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Elucidation of spermidine interaction with nucleotide ATP by multiple NMR techniques

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Abstract

Interaction of polyamines with nucleotides plays a key role in many biological processes. Here we use multiple NMR techniques to characterize interaction of spermidine with adenosine 5′-triphosphate (ATP). Two-dimensional ^{1}H-^{15}N spectra obtained from gs-HMBC experiments at varied pH show significant shift of N-1 peak around pH 2.0-7.0 range, suggesting that spermidine binds to N-1 site of ATP base. The binding facilitates N-1 deprotonation, shifting its pK_a from 4.3 to 3.4. By correlating ^{15}N and ^{31}P chemical shift data, it is clear that spermidine is capable of concurrently binding to ATP base and phosphate sites around pH 4.0-7.0. The self-diffusion constants derived from ^{1}H PFG-diffusion measurements provide evidence that binding of spermidine to ATP is in 1:1 ratio, and pH variations do not induce significant nucleotide self-association in our samples. ^{31}P spectral analysis suggests that at neutral pH, Mg^{2+} ion competes with spermidine and shows stronger binding to ATP phosphates. From ^{31}P kinetic measurements of myosin catalyzed ATP hydrolysis, it is found that binding of spermidine affects the stability and reactivity of ATP. These NMR results are important for advancing the studies on nucleotide-polyamine interaction and its impact on nucleotide structures and activities under varied conditions.

Keywords

NMR; ^{1}H; ^{15}N; ^{31}P; nucleotide; polyamine; HMBC; PFG-diffusion; kinetic

Introduction

Nucleotide adenosine 5′-triphosphate (ATP) plays a key role in all aspects of metabolism. It serves as an important energy source for various cellular processes, such as biosynthesis, signal transduction, ion transportation and cell division.[1-3] Polyamines (spermidine, spermine and putrescine, etc.) are a group of essential substances which exist in all mammalian cells and are involved in cell growth, differentiation and proliferation. [4] Among other things, it has been found that the presence and interplay of polyamines and ATP in cells may strongly affect the function of ATP-dependent transport proteins,[5-8] the ATPase activity of enzymatic proteins,[9,10] and the compact state and genetic activity of DNA.[11,12]

Under physiological pH condition (around 7.0), polyamines, including spermidine (Fig.1b), exist as polyvalent cations, therefore are capable of direct coordination to ATP (Fig.1a) through electrostatic interaction. In intracellular concentrations, polyamines (~3 mM) also compete with naturally occurring metal ions such as K^{+} (~140 mM) and Mg^{2+} (~1 mM) for binding to ATP. Günther et al. demonstrated using mag-fura-2 and ^{31}P NMR that

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polyamines bound to ATP under physiological conditions.[13] They also used Mg^{2+}-selective electrodes to verify that the capacities of polyamine binding to ATP were in an order of spermine > spermidine > putrescine; and interaction of polyamines with ATP resulted in reduced Mg^{2+} binding on ATP, in the case of spermine the free Mg^{2+} concentration was almost doubled.[14] Lüthi et al. measured apparent binding constants for spermine binding to ATP under varied conditions; they found that the apparent binding constants of spermine were increased at higher spermine concentrations, which lead to increased amount of free Mg^{2+}, as detected using Mg^{2+}-macroelectrodes.[15] The formation of ternary ATP-polyamine-metal ion complexes is believed to play an important role in some ATP-dependent reactions in vivo.[16] Binding of polyamines to ATP may also accompanied by conformational changes of polyamines or ATP.[16,17]

Nuclear magnetic resonance (NMR) spectroscopy, in combination with other techniques such as spectrophotometry and potentiometry, has been successfully applied to investigate interaction of polyamines with nucleotides.[16-19] However, certain ambiguity and disagreement remain. Meksuriyen et al. conducted ^{31}P and ^{1}H NMR studies on ATP-spermine complexes in the presence and absence of Mg^{2+}. They found that the ^{31}P peaks were broadened upon spermine binding to ATP at pH 7.8, but the ^{1}H resonances of ATP base were essentially unchanged. Thus they concluded that spermine predominantly interacts with β- and γ-phosphates of ATP.[16] On the other hand, Gasowska applied ^{13}C and ^{31}P NMR to study interaction of nucleotides (ADP and ATP) with polyamines (spermine and 3,3,3-tet). He observed slight peak-shift for carbons adjacent to N-1 and N-7 of nucleobase, but no significant changes in phosphate spectra. He concluded that spermine mainly binds to N-1 and N-7 of nucleobase although 3,3,3-tet may bind to both nucleobase and phosphates.[18] The discrepancy in these findings tells us that interaction of polyamines with nucleotides may strongly depend on sample conditions, and further NMR investigations are necessary in this regard.

For this investigation, we implemented a gradient-selected heteronuclear multi-bond connectivity (gs-HMBC) pulse sequence [20] to obtain two-dimensional (2D) ^{1}H-^{15}N spectra of ATP and ATP-spermidine complexes under varied sample pH. This NMR technique correlates abundant ^{1}H spins (H-2, H-8) with dilute ^{15}N spins (N-1, N-3, N-7, N-9) of ATP base through two-bond spin couplings. It provides significantly enhanced sensitivity than conventional one-dimensional (1D) ^{15}N NMR, and makes us possible to acquire rather weak ^{15}N signals using natural abundant ATP in relatively low concentration (20mM) without ^{15}N-enrichement. We believe that the ^{15}N chemical shift data from HMBC spectra are more straightforward and indicative of spermidine interaction with nucleobase, compared to the shift data of adjacent ^{13}C or ^{1}H. We also measured 1D ^{31}P spectra to verify the interaction of spermidine with ATP phosphates, and binding competition between Mg^{2+} and spermidine. Furthermore, ^{1}H pulsed-field-gradient (PFG) diffusion experiments were implemented, using the standard stimulated-echo (STE) pulse sequence,[21,22] to derive the diffusion constants of ATP and ATP-spermidine complex. Finally, ^{31}P NMR kinetic studies were conducted for myosin catalyzed ATP hydrolysis, to characterize the effect of complex formation on stability and reactivity of ATP.

Experimental

All chemicals including Na_2ATP, spermidine ($C_7H_{19}N_3$), $MgCl_2{\cdot}6H_2O$ and calcium-activated myosin from chicken muscle (dissolved in aqueous glycerol solution) were purchased from Sigma-Aldrich without further purification. NMR samples were prepared in D_2O solution for purpose of filed-lock, with typically 20 mM ATP in the presence or absence of equal-molar spermidine or $MgCl_2$ (For accuracy, the molar quantities of $MgCl_2$ were controlled by preparing a 0.1 M stock-solution and diluting it to desired

concentrations). Samples for myosin catalyzed ATP hydrolysis contained 0.79 mg myosin (in 0.1 ml glycerol solution) and 5 mM ATP (with or without 5 mM spermidine and Mg^{2+}) at pH 7.0. Sample pH was adjusted using NaOH and HCl solutions (accurate to pH 0.1). No other pH buffer substances were used to avoid the interference of impurities.

NMR measurements were conducted at room temperature, using JEOL ECX-300 spectrometer and a 5 mm broadband auto-tune probe. The π/2 pulse-widths of ^{1}H, ^{15}N and ^{31}P were 11.7, 21.3 and 10.75 μs, respectively. In 2D gs-HMBC experiments, the two-bond coupling constant (J) of ^{1}H and ^{15}N was 14 Hz, corresponding to an evolution time 1/(2J) = 35.7 ms; the signals were acquired with 128 scans, 2 s repetition delay, and 128 increments in t_1 dimension. In ^{1}H PFG-STE experiments, diffusion time Δ = 100 ms; gradient-pulse duration δ = 5 ms; gradient-pulse strength G was incremented from 0.003 to 0.283 Tesla/m in 10 steps; and signals were acquired with 16 scans and 10 s repetition delay. Based on the well-known exponential relationship between signal S and diffusion constant D, $S \propto \exp[-D(\gamma G\delta)^2(\Delta - \delta/3)]$, diffusion spectra were integrated and natural logarithms of the signals (ln S) were plotted vs. G^2. By linear fitting, diffusion constants were derived as $D = \text{slope} / [-\gamma^2\delta^2(\Delta - \delta/3)]$. ^{31}P spectra were recorded with 128 scans, 3 s repetition delay; and ^{31}P kinetic measurements for ATP hydrolysis were performed at preset timings (once per hour for total 18 hours). The kinetic spectra were analyzed from decreasing β-integrals over time. We found that it was necessary to use peak integrals instead of heights for sufficiently accurate data fitting (with correlation coefficients R > 0.99) in both diffusion and kinetic analyses.

Results and Discussion

A. ^{15}N Chemical Shifts Obtained from ^{1}H-^{15}N Spectra

Fig. 2 is a representative 2D ^{1}H-^{15}N HMBC spectrum, obtained with 20 mM ATP at pH 3.2. A high-resolution ^{1}H spectrum is also displayed in x-dimension, along with a y-projection for nitrogen peaks and peak assignments according to earlier studies [23,24]. The 2D spectrum shows well-resolved four correlation peaks for ATP base, i.e. H-8 is correlated with N-7 and N-9; H-2 is correlated with N-1 and N-3. Since all four correlation peaks are observed, it is evident that using a compromised J-coupling constant of 14 Hz for spin evolution is adequate here, although the actual two-bond J-coupling constants of adenine base are such divergent: 15.8 Hz (H-2 and N-1), 17.1 Hz (H-2 and N-3,) 11.0 Hz (H-8 and N-7), and 5 Hz (H-8 and N-9), as reported earlier.[25] Besides, there is no amino (NH_2) peak in the spectrum, which can be attributed to two possible reasons: (i) the amino protons exchange rather rapidly with water protons; (ii) the signals evolved via one-bond J-coupling of ^{1}H-^{15}N are effectively suppressed by the gs-HMBC sequence.

From various ^{1}H-^{15}N HMBC spectra acquired for ATP or ATP-spermidine samples at varied pH, chemical shift changes of N-1, N-3, N-7 and N-9 are clearly seen. As shown in Fig. 3, N-1 peak has dramatic up-field shift (about 70 ppm) when sample pH is reduced from 7.0 to 2.0, while N-3, N-7 and N-9 are only slightly down-field shifted (about 4-6 ppm). This agrees with the 1D ^{15}N NMR results reported by Major et al.[26] In fact, N-1 is the most basic site in adenine ring.[26,27] The dramatic up-field shift of N-1 at lowing pH is apparently associated with increased N-1 protonation, i.e. the protonation makes N-1 nucleus more shielded.[25] But in pH >7.0 range, like N-3, N-7 and N-9, N-1 peak is almost unaffected by pH changes due to its full deprotonation at high pH.

It can be known from Fig. 3 that binding of spermidine to ATP has no significant effect on chemical shifts of N-3, N-7 and N-9. Relative to ATP, however, ATP-spermidine has significant down-field shift of N-1 peak in pH 2.0-7.0 range. The largest effect occurs around pH = 3.0, with ~30 ppm shift difference between ATP and ATP-spermidine. Such

effect must be due to strong spermidine binding to N-1, and the net down-field shift is associated with reduced nuclear shielding when spermidine replaces H^+ at N-1 site. The spermidine effect on N-1 is also more pronounced comparing to some divalent metal ions. In Mg-ATP and Zn-ATP, for instance, Mg^{2+} and Zn^{2+} were found to interact with N-1 at lower pH, with maximum down-field shifts of 10 ppm (Mg-ATP around pH 4.0) and 24 ppm (Zn-ATP around pH 3.7), respectively.[24,28]

In contrast, the ^1H chemical shifts of ATP and ATP-spermidine are much less sensitive to pH variations. When sample pH was reduced from 7.0 to 2.0, for instance, H-8 and H-2 peaks in various ^1H-^{15}N HMBC spectra were only down-field shifted by ~0.2-0.3 ppm (the graph is not shown). These results are in consistent with earlier ^1H-NMR measurements of ATP.[29] However, the ^1H chemical shift data are difficult to analyze because of too small changes over the entire pH range.

We apply Eq. 1 to evaluate pK_a values for N-1 protonation / deprotonation of ATP and ATP-spermidine.[30]

$$pK_a = \sum_{k(\min)}^{k(\max)} 0.5\,(pH_\kappa + pH_{\kappa+1})\,(\delta_{\kappa+1} - \delta_\kappa) \,/\, (\delta_{\kappa(\max)} - \delta_{\kappa(\min)}) \qquad [1]$$

In this summation, $\delta_{\kappa(max)}$ and $\delta_{\kappa(min)}$ refer to maximum and minimum shift values, estimated from the start and end on N-1 curve-rise; pH_κ and $pH_{\kappa+1}$ are two adjacent pH data within *k*(min) and *k*(max) range, δ_κ and $\delta_{\kappa+1}$ are corresponding shifts. The pK_a value calculated from Eq.1 virtually matches the midpoint on N-1 curve-rise. As indicated in Fig. 3, pK_a for N-1 of ATP is 4.3, which is very close to the literature value (4.16) obtained by sigmoid fitting of the titration curve.[26] In contrast, pK_a for N-1 of ATP-spermidine is 3.1. This significantly lowered pK_a suggests that spermidine is capable of binding to N-1 site, and the binding promotes N-1 deprotonation.

B. ^{31}P Chemical Shift Variations

While ^{15}N data are indicative of spermidine interaction with ATP base, ^{31}P data are indicative of spermidine interaction with ATP phosphates. Fig. 4 shows shift variations of α-, β-, γ-phosphates of ATP and ATP-spermidine, obtained from various 1D acquisitions at different pH. Although in the physiologically relevant cellular pH range (6.86-7.21)[31] there are no appreciable changes of α-, β- and γ-shift, some useful information can still be obtained from the shift changes outside the physiological pH range. Unlike binding of divalent metal ions (Mg^{2+} etc.) on ATP, which induces significant down-field shift of β- and γ-peak but has little effect on α-shift,[30] effect of spermidine on α- and β-peak is small. However, spermidine has significant effect on γ-shift around pH 4.0-7.0. This suggests that spermidine interacts more strongly with ATP at γ-site. The pK_a values for γ-phosphate of ATP and ATP-spermidine are obtained as 6.0 and 5.0 respectively, according to Eq. 1. These pK_a values are related to protonation / deprotonation of the secondary H^+ in γ-phosphate.[27,30] The lowered pK_a of ATP-spermidine suggests that binding of spermidine facilitates the release of secondary H^+ from γ-ATP.

It can be realized from ^{15}N and ^{31}P data (Figs. 3-4) that over pH ~ 4.0-7.0, both N-1 peak and γ-peak of ATP-spermidine are down-field shifted compared to ATP. This means that in this pH range, spermidine is capable of concurrently binding to ATP base and phosphate sites. Such binding mode differs significantly from binding of some metal ions. In Mg-ATP and Zn-ATP, for instance, Mg^{2+} and Zn^{+2} ions are believed to bind to N1 at pH < 4.0 but switch from N-1 to the triphosphate end at elevated pH,[24,28] although to a small extent,

phosphate-bound metal ions may interact with N-7 to form intramolecular chelates.[27] The distinction suggests that the linear spermidine molecule has its inherent capacity to reach the two terminal sides (γ-P and base N-1) across the ATP molecule, although ATP predominantly takes an anti conformation (i.e. the base and sugar are on the opposite sides with respect to N9-C1' bond)[27] which makes N-1 farther than N-7 from phosphates. Indeed, upon interacting with ATP or some proteins spermidine may exist primarily in a linear conformation or take a more bent form, depending on binding conditions.[16,17,32]

C. ^{1}H NMR Diffusion Measurements

NMR PFG-diffusion measurements provide valuable information on molecular interaction and mobility.[22] Fig. 5 compares diffusion constants of spermidine, ATP, and ATP-spermidine samples (obtained at 20 mM, 25°C, and varied pH). Over the pH range ~2.0-9.0, the diffusion constants are about 2.4×10^{-10} to 2.6×10^{-10} m^2/s for spermidine, 1.4×10^{-10} to 1.5×10^{-10} m^2/s for ATP, and 1.1×10^{-10} to 1.3×10^{-10} m^2/s for ATP-spermidine. The molecular self-diffusion is known to inversely related to the apparent molecular mass and viscosity.[33,34] Here the trend of $D_{ATP\text{-}spermidine} < D_{ATP} < D_{spermidine}$ is consistent with their apparent masses, i.e. ATP-spermidine > ATP > spermidine. However, it is unclear whether these diffusion data have the implication to the cellular physiology.

It can be noticed from Fig. 5 that the diffusion curves fluctuate under varied pH in range of ~0.2×10^{-10} m^2/s. Such irregularity was also observed in earlier ^{1}H-diffusion measurements of ATP and Mg^{2+}-bound ATP by Jiang et al.[24] The irregularity can probably be attributed to two major factors: (i) the measurements had ~2-3% experimental uncertainties (estimated with the correlation coefficient $R \approx 0.99$ in the data fitting); (ii) the pH variations could induce protonation / deprotonation of ATP at the phosphates and/or N-1 site, which more or less affect the tendency for nucleotide self-association according to Sigel et al.,[27] thus affecting its self-diffusion. Nevertheless, the pH variations cause no dramatic changes in these measured diffusions. The ratios of diffusion constants, $D_{ATP} : D_{ATP\text{-}spermidine}$, are about 1.2 over pH 2.0-7.0. This value is close to the mass ratio of $M_{ATP\text{-}spermidine} : M_{ATP}$, suggesting that (i) binding of spermidine to ATP is in 1:1 ratio throughout this pH range; (ii) there is no significant pH-related nucleotide self-association in these samples. The differences between D_{ATP} and $D_{ATP\text{-}spermidine}$ are smaller when pH is above 8.0-9.0. This indicates reduced binding capacity of spermidine due to increased spermidine deprotonation at high pH.

D. Competitive Binding of Mg^{2+} and Spermidine

Chemical shift data can be utilized to analyze binding competition of different metal ions in nucleotide systems.[35-37] To investigate whether Mg^{2+} and spermidine compete for ATP binding, we also acquired ^{1}H-^{15}N HMBC and ^{31}P spectra of ATP and ATP-spermidine in the presence of equal amount Mg^{2+} (20mM) at pH 7.0. While the ^{15}N shifts were essentially unaffected by adding Mg^{2+}, major differences were observed in phosphate spectra.

Fig. 6 compares ^{31}P resonances of ATP, ATP-spermidine, ATP-spermidine-Mg and ATP-Mg samples, with four spectra aligned along α-peak. The spectra of ATP (d) and ATP-spermidine (c) are alike. This result is consistent with Fig. 4, where α-, β- or γ-curves of ATP and ATP-spermidine are nearly overlapped in pH 7.0-10.5 range. However, addition of Mg^{2+} to ATP and ATP-spermidine induces significant down-field shift of β- and γ-peak, as shown in spectra of ATP-Mg (a) and ATP-spermidine-Mg (b). This indicates that strong Mg^{2+} binding occurs at β- and γ-site. The β-peak of ATP-spermidine-Mg is also slightly broadened, confirming the existence of chemical exchange between ATP-Mg and ATP-spermidine caused by competitive binding of Mg^{2+} and spermidine at β-site. We estimate the relative amount of these two ATP forms by

$$X_{ATP-Mg} = \left(\Delta_{ATP} - \text{spermidine} - \Delta_{ATP-spermidine-Mg}\right) / \left(\Delta_{ATP-spermidine} - \Delta_{ATP-Mg}\right) \quad [2]$$

where $\Delta_{ATP\text{-}spermidine}$, $\Delta_{ATP\text{-}spermidine\text{-}Mg}$, and $\Delta_{ATP\text{-}Mg}$ are peak separations between α- and β-resonances in spectra of ATP-spermidine (c), ATP-spermidine-Mg (b) and ATP-Mg (a), respectively. The calculated $X_{ATP\text{-}Mg}$ = 0.88 and $X_{ATP\text{-}spermidine}$ = 0.12, indicating that ATP phosphates are predominantly bound by Mg^{2+} at neutral pH.

E. Effect of Spermidine on Myosin Catalyzed ATP Hydrolysis

Formation of ATP-spermidine complex may significantly affect the stability and activity of ATP. Fig. 7 describes ^{31}P NMR kinetic studies of myosin catalyzed ATP hydrolysis in the absence or presence of spermidine and Mg^{2+}. From β-peak decreases over time (Fig. 7a), it is clear that the rates of myosin catalyzed hydrolysis are ATP > ATP-Mg > ATP-spermidine > ATP-spermidine-Mg. The natural logarithms of signals (ln S_β) vs. time are linear (Fig. 7b), characterizing the reaction as pseudo first order. From slopes of straight-lines, the apparent rate constants (k= − slope) are obtained: 0.3319 h^{-1} for ATP, 0.2166 h^{-1} for ATP-Mg, 0.0738 h^{-1} for ATP-spermidine, and 0.0666 h^{-1} for ATP-spermidine-Mg, respectively. One should also bear in mind that in a sample containing ATP, spermidine and Mg^{2+}, the competitive binding of spermidine to ATP can somewhat decrease Mg^{2+} binding, increasing the free Mg^{2+}; and this factor may contribute to the hydrolysis rate as well. As the result, there is only slight difference between the measured hydrolysis rates of ATP-spermidine and ATP-spermidine-Mg.

Myosin catalyzed ATP hydrolysis involves the role of several amino acid residues in myosin, including interaction of β- and γ-phosphate with Lys185 through hydrogen bonds; coordination of Thr186 and Ser237 with cation; and a water molecule nucleophilically attacks γ-P.[38] The hydrolysis can be inhibited if binding of γ-phosphate on active site of myosin is interrupted.[39] Spermidine may increase or decrease ATPase activity of some proteins.[9,10,16] It is apparent from our data that both spermidine and Mg^{2+} contribute to the stability of ATP in myosin system. But they may function differently. Mg^{2+} is capable of β-, γ-bidentate and α-, β-, γ-tridentate binding to ATP, while the tridentate binding mode of cation may somewhat inhibit nucleotide hydrolysis, as proposed by Pelletier.[40] We believe that spermidine interacts with ATP phosphates and nucleobase concurrently at neutral pH; this explains the larger effect of spermidine than Mg^{2+} on stabilizing ATP.

In summary, the combination of multiple NMR techniques in this investigation, including ^{1}H-^{15}N HMBC, ^{1}H PFG-diffusion, ^{31}P NMR and kinetic measurements, enables us to get insight into specific interaction of spermidine with ATP at nucleobase and phosphate sites. The formation of ATP-spermidine or ATP-spermidine-Mg complexes is found to affect the stability and reactivity of ATP. We will apply these methods to explore other nucleotide-polyamine systems as well. The NMR results are important for advancing the studies on nucleotide-polyamine interaction and assessing its impact on nucleotide structures and activities under varied conditions.

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References

[1]. Surprenant A, Evans RJ. Nature. 1993; 362:211. [PubMed: 8384698]

[2]. Boyer PD. Biochemistry. 1987; 26:8503. [PubMed: 2894841]

[3]. Sigel, A.; Sigel, H. Metal Ions in Biological Systems, Interactions of Metal Ions with Nucleotides, Nucleic Acids, and Their Constituents. M. Dekker, Inc.; New York: 1996.

[4]. Pegg AE. Cancer Res. 1988; 48:759. [PubMed: 3123052]

[5]. Yamada M, Kurachi Y. J. Biol. Chem. 1995; 270:9289. [PubMed: 7721849]

[6]. Kosterin SO, Babich LH, Shlykov SH, Titus OV, Borysova LA. Ukr. Biokhim. Zh. 2005; 77:76. [PubMed: 16566133]

[7]. Babich LG, Borisova LA, Shlykov SG, Titus OV, Kosterin SA. Ukr. Biokhim. Zh. 2004; 76:55. [PubMed: 16350744]

[8]. Babich LG, Borisova LA, Shlykov SG, Titus OV, Kosterin SA. Ukr. Biokhim. Zh. 2004; 76:52. [PubMed: 16100897]

[9]. Kashiwagi K, Endo H, Kobayashi H, Takio K, Igarashi K. J. Biol. Chem. 1995; 270:25377. [PubMed: 7592703]

[10]. Veklich TO, Shkrabak OA, Kosterin SO. Ukr. Biokhim. Zh. 2008; 80:42. [PubMed: 19140449]

[11]. Kuramochi H, Yonezawa Y. J. Biosci. Bioeng. 2003; 95:225. [PubMed: 16233397]

[12]. Makita N, Yoshikawa K. FEBS Lett. 1999; 460:333. [PubMed: 10544259]

[13]. Günther T, Vormann J, Konstanczak P, Schafer A. Biochim. Biophys. Acta. 1994; 1192:281. [PubMed: 8018709]

[14]. Günther T, Vormann J, McGuigan JA, Luthi D, Gerber D. Biochem. Mol. Biol. Int. 1995; 36:51. [PubMed: 7663422]

[15]. Lüthi D, Gunzel D, McGuigan JA. Exp. Physiol. 1999; 84:231. [PubMed: 10226168]

[16]. Meksuriyen D, Fukuchi-Shimogori T, Tomitori H, Kashiwagi K, Toida T, Imanari T, Kawai G, Igarashi K. J. Biol. Chem. 1998; 273:30939. [PubMed: 9812989]

[17]. Maruyoshi K, Nonaka K, Sagane T, Demura T, Yamaguchi T, Matsumori N, Oishi T, Murata M. Chem. Eur. J. 2009; 15:1618.

[18]. Gasowska A. J. Inorg. Biochem. 2003; 96:346. [PubMed: 12888270]

[19]. Lomozik L, Gasowska A, Krzysko G. J. Inorg. Biochem. 2006; 100:1781. [PubMed: 16899296]

[20]. Willker W, Leibfritz D, Kerssebaum R, Bermel W. Magn. Reson. Chem. 1993; 31:287.

[21]. Tanner JE. J. Chem. Phys. 1970; 52:2523.

[22]. Johnson CS Jr. Progr. NMR. Spectr. 1999; 34:203.

[23]. Happe JA, Morales M. J. Am. Chem. Soc. 1966; 88:2077.

[24]. Jiang L, Mao XA. Spectrochim. Acta A Mol. Biomol. Spectrosc. 2001; 57:1711. [PubMed: 11471723]

[25]. Markowski V, Sullivan GR, Roberts JD. J. Am. Chem. Soc. 1977; 99:714. [PubMed: 833384]

[26]. Major DT, Laxer A, Fischer B. J. Org. Chem. 2002; 67:790. [PubMed: 11856021]

[27]. Sigel H, Griesser R. Chem. Soc. Rev. 2005; 34:875. [PubMed: 16172677]

[28]. Du F, Mao XA. Spectrochim. Acta A Mol. Biomol. Spectrosc. 2000; 56A:2391. [PubMed: 11075679]

[29]. Corfu NA, Sigel H. Eur. J. Biochem. 1991; 199:659. [PubMed: 1868851]

[30]. Song Z, Sims A, Eady J, Zhao H, Olubajo O. Can. J. Anal. Sci. Spectrosc. 2008; 53:45.

[31]. Blatter LA, McGuigan JA. Circ. Res. 1991; 68:150. [PubMed: 1845852]

[32]. Sugiyama S, Vassylyev DG, Matsushima M, Kashiwagi K, Igarashi K, Morikawa K. J. Biol. Chem. 1996; 271:9519. [PubMed: 8621624]

[33]. Dingley AJ, Mackay JP, Chapman BE, Morris MB, Kuchel PW, Hambly BD, King GF. J. Biomol. NMR. 1995; 6:321. [PubMed: 8520223]

[34]. Dingley AJ, Mackay JP, Shaw GL, Hambly BD, King GF. J. Biomol. NMR. 1997; 10:1. [PubMed: 20683727]

[35]. Amari L, Layden B, Nikolakopoulos J, Rong Q, Mota de FD, Baltazar G, Castro MM, Geraldes CF. Biophys J. 1999; 76:2934. [PubMed: 10354421]

[36]. Amari L, Layden B, Rong Q, Geraldes CF, Mota de FD. Anal. Biochem. 1999; 272:1. [PubMed: 10405286]

[37]. Mota de FD, Amari L, Srinivasan C, Rong Q, Ramasamy R, Abraha A, Geraldes CF, Boyd MK. Biochemistry. 1994; 33:4101. [PubMed: 8155627]

[38]. Okimoto N, Yamanaka K, Ueno J, Hata M, Hoshino T, Tsuda M. Biophys. J. 2001; 81:2786. [PubMed: 11606291]

[39]. Henry GD, Maruta S, Ikebe M, Sykes BD. Biochemistry. 1993; 32:10451. [PubMed: 8399190]

[40]. Pelletier H. Science. 1994; 266:2025. [PubMed: 7801132]

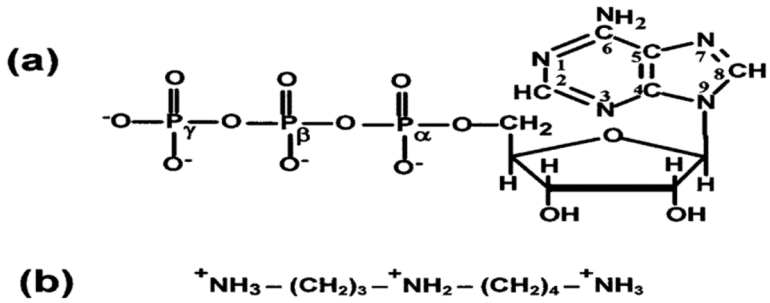

Figure 1.
ATP (a) and spermidine (b).

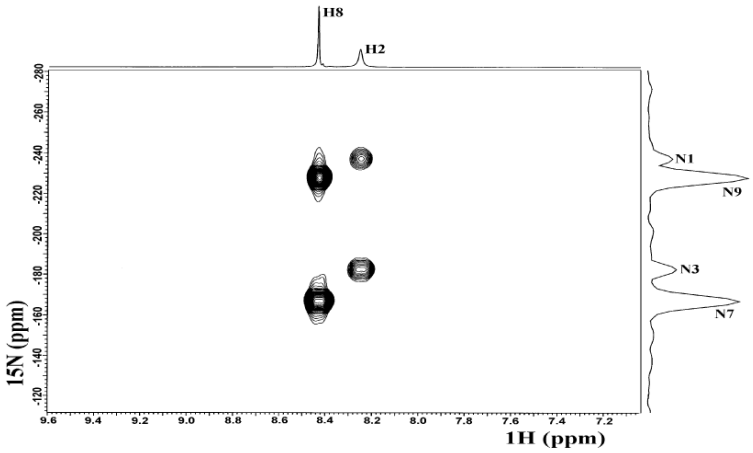

Figure 2.

A typical ^{1}H-^{15}N HMBC spectrum acquired with 20 mM ATP at pH 3.2. It shows well-resolved correlation peaks of H-8 and N-9, H-8 and N-7, H-2 and N-1, H-2 and N-3.

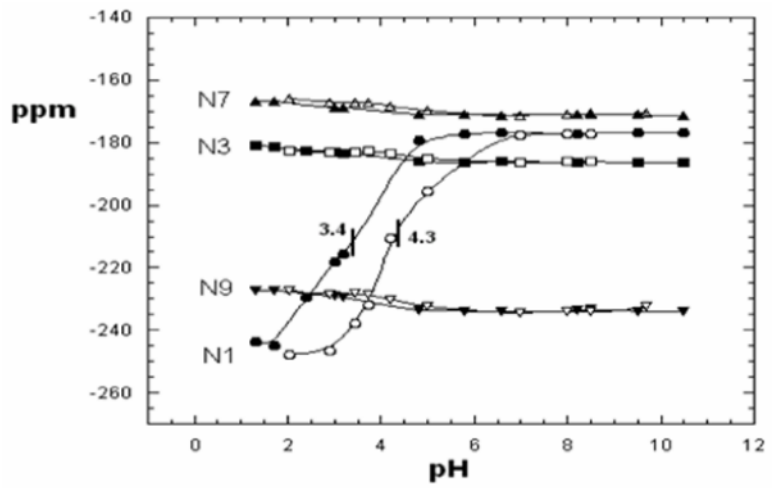

Figure 3.

^{15}N chemical shifts of nucleobase of ATP and ATP-spermidine. The data were from ^{1}H-^{15}N HMBC spectra measured at varied sample pH, and ^{15}N shift values were externally referenced to 90% formamide in DMSO (108.2 ppm). ATP: N-1 (○), N-3 (□), N-7 (△), N-9 (▽); ATP-spermidine: N-1 (●), N-3 (■), N-7 (▲), N-9 (▼). The pK_a values for N-1 protonation / deprotonation are 4.3 and 3.4 respectively.

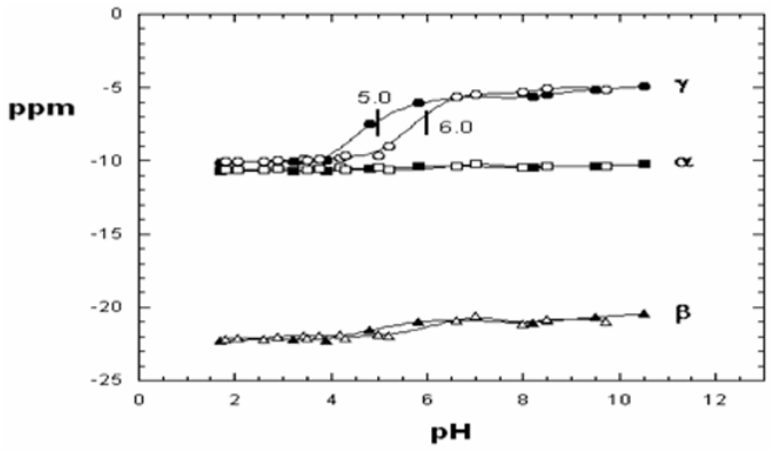

Figure 4.

^{31}P chemical shifts of α-, β-, γ-phosphates of ATP and ATP-spermidine at varied sample pH, with external reference of 85% H_3PO_4. ATP: α (□), β (△), γ (○); ATP-spermidine: α (■), β (▲), γ (●). The pK_a values for γ-phosphate are 6.0 and 5.0 respectively.

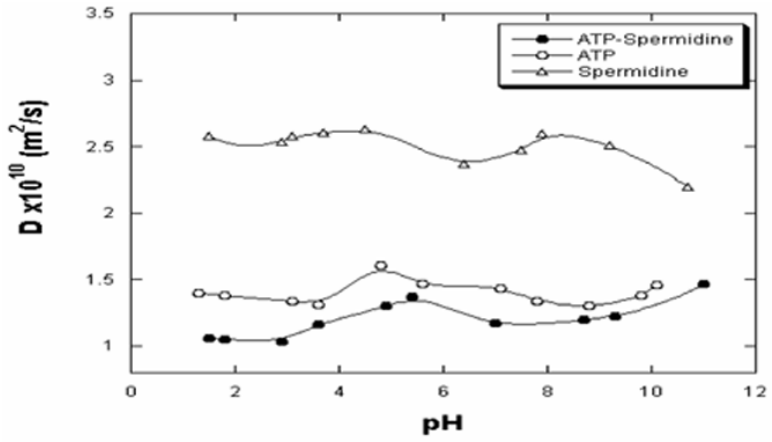

Figure 5.
Diffusion constants of spermidine (△), ATP (○), and ATP-spermidine (●) at varied pH. The data were derived from PFG-STE measurements with 20 mM samples at 25°C.

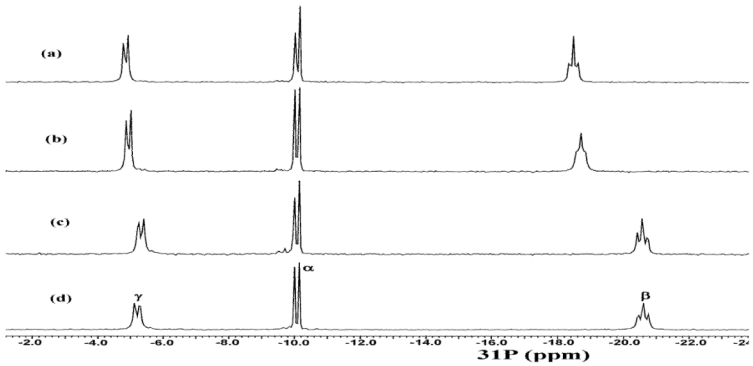

Figure 6.
^{31}P spectra of ATP-Mg (a), ATP-spermidine-Mg (b), ATP-spermidine (c) and ATP (d) measured at pH 7.0.

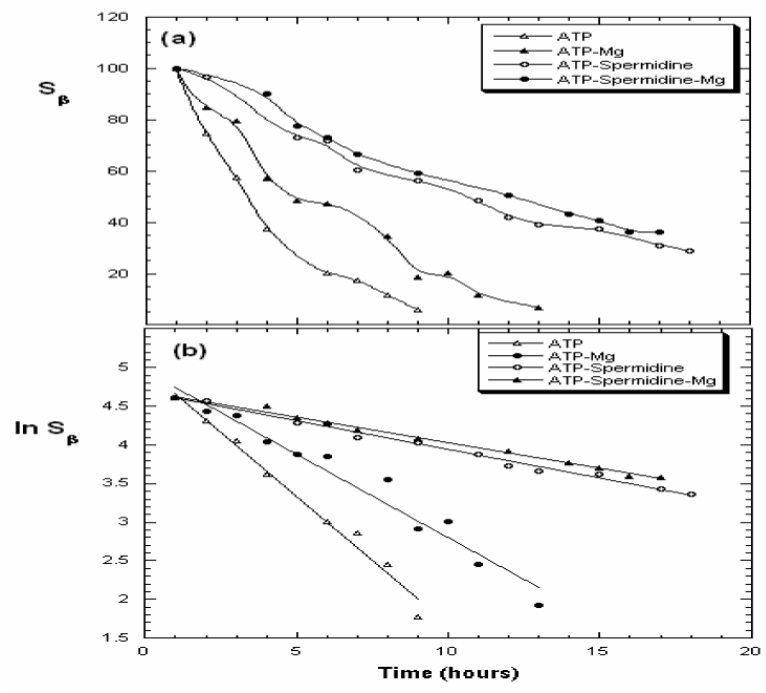

Figure 7.
(a) Myosin-catalyzed hydrolysis of ATP (△), ATP-Mg (▲), ATP-spermidine (○) and ATP-spermidine-Mg (●), characterized by decreasing β-signals; (b) Linear relationships of natural logarithms of β-signals vs. time.