bilayers which contain only the phospholipid DMPC, but binds strongly to membranes when the negatively charged lipid DMPG is present. This is seen in the NMR spectra for DMPC bilayers where no observable spectral changes could be detected for various protein concentrations, while for mixed DMPC/DMPG membranes the line shapes significantly changed upon addition of the protein. Analysis of the various NMR spectra clearly suggest an electrostatic interaction of the protein with charged bilayers. The results obtained in this study support previous reports of type I myosin interactions with lipids, where less sensitive techniques were employed. These previous studies demonstrated that charged lipids were always necessary for the lipid−protein interaction to occur (*1*).

Table 1: ^{2}H NMR Quadrupole Splittings ($\Delta\nu_Q$) and Relaxation Times (T_{1Z}) for the Three Segments (α-, β-, and γ-) of DMPG-d_5 in Mixed DMPC/DMPG Liposomes in the Absence and Presence of Myosin (8000:1 L/P Molar Ratio) at 303 K

^{2}H segment	DMPC/DMPG-d_5	DMPC/DMPG-d_5 + myosin
α-position	$\Delta\nu_Q = 9606$ Hz; $T_{1Z} = 10$ ms	$\Delta\nu_Q = 0$ Hz; av $T_{1Z} = 10$ ms
β-position	$\Delta\nu_Q = 3149$ Hz; $T_{1Z} = 10$ ms	as above
γ-position	$\Delta\nu_Q = 671$ Hz; $T_{1Z} = 13$ ms	as above

Deuterium NMR. Using ^{2}H NMR of headgroup-deuterated phospholipids has permitted us to detect changes of the membrane surface charge of various lipid bilayer suspensions upon interaction with the protein myosin. This ^{2}H NMR approach had already been established earlier by us for myelin basic protein (*32*, *33*). The headgroup of PC in liquid crystalline bilayers undergoes a conformational change in response to a variation in membrane surface charge, a change reflected in the ^{2}H NMR spectra arising from α- and β-deuterons. The effect is often described as the "molecular voltmeter" (*9*, *18*, *22*, *34*).

Interaction with Neutral Membranes. Adding myosin to pure DMPC-d_4 bilayers at various protein concentrations did not lead to any change of either the measured ^{2}H NMR line shapes (Figure 1) or the T_{1Z} values. In the case of DMPC-d_4 membranes, the overlapping β- and α-deuterons (*31*) have the same quadrupole splitting for all used protein concentrations at the measured temperature. Any possible binding of myosin containing positively charged residues to the neutral membrane would have caused a response of the lipid headgroup in two ways: either (i) a conformational change visible in a counter directional change in the splittings of the α- and β-deuterons (*9*, *12*), or (ii) a simple nonspecific response, such as a change in headgroup flexibility, which would effect all headgroup segments in the same way (*34*, *35*). On the basis of these measurements it is possible to exclude any binding of myosin to neutral bilayers on the time scale of our NMR experiments. This result confirms previous observations on the same system using DMPC-d_9 to probe the outermost region of the lipid−water interface (*21*). Also T_{1Z} relaxation data obtained for DMPC-d_9 do not vary upon addition of protein to the DMPC vesicles, thereby suggesting no change at all in the headgroup flexibility through the protein (*21*).

Interaction with Charged Membranes. In contrast to the results obtained on neutral DMPC-vesicles, the spectra obtained from negatively charged DMPG/DMPC bilayers show a strong direct interaction of myosin with the charged membrane surface. While in mixed vesicles where the deuterated neutral lipid DMPC was used (Figure 2), elec-

trostatic interactions between myosin and the negative bilayer could only be monitored indirectly, permitted the use of headgroup-deuterated DMPG to explore directly the interaction of the protein with the charged lipid component (Figure 6). As seen in Figure 6, the DMPG-d_5 spectrum changes dramatically upon addition of protein, leading to a collapse of all resonances into a single broad peak. This change in the spectrum, as previously seen with myelin basic protein (*15*), indicates a strong direct electrostatic interaction between myosin and the acidic polar headgroup of phosphatidylglycerol, affecting its orientation. Additionally, for the γ-deuterons the rate and/or amplitude of molecular motion is also affected, as the measured shorter T_{1Z} values for these deuterons indicate.

However, compared with the observed changes in the NMR spectrum for DMPG-d_5 the changes seen in the spectra of DMPC at α-, β-, or γ-position are quite moderate, suggesting that the DMPC headgroup cannot bind directly to the protein and reacts only upon a general change in the membrane surface charge. Adding the lipid DMPG which carries one negative charge at its headgroup to the DMPC bilayers substantially alters the ^{2}H NMR of DMPC-d_4 spectra (Figure 2) in a way expected for the concept of the molecular voltmeter (*9*, *18*, *22*, *35*). The observed counter directional change in the quadrupolar splitting from α-methylene to β-methylene (Figure 3) reflects the conformational change in the DMPC headgroup as a response to the presence of a negative surface charge. Adding myosin to the negatively charged DMPC/DMPG bilayers displays a clear concentration dependence of the quadrupole splitting for the α- and β-deuterons toward a reversed counter directional way (Figure 3) as expected when positive charges are added. In this case myosin must act through positively charged residues with the membrane, thereby partially neutralizing the membrane's negative charge. The observed "differential response" for each DMPC headgroup segment is characteristic for an electrostatic interaction between the protein and the membrane and cannot be explained in a simple structural perturbation of the headgroup. If the latter would be the case, both segments would be affected in the same direction, as seen in the case of mellitin (*35*).

The observed strong electrostatic interactions of myosin with charged liposomes are very similar to others described earlier for several classes of peripheral membrane proteins (*21*, *33*, *36*). These proteins bind preferentially to negatively charged lipids through their net positive charges where residues from the protein molecule interact with negatively charged phospholipids, such as DMPG. In the case of myosin, this type of interaction would preferably occur at the outer part of the super-coiled coil rod where positively charged lysine residues are distributed in a periodic way (*3*), leaving free the head domain, where the ATPase active site is located, for further interactions. This conclusion is further supported by the recent description of a whole family of unconventional nonmuscle myosins which probably move organelles within the cells by direct interaction with the membrane (*4*). However, the interaction between myosin and negatively charged membranes cannot be reduced solely to the rod part of the molecule, as the binding studies in Figure 5 reveal. Here the previous complexation of myosin with ATP at the head domain resulted in an attenuation of the observed quadrupole splittings and T_{1Z} values compared to those of untreated myosin. The myosin head domain, where ATP is located, contains the active site for ATPase activity and another site for actin interaction during muscle contraction and relaxation. It was postulated that single head type I myosins could have an extra hydrophobic site in this domain close to its link with the tail domain (*4*). This hydrophobic site would explain the observed type I myosin interactions with membranes. The changes in quadrupole splittings and T_{1Z} presented in Figure 5 could then be explained by the presence of the ATP molecule in this region affecting this close hydrophobic site. This observation would therefore suggest a participation of this part of the molecule in the association with charged liposomes. However, this participation of the headgroup in binding might be not direct, but indirect, by propagating the interaction occurring in the headgroup through the molecule to a site which interacts more directly with the bilayer. This propagation could happen in a way (*37*) that the ATP and Mg^{2+} interaction with the headgroup leads to a folding of the tail domain to a hairpin-like structure which would reduce the available binding sites at the myosin tail. Nevertheless, these interactions in the headgroup domain might potentially modulate the binding of myosin to membranes during its action as a biological motor powered by the binding and the hydrolysis of ATP.

As this study has shown, the protein myosin is readily seen to perturb the lipid headgroups in negatively charged membrane systems through interaction with the bilayer surface. It is now important to determine whether this interaction is highly specific for the charged lipid DMPG only or whether it is averaged out through the whole membrane system.

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