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Phosphorus-31 Nuclear Magnetic Resonance Studies of the Adenosine 5′-Triphosphate–Calcium–G-actin Complex†

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ABSTRACT: Phosphorus-31 nuclear magnetic resonance spectra of the adenosine 5′-triphosphate–calcium–G-actin complex were obtained, and the resonances of the three phosphates of the protein-bound ATP were detected. The exchange of the ATP between its protein-bound and free states was found to be slow on the NMR time scale, with an exchange rate of less than 480 s^{-1} at pH* 7.8, 4 °C. The line width of the protein-bound γ-phosphate resonance (corrected for spin–spin splitting by the β phosphate) was used to calculate a rotational correlation time for the G-actin-bound ATP. With the assumption that chemical shift anisotropy is the dominant relaxation mechanism at 109.29 MHz and that the chemical shielding tensor for pyrophosphate serves as a good model for the γ phosphate of the bound ATP, a correlation time of 60 ns was estimated. Since the theoretical correlation time of a globular protein the size of G-actin is 36 ns, the line width of the bound γ-phosphate resonance is consistent with that expected for ATP bound to G-actin without large-scale rapid internal mobility. The addition of 1.5 M urea to the ATP–Ca–G-actin complex caused exchange broadening of the γ and β phosphates, but no effect on the α phosphate. This indicates an increase in the rate of exchange for the β and γ phosphates between the protein-buried and solvent-exposed environments at 1.5 M urea. At 6 M urea, the intensities of the protein-bound ATP resonances were greatly reduced, and the intensities of the free ATP resonances were greatly increased, indicative of complete protein unfolding and liberation of protein-bound ATP.

The necessity of a bound nucleotide and divalent cation in the maintenance of the native conformation of G-actin has been previously demonstrated (Laki et al., 1950; Straub & Feuer, 1950; Oosawa & Kasai, 1971). However, the roles of the bound nucleotide in the physiological functions of actin, such as cross-bridge formation with myosin heads (Huxley, 1969; Mannherz & Goody, 1970), polymerization to form thin filaments (Oosawa & Kasai, 1971; Engel et al., 1977), and binding with tropomyosin and troponin (Mannherz & Goody, 1970; Ebashi & Ebashi, 1965), are not clearly understood. ^{31}P NMR[1] spectroscopy offers a noninvasive probe to monitor the microenvironments of each of the three phosphates of ATP (Cohn & Hughes, 1960, 1962). The utility of ^{31}P NMR in studying ligand binding to various proteins, such as alkaline phosphatase (Hull et al., 1976), glycogen phosphorylase (Withers et al., 1979), and pyruvate, phosphoglycerate, and arginine kinases (Nageswara Rao et al., 1978, 1979; Nageswara Rao & Cohn, 1977), has already been well shown. Herein, we report the first ^{31}P NMR spectra of G-actin-bound ATP, with an interpretation of the spectral features in terms of protein structure and ATP binding.

Materials and Methods

Actin was prepared from rabbit skeletal muscle by the method of Spudich & Watt (1971). The protein migrated in one band on sodium dodecyl sulfate gel electrophoresis (Weber & Osborn, 1969). G-actin, after dialysis against 2 mM Tris, 0.2 mM $CaCl_2$, 0.5 mM dithiothreitol (DTT), and 0.2 mM ATP at pH 7.8, 4 °C, was diluted with an equal volume of D_2O and concentrated to 0.3–0.4 mM by using a Minicon B15

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[1] Abbreviations used: NMR, nuclear magnetic resonance; ATP, adenosine 5′-triphosphate; Tris, tris(hydroxymethyl)aminomethane; EDTA, ethylenediaminetetraacetate; T_1, spin–lattice relaxation time; T_2, spin–spin relaxation time; D_2O, deuterium oxide.

0006-2960/81/0420-2060$01.25/0

Table I: ^{31}P Chemical Shifts of Free and G-actin-Bound ATP at pH* 7.8, 4 °C

	chemical shift (ppm)					
	γP (free)	γP (bound)	αP (free)	αP (bound)	βP (free)	βP (bound)
20 mM ATP + 5 mM EDTA	−5.7		−10.4		−21.3	
4 mM ATP + 20 mM $CaCl_2$	−4.8		−10.1		−18.8	
ATP–Ca–G-actin (Figure 1A)		−5.8	−10.1	−8.7		−20.6
ATP–Ca–G-actin + free ATP (Figure 1B)	−5.0	−5.7	−10.1	−8.6	−19.2	−20.5
ATP–Ca–G-actin in 1.5 M urea (Figure 1C)		−5.4	−10.0	−8.6	−19.0	−20.7
ATP–Ca–G-actin in 6 M urea (Figure 1D)	−4.8	−5.4	−9.9	−8.6	−18.8	−20.6

concentrator. Trace metal ions were removed from the D_2O via pretreatment with Chelex-100 (50–100 mesh). After concentration, a typical sample was found to contain 84% G-actin, based on the optical density of the supernatant after centrifugation at 80000*g* for 3 h at 4 °C. Determinations of pH, indicated as pH*, were uncorrected for the deuterium isotope effect. Concentrations of G-actin and free ATP were based on the following molar absorption coefficients: G-actin (280 nm) 46 500 (Johnson & Taylor, 1978); ATP (259 nm) 15 400 (Dunn & Hall, 1970). Urea solutions were run through a mixed-bed resin (AG-501-X8) to remove cyanates (Stark et al., 1960).

^{31}P NMR spectra (109.29 MHz) were obtained at 4 °C with a Bruker HXS 270 NMR spectrometer, operating in the Fourier-transform mode with quadrature detection. Typical spectra were taken with a spectral width of ±2500 Hz, an acquisition time of 0.409 s, a pulse delay of 1.6 s, and a pulse width of 22 μs (78° pulse angle). Exponential multiplication of the free-induction decay was used to reduce spectral noise, resulting in 10-Hz line broadening. Samples of 1.5-mL volume were run in 10-mm NMR tubes equipped with a Teflon vortex plug. Chemical shifts were reported in parts per million (ppm) upfield from phosphoric acid (85%).

Results

The ^{31}P NMR spectrum of ATP in an ATP–Ca–G-actin complex is shown in Figure 1A, and the chemical shifts are given in Table I. The three resonances at −5.8, −8.7, and −20.6 ppm were assigned respectively to the γ-, α-, and β-phosphorus atoms of the protein-bound ATP, based on the assignments of the resonances in free ATP (Cohn & Hughes, 1960, 1962). The sharp resonance at +2.7 ppm was assigned to free inorganic phosphate (Busby et al., 1978; Burt et al., 1979). This inorganic phosphate was likely the result of some polymerization of the G-actin during the concentration step prior to the running of the NMR spectrum, since the polymerization reaction always results in ATP hydrolysis and inorganic phosphate liberation (Oosawa & Kasai, 1971). Additional ATP, in a ratio of 0.5 mol of free ATP to 1.0 mol of G-actin, was added to the sample in Figure 1A; the resultant spectrum is shown in Figure 1B.[2] The chemical shifts of the resonances of the protein-bound ATP remained essentially unchanged (see Table I), with new sharp resonances appearing at −5.1, −10.2, and −19.3 ppm. These resonances were assigned, respectively, to the γ-, α-, and β-phosphorus atoms of free ATP (Cohn & Hughes, 1960, 1962). The chemical shifts of metal-free ATP and Ca-saturated ATP at pH* 7.8, 4 °C, are also given in Table I.

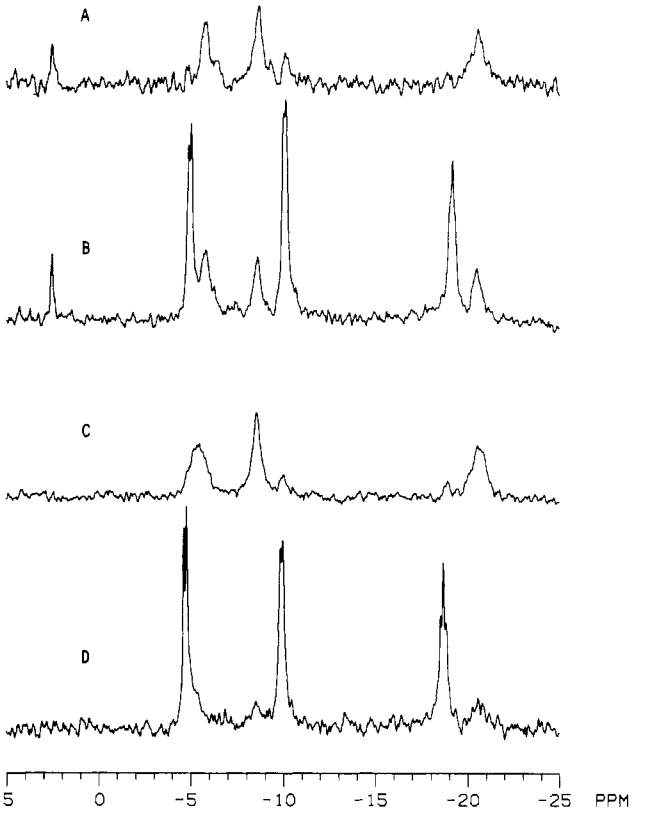

FIGURE 1: ^{31}P NMR spectra at 109.29 MHz of the ATP–Ca–G-actin complex. All samples were run at pH* 7.8, 4 °C, in 1 mM Tris, 0.25 mM DTT, 0.1 mM $CaCl_2$, and 50% D_2O. (A) 0.395 mM ATP–Ca–G-actin (26 800 scans). (B) 0.395 mM ATP–Ca–G-actin + 0.183 mM ATP (35 300 scans). Molar ratio for ATP–Ca–G-actin/free ATP of 1:0.46; same sample as used for (A). (C) 0.718 mM ATP–Ca–G-actin in 1.5 M urea (23 000 scans). New sample. (D) 0.598 mM ATP–Ca–G-actin in 6 M urea (8100 scans). Same sample as used for (C); the addition of urea caused some dilution of the sample.

For demonstration that the resonances at −5.8, −8.7, and −20.6 ppm do correspond to ATP bound to the native conformation of G-actin, urea-induced denaturation studies were conducted. The ^{31}P NMR spectrum of the ATP–Ca–G-actin complex (with no added free ATP) in 1.5 M urea is shown in Figure 1C. The large resonances at −8.6 and −20.7 ppm correspond well with the α and β resonances of the protein-bound ATP (compare to Figure 1A and Table I) while the low-intensity peaks at −10.0 and −19.0 ppm correspond fairly well with the α and β resonances of free ATP (compare to Figure 1B and Table I). The large resonance at −5.4 ppm likely represents the γ phosphorus of protein-bound ATP which has been shifted from its previous position at −5.8 ppm. The resonance for the γ phosphorus of the free ATP is not resolved

[2] The ratios of the intensities of the free to bound ATP resonances were 2.4 for the γ phosphorus, 2.6 for the α phosphorus, and 2.3 for the β phosphorus. The relative intensities of the free and bound ATP resonances are not directly proportional to their relative concentrations if the T_1 values for the free and bound ATP resonances are different and the repetition rate for data accumulation is $<5T_1$ (Hull et al., 1976). Preliminary results with a nitrated G-actin derivative indicate that the T_1 values of the free ATP resonances were 0.6–0.8 s while the T_1 values for the bound ATP resonances were 3.0–4.3 s. Our normal repetition rate for data accumulation was one scan per 2.0 s. Thus, rapid T_1 values for the free ATP resonances relative to the bound ATP resonances for unmodified G-actin could readily account for the high relative intensities of the free ATP resonances.

from the large protein-bound resonance at −5.4 ppm. Generally, the effects of the addition of 1.5 M urea to the ^{31}P NMR spectrum of the Ca–ATP–G-actin complex are relatively small, indicating that no major unfolding processes have occurred. However, the addition of 6 M urea dramatically altered the spectrum of the G-actin complex (see Figure 1D and Table I). The protein-bound ATP resonances at −5.4, −8.6, and −20.6 ppm had decreased appreciably in intensity, and the free ATP resonances at −4.8, −9.9, and −18.8 ppm had greatly increased in intensity. This indicates that at 6 M urea the G-actin has largely unfolded, liberating its ATP.

Discussion

When a nucleus in a molecule exists in two or more forms having different chemical shifts, the nucleus is involved in an exchange process (Sykes & Scott, 1972). If the rate of the exchange process (τ_{ex}^{-1}) between two sites A and B is much less than the chemical shift difference (in radians per second) between the two sites $|\omega_A - \omega_B|$, then the exchange is slow on the NMR time scale, and one sees individual resonances at ω_A and ω_B corresponding to the two states. If the rate of exchange (τ_{ex}^{-1}) is much greater than the chemical shift difference $|\omega_A - \omega_B|$, the exchange is fast on the NMR time scale. One then sees a single exchange-averaged resonance, at a resonance frequency $\omega = P_A\omega_A + P_B\omega_B$, where P_A and P_B are the populations at sites A and B. Exchange broadening occurs at intermediate exchange rates where $\tau_{ex}^{-1} \simeq |\omega_A - \omega_B|$.

The free ATP in these experiments is in equilibrium with the Ca–ATP complex, since 0.1 mM $CaCl_2$ is present in the dialysis buffer of the sample. The exchange of ATP between its metal complex and its metal-free form is known to be rapid on the NMR time scale (Cohn & Hughes, 1960, 1962; Busby et al., 1978; Burt et al., 1979). Hence, the chemical shifts of the free ATP resonance will be a weighted average of the chemical shift of the metal-free and metal-complexed forms. From the chemical shifts of the γ- and α-phosphorus resonances of the free ATP in Figure 1B, the ATP was calculated to be 78% and 84% calcium bound, respectively. This yields an apparent log K_A of 4.55–4.72 for the binding of Ca to ATP at pH 7.8, 4 °C, at ionic strength <0.05, neglecting the contribution of free Ca^{2+} from the low-affinity binding sites for Ca on G-actin (Oosawa & Kasai, 1971; Engel et al., 1977). Literature values for the log K_A of Ca binding to ATP range from 4.10 (Taqui Khan & Martell, 1966) to 4.60 (O'Sullivan & Perin, 1964). The free ATP resonances in figure 1D, where the G-actin has been largely denatured by urea, are at chemical shifts corresponding to 100% Ca-bound ATP (see Table I). This likely reflects the unfolding of the G-actin by urea, liberating Ca from its four to seven low-affinity (K_A of 10^3–10^4 M^{-1}) (Strzelecka-Golaszewska et al., 1978) and one high-affinity (K_A of about 10^5 M^{-1}) binding sites; this Ca is now free to bind to ATP.

The free ATP in these experiments is also in equilibrium with G-actin-bound ATP (Oosawa & Kasai, 1971; Engel et al., 1977). The fact that separate resonances for the protein-bound and free ATP can be readily observed (see Figure 1B) indicates that the exchange of ATP between these two states is slow on the NMR time scale. The lower limit of the exchange rate can be determined by selecting the phosphorus of ATP for which the bound and free resonances are closest together, i.e., $|\omega_A - \omega_B|$ is the smallest. From Table I, the difference between the bound and free resonances for the γ phosphorus of ATP is 0.7 ppm or 480 rad/s; thus, the rate of exchange for ATP binding to G-actin must be slower than 480 s^{-1} at pH 7.8, 4 °C. This conclusion is in agreement with studies of ATP binding to G-actin monitored by other methods. Strzelecka-Golaszewska (1973), using [^{14}C]ATP, calculated an apparent rate constant of 5.97×10^{-5} s^{-1} for the dissociation of ATP from the ATP–Ca–G-actin complex at 0 °C. Via nucleotide exchange studies with a fluorescent ATP analogue (ϵ-ATP), Neidl & Engel (1979) determined a dissociation rate constant for G-actin-bound ATP of 8×10^{-4} s^{-1} at 21 °C, pH 8.2, in 0.8 mM $CaCl_2$.

Previous ^{31}P NMR studies of the ATP–Ca–G-actin complex (Cozzone et al., 1974; Nelson et al., 1974) indicated that fast exchange occurred between free and protein-bound ATP. These ^{31}P NMR studies were done at 40.5 MHz on samples containing 2 mM free ATP, 0.4 mM G-actin, and 0.2 mM $CaCl_2$ at 17 °C, while our studies were done at 109.3 MHz on samples containing 0–0.2 mM free ATP, 0.4 mM G-actin, and 0.1 mM $CaCl_2$ at 4 °C. A number of factors, such as differences in free Ca^{2+} concentration, temperature, and resonance frequency, might account for the fast exchange of ATP seen by the previous workers. The earlier study used an excess of free ATP over $CaCl_2$. Since free ATP readily binds calcium (Taqui Khan & Martell, 1966; O'Sullivan & Perin, 1964), the concentration of free calcium would be very low [(3–9) × 10^{-6} M] in this system. Kuehl & Gergely (1969) found that the rate of ATP exchange varied inversely with the concentration of free calcium. The rate constant for the dissociation of ϵ-ATP from G-actin increased by a factor of 2–3 as the calcium concentration was decreased (Waechter, 1975). Waechter also proposed that increasing concentrations of free ATP increase the rate of ϵ-ATP dissociation by a direct effector-like action of ATP, independent of its binding to free calcium (Waechter, 1975). The dissociation rate constant is also increased with increasing temperature (Asakura, 1961; Waechter & Engel, 1975). While it is difficult to quantitate the effects of temperature and ATP and Ca concentrations from one set of experimental conditions to another, it is clear that an increase in temperature and free ATP concentration (with a resultant decrease in free Ca levels) would increase the dissociation rate constant of ATP from G-actin. However, the dissociation rate constant would need to be increased approximately 5 orders of magnitude to place the NMR spectrum in the fast exchange limit. Also, the previous ^{31}P NMR studies of Cozzone et al. and Nelson et al. were done at lower magnetic field strength (40.5 MHz) than was this present study (109.3 MHz), so that the chemical shift difference (in radians per second) between free and bound ATP resonances, $|\omega_A - \omega_B|$, would be smaller by a factor of 2.70, assuming all other factors were equal. This effect would favor the observation of fast exchange at 40.5 MHz relative to 109.3 MHz, without any actual effect on the dissociation rate constant of the sample. Thus, the observation of fast exchange between free and G-actin-bound ATP observed by Cozzone et al. (1974) and Nelson et al. (1974) is not incompatible with our observations of slow exchange, given the differences in sample conditions and spectrometer field strengths.

The addition of 6 M urea to the ATP–Ca–G-actin complex (Figure 1D) leads to the liberation of ATP and a large decrease in the intensity of the bound ATP resonances. Separate resonances are, however, still seen for the liberated ATP and the bound ATP, because of the slow exchange between these two states. Once the protein-bound ATP has dissociated from the G-actin, the nucleotide-free G-actin can be readily and irreversibly denatured by the 6.0 M urea (Asakura, 1961; Oosawa & Kasai, 1971). Thus, the overall sequence of events in 6.0 M urea is probably the reversible dissociation of ATP from the native conformation of G-actin (slow exchange) followed by irreversible denaturation of the nucleotide-free

G-actin. The fact that bound resonances are still observed after several hours is consistent with the very long lifetime of the ATP–Ca–G-actin complex. The lifetime is calculated to be 3.2 h from the literature values of the dissociation rate constant (Strzelecka-Golaszewska, 1973).

The line widths of the protein-bound ATP resonances can also provide information about the binding of ATP to G-actin. The line widths (± standard deviation) for the bound ATP resonances (as in Figure 1A) were 52 ± 3, 49 ± 6, and 67 ± 5 Hz for the γ-, α-, and β-phosphorus resonances, respectively, based on three separately prepared samples of ATP–Ca–G-actin. These line widths include homonuclear spin–spin splitting contributions. For 20 mM ATP + 5 mM EDTA, pH* 7.8 at 4 °C, the $^2J_{P\beta,P\gamma}$ and $^2J_{P\alpha,P\beta}$ splittings were found to be 19.6 Hz. The $^2J_{P\beta,P\gamma}$ and $^2J_{P\alpha,P\beta}$ splittings for 4 mM ATP in 20 mM $CaCl_2$, pH* 7.8 at 4 °C, were found to be 16.9 Hz. If we assume that the protein-bound ATP resonances undergo the 19.6-Hz splitting of the free ATP rather than the 16.9-Hz splitting of the Ca–ATP, then the corrected line widths for the γ, α, and β resonances are 32, 29, and 28 Hz, respectively. [For the β phosphate, the corrected line width was determined by subtracting 39.2 Hz (19.6 Hz × 2) to take into account the apparent AX_2 splitting pattern. Determination of line width by line-shape analysis was not justified by the signal-to-noise ratio of the resonance.] Thus, the T_2 values for the γ-, α-, and β-phosphorus resonances of G-actin-bound ATP are 10, 11, and 11 ms, respectively.

From the T_2 values determined from line width measurements, one can estimate the rotational correlation times of the protein-bound ATP. At 109.29 MHz, the predominant relaxation mechanism of ^{31}P nuclei is chemical shift anisotropy (Hull & Sykes, 1975).[3] If one assumes that only chemical shift anisotropy contributes to the line width of the bound resonances, the correlation time (τ_c) is related to the T_2 value via the relationship:

$$\frac{1}{T_2} = \frac{1}{90}\omega_0^2(\delta_{\parallel} - \delta_{\perp})^2\left[8\tau_c + \frac{6\tau_c}{1 + \omega_0^2\tau_c^2}\right]$$

where ω_0 is the Larmor frequency, and $\delta_{\parallel}$ and $\delta_{\perp}$ refer to the chemical shielding factors along and perpendicular to the symmetry axis of a nucleus. If we assume that $\omega_0^2\tau_c^2 \gg 1$, then

$$\frac{1}{T_2} = \frac{1}{90}\omega_0^2(\delta_{\parallel} - \delta_{\perp})^2[8\tau_c]$$

The factor $|\delta_{\parallel} - \delta_{\perp}|$ for sodium and potassium pyrophosphate has been found to be 200 ± 100 ppm, assuming axial symmetry (Derbyshire et al., 1969). If we assume that pyrophosphate is a good model compound for the γ phosphorus of G-actin-bound ATP, one can calculate a τ_c of 60 ns.

It should be emphasized that this calculated τ_c is based on several assumptions: (1) a homonuclear splitting contribution to the line width of 19.6 Hz, based on the splitting of free ATP; (2) chemical shift anisotropy as the dominant relaxation mechanism at 109.29 MHz; (3) the chemical shielding tensor for pyrophosphate ($\delta_{\parallel} - \delta_{\perp}$ of 200 ± 100 ppm) as a model for the γ phosphate of bound ATP; (4) axial symmetry for the bound ATP. In spite of these assumptions, the estimated τ_c of 60 ns is in line with the rotational correlation times determined by other methods. By use of the fluorescence anisotropy decay of ϵ-ATP bound to G-actin, a τ_c of 39 ns at 5 °C was determined (Mihashi & Wahl, 1975). When the decay of *N*-iodoacetyl-*N'*-(5-sulfo-1-naphthyl)ethylenediamine reacted to Cys-373 of G-actin was monitored, τ_c was determined to be 45 ns at 3.5 °C in one study (Ikkai et al., 1979) and 55 ns (corrected to 4 °C in water) in another (Tao, 1978). One can determine the theoretical τ_c for the rotation of a sphere the size of G-actin, using the Stokes–Einstein equation:

$$\tau_c = \frac{4\pi\eta r^3}{3kT}$$

where η is the viscosity coefficient [0.01567 g s^{-1} cm^{-1} for H_2O at 4 °C (Handbook of Biochemistry, 1968)], r is the radius of a G-actin monomer [27.5 Å (Oosawa & Kasai, 1971)], k is the Boltzman constant, and T is the temperature. From this equation, the theoretical rotational τ_c is 36 ns. The theoretical rotational τ_c for a free ATP molecule in the form of a Ca–ATP complex, assuming a conformation similar to a rigid sphere of 7.5 Å, would be 0.83 ns. Any intramolecular flexibility would further decrease this correlation time. It is obvious that the τ_c of 60 ns for the γ phosphorus of G-actin-bound ATP more closely approximates the rotational motion of the G-actin macromolecule (τ_c of 36 ns) than the motion of the Ca–ATP complex (τ_c of about 0.83 ns). Thus, the phosphates of the ATP in the ATP–Ca–G-actin complex must be bound without large-scale rapid internal mobility.

The line widths of the protein-bound ATP resonances changed appreciably in the presence of 1.5 M urea (see Figure 1C). The line widths, corrected for the spin–spin splitting of 19.6 Hz, of the γ-, α-, and β-phosphorus resonances were 92, 30, and 53 Hz, respectively. The corresponding T_2 values were 3.5, 11, and 6.0 ns. Thus, the γ resonance has been broadened 60 Hz, and the β resonance has been broadened 25 Hz, relative to the line widths under native conditions (e.g., Figure 1A). The α-phosphorus resonance is virtually unchanged (0.5 Hz wider). The effect on the β resonance is indicative of exchange broadening, where the rate of exchange of ATP between its free and protein-bound forms has increased but separate resonances for the two forms are still present, i.e., $\tau_{ex}^{-1} \leq |\omega_A - \omega_B|$. The difference between the line width under slow-to-intermediate exchange, $\Delta\nu$, and the line width at the slow exchange limit, $\Delta\nu_0$, is related to the lifetime of each state, τ_{ex}, according to the equation:

$$\Delta\nu - \Delta\nu_0 = \frac{1}{\pi\tau_{ex}}$$

Thus, for the β-phosphorus resonance, τ_{ex}^{-1} is 80 s^{-1}. Unlike the β resonances, the γ resonances previously at −5.0 and −5.7 ppm have coalesced to a resonance at −5.4 ppm, indicating intermediate exchange, where $\tau_{ex}^{-1} \simeq |\omega_A - \omega_B| \simeq 480$ s^{-1}. Since the protein-bound α and β resonances have far greater intensities than the free reonance, the G-actin must remain in an essentially native conformation at 1.5 M urea. However, the rates of exchange for the β and γ phosphorus of ATP have increased significantly more than the rate of exchange of the α phosphorus. It is tempting to speculate that the urea is selectively disrupting the ionic binding site around the β and γ phosphorus of ATP, allowing a more rapid exchange with a solvent-exposed environment while leaving the more hy-

[3] A possible contribution to the line broadening of the bound resonances is chemical exchange broadening. In the slow exchange limit, this line broadening will be equal to k_{off}/π, where k_{off} is the dissociation rate constant for ATP from the Ca–ATP–G-actin complex. Two lines of evidence suggest that the exchange broadening is negligible. First, the published values of k_{off} are $\approx 10^{-4}$ s^{-1} (Strzelecka-Golaszewska, 1973). Second, the bound line widths do not increase with increasing temperature between 4 and 28 °C. These results were obtained in studies of ATP bound to a nitrated, nonpolymerizable G-actin derivative (M. Brauer, unpublished results).

drophobic binding of the adenine and ribose rings and the adjoining α phosphorus essentially intact.

Conclusion

In this study, we have for the first time observed the protein-bound resonances of the ATP in the ATP–Ca–G-actin complex. From the chemical shift differences of the ATP resonances between their bound and free (largely as a Ca–ATP complex) states, it was determined that the exchange was slow on the NMR time scale, with an exchange rate of less than 480 s^{-1}. This apparently contradicts previous ^{31}P NMR studies (Cozzone et al., 1974; Nelson et al., 1974) where the exchange of free and G-actin-bound ATP was said to be fast on the NMR time scale, but different experimental conditions may account for these results. From the line widths of the protein-bound ATP resonances, a rotational correlation time of 60 ns was estimated. This compares well with the theoretical rotational correlation time of a sphere the size of G-actin (36 ns), indicating that the ATP is bound and undergoes no appreciable rapid internal rotation within the G-actin binding site. The addition of 1.5 M urea causes an increase in the exchange rates of the β and γ phosphates of the protein-bound ATP. This may indicate a gradual and sequential loosening of the bound ATP, starting at the γ phosphate, due to the urea-induced unfolding of the protein structure at the ATP binding site. At 6 M urea, one can see a dramatic decrease in the intensity of the protein-bound ATP resonances, and the corresponding rise in the intensities of the free ATP resonances. This confirms the assignment of the protein-bound ATP resonances and indicates the liberation of the ATP under these denaturing conditions.

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