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Conformations of Spermine in Adenosine Triphosphate Complex: The Structural Basis for Weak Bimolecular Interactions of Major Cellular Electrolytes

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Abstract: Selectively ^{2}H- and ^{13}C-labeled spermines (SPM) were efficiently synthesized and analyzed by NMR spectroscopy to determine the spin–spin coupling constants for six conformationally relevant bonds. SPM that is composed of three alkyl moieties, a butanylene, and two propanylene chains undergoes a conformational change when interacting with multivalent anions (e.g., adenosine triphosphate (ATP), ATP–Mg^{2+}, and tripolyphosphate). Upon interaction with ATP, the C–C bonds, which affect the distance between the neighboring pairs of ammonium groups (i.e., N1/N5 and N5/N5′), increase the population of *gauche* rotamers by 17–20% relative to those in the 4HCl salt of SPM. However, the trend in increments of the *gauche* conformers for the SPM–ATP complex profoundly differs from that of the spermidine (SPD)–ATP complex. This implies that SPM may preferentially recognize the adenyl group of ATP rather than the tripolyphosphate moiety. This may account for the higher affinity of SPM to ATP–Mg^{2+} than with that of SPD, which chiefly interacts with β- and γ-phosphates and is easily replaced by Mg^{2+}. These results may provide a clue for the further understanding of the structural basis of polyamine biological functions.

Keywords: adenosine triphosphate · conformation analysis · NMR spectroscopy · polyamines · spermines

Introduction

Molecular recognition between ligands and their corresponding receptors often exhibit markedly high affinities, which can result in nanomolar or sub-nanomolar dissociation constants, as exemplified by receptor interactions with hormones, drugs, secondary messengers, and toxins. In addition to these strict molecular recognitions, weak interactions between biomolecules often play crucial roles, such as those observed in neurotransmitters reception.[1] However, molecular basis responsible for these weak interactions remains largely unknown since the relevant compounds are flexible and undergo rapid association and dissociation. Due to the dynamic nature of these interactions, the structural elucidation of a given 3D ligand–receptor complex is extremely difficult. As a result, well-defined conformations of highly flexible linear biomolecules remain largely an unmet objective.[2]

Polyamines such as spermine (SPM, **1**) and spermidine (SPD, **2**) play essential roles in diverse biological events. The distribution and functions of these polyamines are known to be largely similar.[3a] Earlier titration experiments for the corresponding aqueous solutions revealed variations in the affinity to nucleotides as a function of the degree of phosphorylation and the species of nucleotide base examined.[3b] Furthermore, the question of why biological polyamines are mainly composed of three compounds (i.e., putrescine (PUT), spermidine, and spermine) remains unanswered. With respect to the stimulation of protein synthesis, three polyamines demonstrate a similar effect though their effective concentrations are different.[4] Additionally, some proteins that specifically recognize PUT, SPD, or SPM are known to be involved in biological functions.[5,6] The different roles of the polyamines should be derived from their structural differences. Therefore, conformation studies of SPM may provide a clue to answer this question. Polyamines, in particular SPM, form a ternary complex with ATP–Mg^{2+} that can affect phosphorylation of proteins. It is reported that SPM predominantly interacts with the β- and γ-phosphates of ATP in the presence of Mg^{2+}.[7] The ATPase activity of PotA, a protein component of a bacterial uptake system of SPD,[8] was greatly enhanced by SPM possibly due to formation of a ternary complex of SPM–ATP–Mg^{2+}. The activity of protein kinase A was also stimulated about twofold by SPM. The ternary complex, therefore, may play an important role in signal transduction during the proliferation stage of eukaryotic cells. To gain a better under-

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standing of the functional differences among polyamines, we examined in this study the conformation change of SPM in the presence and absence of ATP and compared the results with those of SPD.

Results

Molecular design of ^{2}H-labeled spermines: Molecular probes **3**–**8** were designed to examine the spin–spin coupling constants for the six conformationally relevant bonds of a C2-symmetric SPM molecule. With the *erythro*-deuterated SPMs **3**, **4**, **7**, and **8**, the rotational conformation around C2−C3, C3−C4, C6−C7, and C7−C7′ can be elucidated by the measurement of the respective ^{1}H–^{1}H coupling constant;[9] additional ^{13}C labeling at C7 in **8** was necessary to resolve H7 and H7′ signals in the ^{1}H NMR spectra. The remaining ^{13}C-labeled SPMs **5** and **6** were prepared to analyze the rotamer about the C4−N5 and N5−C6 bonds, respectively, by measuring ^3J(C,H) coupling. Synthetic routes were similar to those employed in the synthesis of SPD as we have reported previously.[9] Briefly, *erythro*-deuteration was effected by a *cis*-selective deuteration of the α,β-unsaturated δ-lactone. A successive Curtius rearrangement provided the propanylene moiety, whereas a reduction/azidation/hydrogenation sequence furnished the butanylene moiety. Coupling between the two parts was carried out using the Fukuyama method.[10] ^{13}C labeling was introduced by S_N2 displacement with $^{13}CN^-$ or a Wittig olefination (Scheme 1; see the Supporting Information for details).

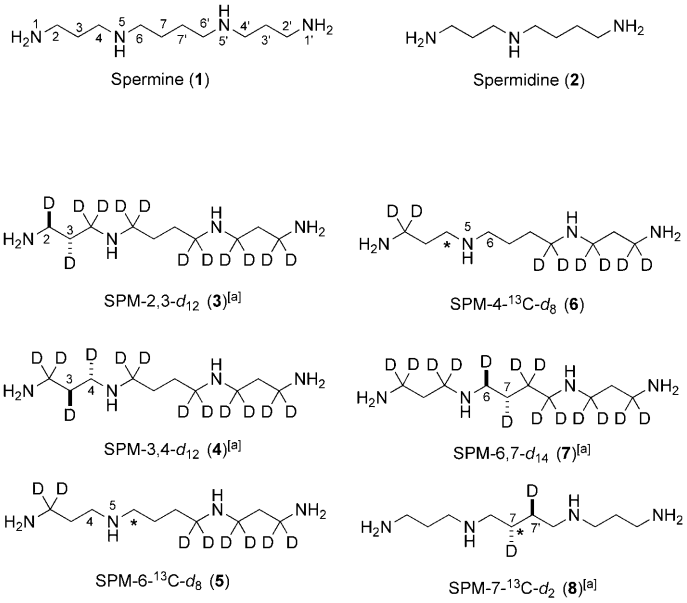
Table 1. Populations of *gauche* rotamers of SPM in complexation with ATP.

Labeled SPM	Bond (torsion angle)	$J_{obsd.}$	Counterion	^3J [Hz]	*gauche* [%]	Increment[a] [%]
SPM-2,3-d_{12} (**3**)	C2−C3 (N1/C4)	^3J(H2,H3)	4 HCl	9.4	18	
			pH 3.3 (ATP^{2-})	8.5	27	9
			pH 5.6 (ATP^{3-})	7.4	38	20
			pH 7.3 (ATP^{4-})	7.4	38	20
SPM-3,4-d_{12} (**4**)	C3−C4 (C2/N5)	^3J(H3,H4)	4 HCl	10.1	11	
			pH 3.3 (ATP^{2-})	9.5	17	6
			pH 5.6 (ATP^{3-})	8.3	29	18
			pH 7.3 (ATP^{4-})	8.3	29	18
SPM-6-^{13}C-d_8 (**5**)	C4−N5 (C3/C6)	^3J(C6,H4)	4 HCl	2.33	26	
			pH 3.3 (ATP^{2-})	2.35	26	0
			pH 5.6 (ATP^{3-})	2.34	26	0
			pH 7.3 (ATP^{4-})	2.36	27	1
SPM-4-^{13}C-d_8 (**6**)	N5−C6 (C4/C7)	^3J(C4,H6)	4 HCl	2.57	34	
			pH 3.3 (ATP^{2-})	2.51	32	−2
			pH 5.6 (ATP^{3-})	2.55	33	−1
			pH 7.3 (ATP^{4-})	2.55	33	−1
SPM-6,7-d_{14} (**7**)	C6−C7 (N5/C8)	^3J(H6,H7)	4 HCl	10.1	11	
			pH 3.3 (ATP^{2-})	9.4	18	7
			pH 5.6 (ATP^{3-})	8.5	28	17
			pH 7.3 (ATP^{4-})	8.5	28	17
SPM-7-^{13}C-d_2 (**8**)	C7−C7′ (C6/C6′)	^3J(H7,H7′)	4 HCl	10.3	24	
			pH 3.3 (ATP^{2-})	10.3	24	0
			pH 5.6 (ATP^{3-})	10.3	25	1
			pH 7.3 (ATP^{4-})	9.9	27	3

[a] Increment of the *gauche* population from that of the 4 HCl salt of SPM.

Binding affinity and stoichiometry of labeled SPMs to ATP: The stoichiometry of the SPM–ATP complex was determined to be one to one by examination of Job's plot (Figure S1 in the Supporting Information).[11] The Stokes radius, *d*, was calculated from the experimental data of the diffusion coefficients (*D* values) by using the Stokes–Einstein equation (Supporting Information).[12] The Stokes radius of the 4 HCl salt of SPM (1.21 nm) appeared to be smaller than the length of SPM in the extended conformation (≈1.6 nm). The Stokes radius of SPM in the presence of ATP is slightly larger than that of SPM. These results rule out the formation of larger multivalent complexes such as $(SPM)_2$–$(ATP)_2$ or $(SPM)_3$–$(ATP)_3$, thereby inferring their one-to-one relationship.

The association constant (K_a) of nondeuterated SPM with ATP was determined to be 1722 M^{-1} by NMR spectroscopic titration experiments, which agrees well with the reported data.[13] The value indicates that 99% of SPM is bound to ATP under the conditions of the NMR spectroscopic experiments described later. Thus the spin coupling constants in Table 1 should be regarded as those of the SPM–ATP complex. Next, the binding affinity of the polydeuterated SPM to ATP was estimated by NMR spectroscopic titrations to ensure that the deuterium substitutions did not influence



Scheme 1. Labeled spermines. [a] These compounds are racemic. [*] Position is labeled with ^{13}C.

the complexation of SPM with ATP. The association constant K_a between SPM-6,7-d_{14} (**7**) and ATP was estimated to be 1670 M^{-1} by the nonlinear least-square fit of the SPM H7 chemical shift (Figure S2 in the Supporting Information). This data supports the notion that deuterium incorporation does not appreciably affect the stability of the SPM–ATP complex.

Determination of the spin–spin coupling constants for SPM as the HCl, ATP, ATP–Mg^{2+}, and tripolyphosphate (TPP) salts: The spin–spin coupling constants, ^3J(H,H), were measured for the labeled SPMs. The SPMs as HCl salts (Figure 1, series A) and those of SPM in the presence of ATP were subjected to NMR spectroscopic measurements at various pH values (Figure 1, series B–D). For the labeled SPMs **3**, **4**, **7**, and **8**, the ^3J(H,H) values could be determined with an accuracy greater than 0.1 Hz despite the fact that the relevant ^{1}H signals were somewhat broadened as a result of the association–dissociation equilibrium, for which spectral simulations were carried out to obtain accurate ^3J-(H,H) values. The ^3J(H,H) values of **3**, **4**, **7**, and **8** as 4 HCl salts of SPM ranged between 9.4–10.1 Hz, which revealed that the dihedral angles of N1/C4, C2/N5, N5/C8, and C6/C6′ predominantly adopt the *anti* orientation (Table 1). When compared with the HCl salt, the ^3J(H,H) values of the ATP salts notably decreased for deuterated SPMs **3**, **4**, and **7**. Regarding the ionization state of ATP, the pK_a values in H_2O were reported at 4.00 and 6.50 under conditions similar to the present experiments.[14] By converting pK_a from H_2O to D_2O solutions according to a reported method,[15] these values become 4.25 and 6.94, respectively, thereby indicating that ATP is largely divalent at pH 3.3, trivalent at pH 5.6, and tetravalent at pH 7.3. At neutral pH, the ^3J-(H,H) value was the smallest, which clearly indicates that SPM undergoes conformational changes that serve to increase the bent conformation (equal to *gauche*-rich) by 17–20 %. These conformational alterations are further observed to be pH-dependent, an effect that is primarily due to the number of anionic sites on ATP; **3**, **4**, and **5** exhibited higher *gauche* populations as the pH was raised from 3.3 to 5.6. Unlike the other C–C bonds, the *gauche* enhancement for the C7/C7′ rotamers was small in the presence of ATP (Table 1). For analogues **5** and **6**, the *gauche* ratios were determined by measuring the ^3J(C,H) values by means of heteronuclear multiple bond correlation (HMBC) experiments; as shown in Figure 2; the HMBC cross-peak intensities that were obtained by varying the correlation time are in agreement with the theoretical curves calculated for ^3J(C,H)

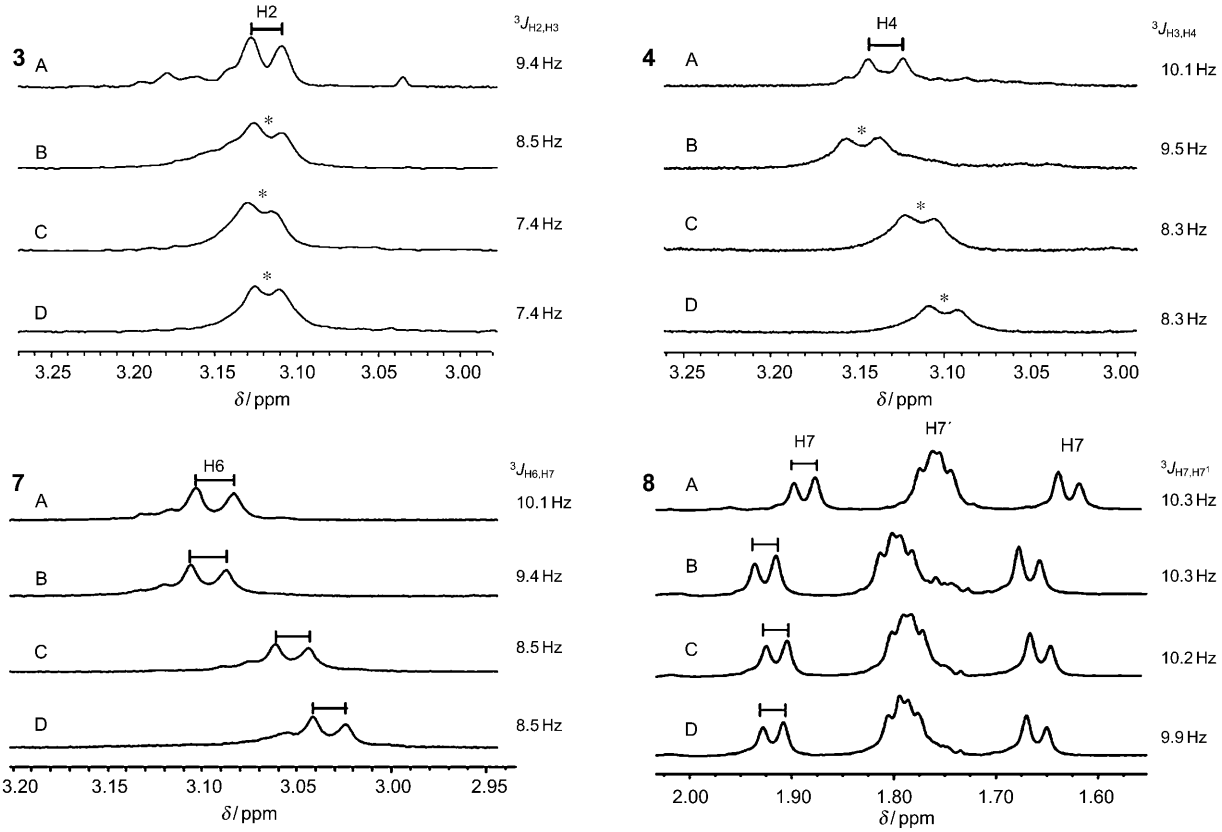


Figure 1. Partial ^{1}H NMR spectra (500 MHz) of the labeled SPMs (**3**, **4**, **7**, and **8**) at 40 °C. NMR spectroscopic experiments were carried out with a 25 mM solution of the 4 HCl salt of deuterated SPM at pH 7.3 in D_2O and combined with: A) no additive, B) a 100 mM ATP solution at pH 3.3, C) a 100 mM ATP solution at pH 5.6, or D) a 100 mM ATP solution at pH 7.3. *J values of these broad peaks with an asterisk were determined by spectral simulations (gNMR) since the peak-to-peak distances of broad signals often led to smaller coupling constants.

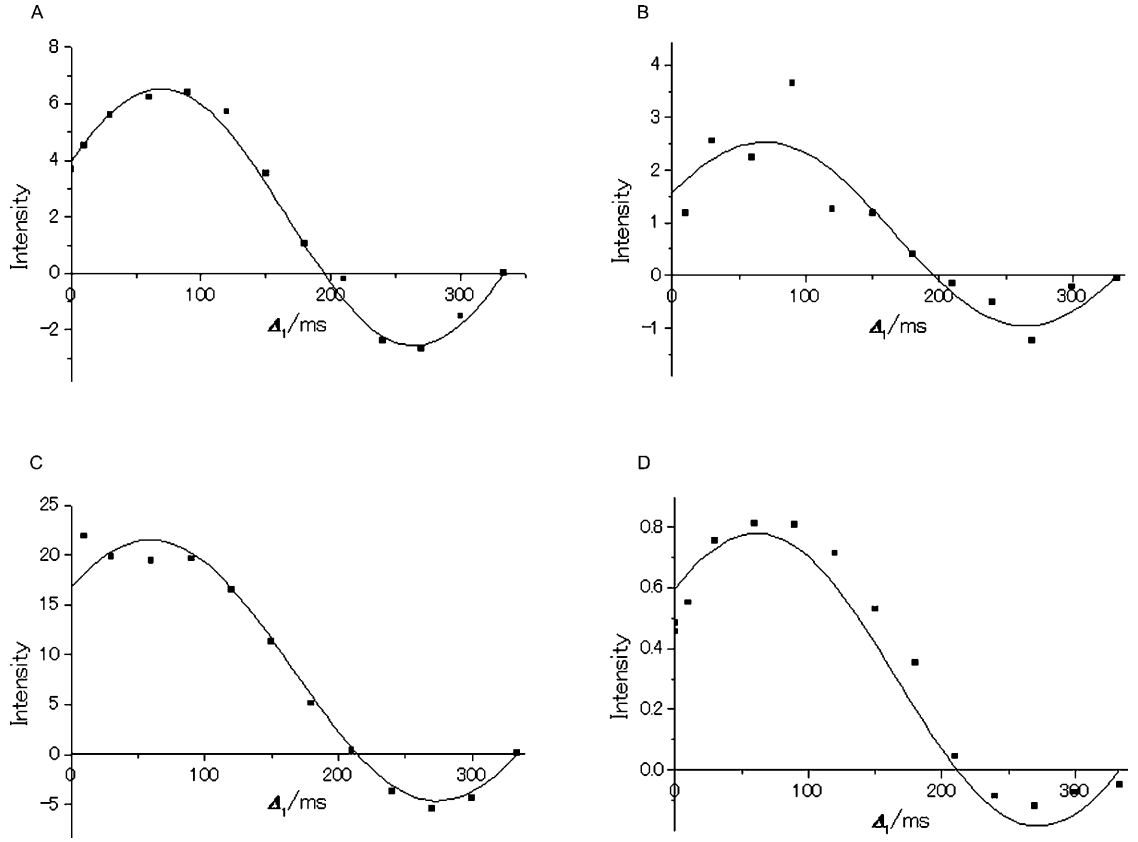


Figure 2. Interval-dependent changes in intensity of HMBC cross peaks due to ^{3}J(C,H) values of ^{13}C-labeled SPMs (**5** and **6**). NMR spectroscopic experiments were carried out: A) 25 mM of the 4HCl salt of labeled SPM **5** at pH 7.3 in D_2O; B) 25 mM of **5** in the presence of 100 mM ATP at pH 7.3; C) 25 mM of 4HCl salt of **6** at pH 7.3 in D_2O; and D) 25 mM of **6** in the presence of 100 mM ATP at pH 7.3. See the Supporting Information for details of the NMR spectroscopic experiments.

values. As is the case with SPD, the *gauche* populations with respect to the C–N bonds (i.e., C4–N5 and N5–C6) are not significantly affected by ATP (Table 1).

We next measured ^{3}J(H,H) values in the presence of TPP and ATP–Mg^{2+} for the C2–C3, C3–C4, C6–C7, and C7–C7′ bonds by using the same methodology since the other two bonds that showed no significant differences upon interaction with ATP were deduced to be unaffected as discussed later. In the presence of Mg^{2+}, which is known to form a complex with ATP with higher affinity than SPM, the conformational changes of SPM are somewhat attenuated (Table 2).

Table 2. Populations of *anti*/*gauche* rotamers of SPM in the presence of Mg^{2+} (100 mM).

Labeled SPM	Bond (torsion angle)	$J_{obsd.}$	Counterion	^{3}J [Hz]	*gauche* [%]	Increment[a] [%]
SPM-2,3-d_{12} (**3**)	C2–C3 (N1/C4)	^{3}J(H2,H3)	pH 3.3 (ATP^{2-})	8.7	25	7
			pH 5.6 (ATP^{3-})	8.7	25	7
			pH 7.3 (ATP^{4-})	8.8	24	6
SPM-3,4-d_{12} (**4**)	C3–C4 (C2/N5)	^{3}J(H3,H4)	pH 3.3 (ATP^{2-})	9.7	15	4
			pH 5.6 (ATP^{3-})	9.4	18	7
			pH 7.3 (ATP^{4-})	9.5	17	6
SPM-6,7-d_{14} (**7**)	C6–C7 (N5/C8)	^{3}J(H6,H7)	pH 3.3 (ATP^{2-})	9.5	17	6
			pH 5.6 (ATP^{3-})	9.2	21	10
			pH 7.3 (ATP^{4-})	9.1	22	11
SPM-7-^{13}C-d_2 (**8**)	C7–C7′ (C6/C6′)	^{3}J(H7,H7′)	pH 3.3 (ATP^{2-})	10.3	24	0
			pH 5.6 (ATP^{3-})	10.2	25	1
			pH 7.3 (ATP^{4-})	10.1	25	1

[a] Increment of the *gauche* population from that of the 4HCl salt of SPM.

Discussion

Conformation of SPM in the presence of ATP: Before discussing the SPM–ATP complex, we should examine the conformation of the 4HCl salt of SPM. The population of the *anti* rotamer about each C–C bond of SPM is approximately 82–89% (Table 1), which indicates that the *anti* conformer of SPM is more populated than that of the alkanes; this is most likely due to the electrostatic repulsion among the four ammonium groups. A similar *anti*-dominant conformation has been observed for SPD.[9]

In the presence of ATP, the ^{1}H–^{1}H spin coupling constants

of SPM were successfully determined for the specific complex that is thought to play a physiological role in ATP hydrolysis.[7] When interacting with ATP, the *gauche* conformers markedly increase for the C2–C3, C3–C4, and C6–C7 bonds; the populations are roughly doubled or tripled (Table 1). These data suggest that SPM tends to assume a bent conformation in the ATP complex, which is also the case with SPD.[9] However, with respect to the remaining bonds (i.e., C4–N5, N5–C6, and C7–C7′) the *gauche*/*anti* ratios are virtually unchanged. This can be explained by the attenuation of the electrostatic repulsion by ATP. The respective distances between the nitrogen atoms in the propanylene and butanylene moieties (i.e., N1–N5 and N5–N5′) are about 0.50 and 0.63 nm in the extended conformation, which is larger than the distance between the neighboring oxygen atoms in the tripolyphosphate portion of ATP (0.41–0.43 nm). Thus, SPM needs to adopt the conformation that arranges each cationic ammonium group in close proximity to the anionic site of ATP (as observed for SPD).[9] On the other hand, the rotation of the C4–N5, N5–C6, or C7–C7′ bond does not affect the distance of neighboring ammonium groups to the same extent as that of the C2–C3, C3–C4, or C6–C7 bond; this is analogous to that of the C3–C4 bond of SPD.[9] The *gauche* conformer with respect to the C7–C7′ bond reduces the distance between N5 and N5′ by 0.05 nm, which is much smaller than the reduction of the distance by 0.11 nm with the rotation about C6–C7 (or C6′–C7′). Rotation of C4–N5 or N5–C6 evidently produces no change in the N1/N5 or N5/N5′ distance.

A different trend in the coupling constants was observed for the SPM complex with TPP (Figure 3), which clearly indicates that the butanylene moiety bends more deeply to facilitate interaction with TPP because of its longer alkyl chain. The difference from those of the SPM–ATP complex in Figure 3A clearly demonstrates that SPM, unlike SPD, recognizes not only the triphosphate group of ATP but also the remaining portion of ATP.

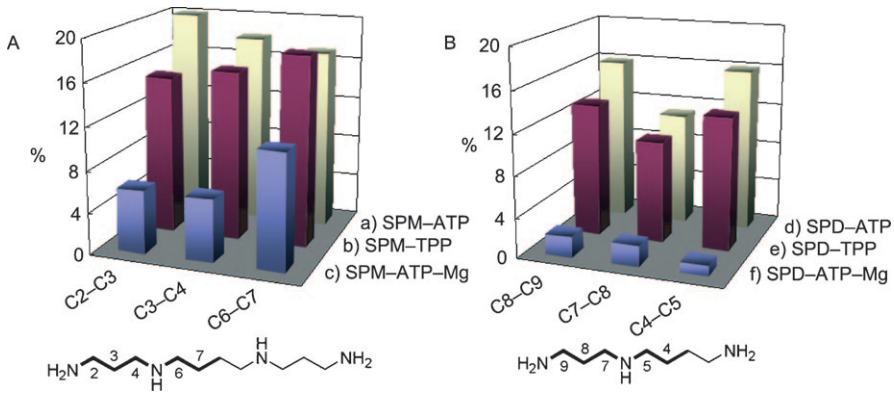


Figure 3. Increments of the *gauche* conformers in a) SPM–ATP, b) SPM–TPP, c) SPM–ATP–Mg^{2+} ternary complex, d) SPD–ATP, e) SPD–TPP, and f) SPD–ATP–Mg^{2+} ternary complex compared with those in the 4 HCl salt of SPM or 3 HCl salt of SPD. All the data were obtained at pH 7.3. Detailed data for a)–f) are provided in Table 1, Table S1 in the Supporting Information, Table 2, and our previous report.[9]

The SPM–ATP interaction at different ionization states was examined by determining the *gauche* populations at various pH values. The optimized structures of ATP with the net charges of −2, −3, and −4 have been deduced previously (see Figure 4).[16,17] The populations of rotamers about each of the C–C bonds did not significantly change between pH 5.6 and 7.3 in the presence of ATP (Table 1). At pH 7.3, the γ-phosphate becomes divalent, thus leading to tetravalent ATP. These unchanged *gauche* populations indicate that SPM has very weak interaction with the γ-phosphate terminus. These assertions are also additionally supported by the pH-dependent change in affinity of SPM with ATP obtained from the current NMR spectroscopic titration experiments; the K_a values at pH 3.3, 5.6, and 7.3 are 912, 1611, and 1722 M^{-1}, respectively.

The present findings are in agreement with the previous report by Meksuriyen et al. in that the conformational changes induced by SPM were observed only for the α-phosphate of ATP and not for the β- and γ-phosphate in the absence of Mg^{2+} as revealed by ^{31}P NMR spectroscopy.[7] Our unpublished data indicated that the K_a value of SPM with cytosine triphosphate (CTP) is appreciably less than that of SPM with ATP, whereas the K_a value between SPD and CTP is nearly equivalent to that between SPD and ATP (data not shown). It is implied that SPM may predominantly interact with the adenosine moiety and/or the α-phosphate group in the absence of Mg^{2+}. The present results may shed light on the differing physiological roles between SPD and SPM. As depicted in Figure 3, SPM exhibited an enhanced population of *gauche* conformers relative to that of SPD in the presence of ATP, which corroborates the higher affinity of SPM to ATP. Specifically, the C2–C3 and C3–C4 bonds, which correspond to the C8–C9 and C7–C8 bonds of SPD, respectively, revealed a significantly higher proportion of the *gauche* rotamers in the presence of ATP. This is not prominent in the SPM–TPP complex (Figure 3). These results again imply that SPM more preferentially recognizes the adenyl group of ATP rather than the γ-phosphate moiety. Another notable difference between SPD and SPM is found in conformation of the TPP complex. Whereas the three relevant bonds in SPD essentially indicated the same populations between the ATP and TPP complexes, SPM revealed a substantially different trend (Figure 3). The discrepancy may be explained by the notion that close interaction of the adenine moiety in ATP with the peripheral N1 amino group of SPM encourages the C2–C3 or C3–C4 bond to more deeply bend (Figure 4C). In the SPM–TPP salt, in which only three out

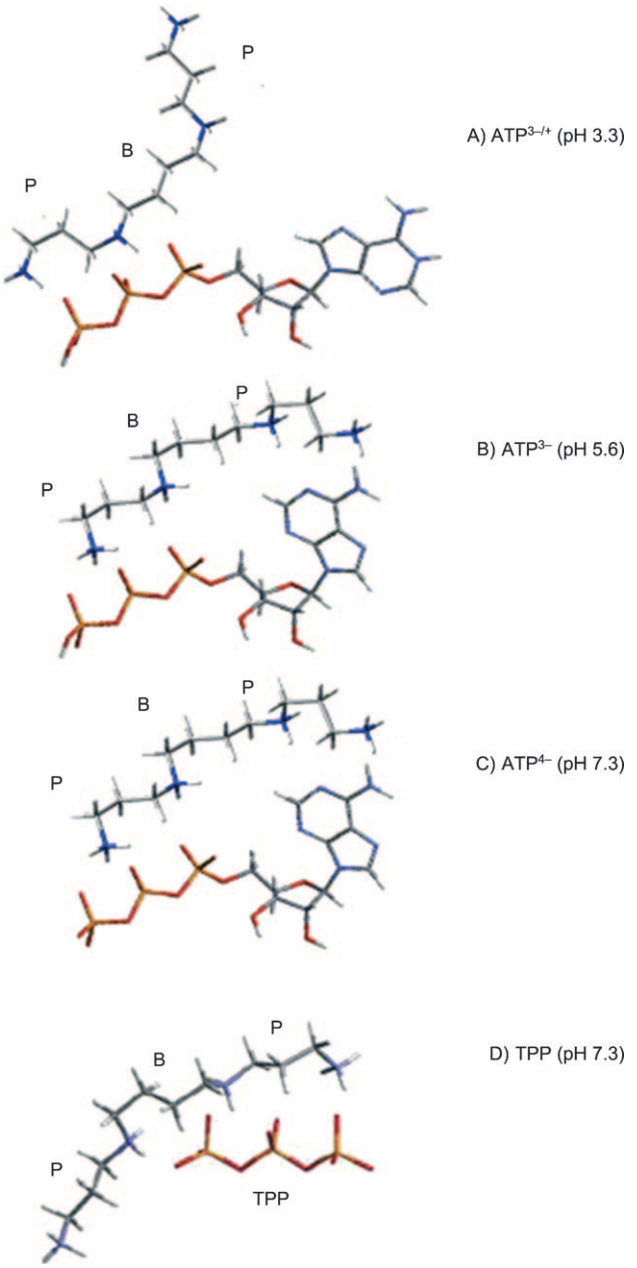


Figure 4. Hypothetical depictions of SPM molecules that account for the pH-dependent conformational change. Three of the possible conformations are present at pH 3.3, 5.6, and 7.3. The conformation of ATP was obtained by the force-field calculation using the optimized potential for liquid simulations (OPLS; B = butanylene part, P = propanylene part). A) SPM mainly interacts with the triphosphate group of ATP at pH 3.3. B) The propanylene portion of SPM recognizes the adenyl moiety while bearing the weak interaction of the terminal γ-phosphate at pH 5.6. C) SPM–ATP interaction at pH 7.3 is essentially the same as that at pH 5.6 (B) although the charge of the γ-phosphate becomes divalent. D) SPM shapes interaction with TPP^{4-}. The conformation of TPP was obtained by the molecular modeling (MM) calculation. The relative position of SPM and ATP (or TPP) derived from the simulation is not based on experimental data.

of the four amino groups in SPM are involved in electrostatic interactions, the internal N5/N5′ amino groups can fully form the salt with the phosphates in TPP, whereas one of the terminal N1/N1′ amino groups is not involved in the salt formation with TPP (Figure 4D). These interactions may cause an increased bent conformation in the butanylene moiety (C6–C7) in comparison with the propanylene (i.e., C2–C3/C3–C4) in the SPM–TPP complex.

Conformation of SPM in SPM–ATP–Mg^{2+} ternary complex: SPM is known to form a ternary complex SPM–ATP–Mg^{2+} with an ATP–Mg^{2+} salt under physiological conditions,[7] and it is thought to play a significant role in ATP-dependent events. Hence, we examined the conformation of the SPM–ATP–Mg^{2+} ternary complex using the same strategy. Under the conditions of NMR spectroscopic measurements with relatively high concentrations of these three electrolytes for Table 2, the NMR spectroscopic data could largely be attributed to the ternary complex since it was reported that the presence of Mg^{2+} does not greatly reduce the affinity of spermine to ATP, and vice versa.[7]

To deduce the conformational change of SPM upon interaction with ATP–Mg^{2+}, the relevant J values (Table 2) were measured under the conditions in which 85% of SPM was deduced to be complexed with ATP–Mg^{2+} on the basis of the K_a value of 71.0 M^{-1} at pH 7.3 (Figure 5). No significant

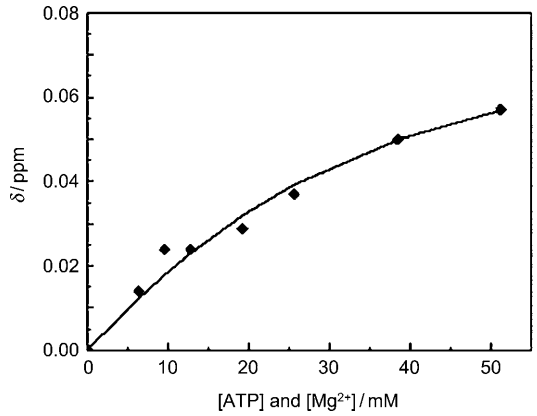
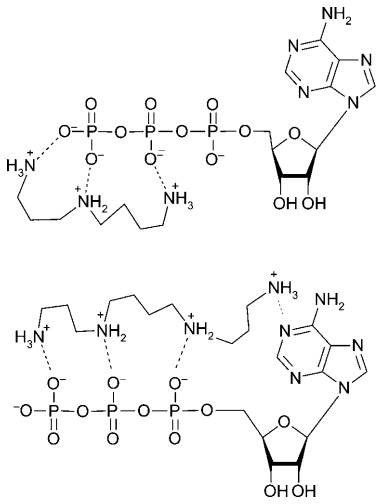


Figure 5. The association constants (K_a) between SPM and ATP–Mg^{2+} in D_2O at pH 7.3 and 40 °C were estimated with the nonlinear least-squares fit of the chemical shift of the H7 signal of SPM (K_a = 71.0 M^{-1}).

conformational change about each C–C bond was observed between pH 5.6 and 7.3 in the presence and absence of Mg^{2+}. Moreover, increments of the *gauche* conformers were smaller than those observed in the absence of Mg^{2+} (Table 2 and Figure 3). However, an 11% increase was observed for the C6–C7 bond, which may suggest that the SPM–ATP–Mg^{2+} complex possesses a different SPM–ATP interaction than that of the SPM–ATP complex. A similar trend in which the C6–C7 bond predominantly adopted the *gauche* conformation was observed for the complex between SPM and TPP (Figure 3). This indicates that SPM preferentially interacts with the tripolyphosphate moiety, particularly with the α- and β-phosphate groups of ATP in the presence of Mg^{2+}. These results are roughly consistent with the previous

observation that Mg^{2+} chiefly interacts with the β-, γ-phosphate group.[18,19] In contrast, SPD tends to bind to the γ-phosphate part as reported previously (Scheme 2). In this



Scheme 2. Simplified models of the SPD–ATP and SPM–ATP complexes in the absence of Mg^{2+}.

case, SPD should be readily repelled by Mg^{2+} to avoid an unfavorable charge–charge interaction. This difference in the binding mode may account for the higher affinity of SPM to the ATP–Mg^{2+} complex relative to that of SPD. To further examine the conformation of SPM in ATP or ATP–Mg^{2+} complexes, molecular modeling should be necessary. Elucidation of 3D structures of these highly flexible molecules, however, is still very difficult since conformation of SPM is very dynamic and depends heavily upon hydration and also the presence of other ions such as K^+ and Cl^-; for example, even upon complexation with structurally firm DNA duplex, SPD is reported to be very flexible and forms no defined complexes.[20]

Conclusion

Using the selectively ^2H- and ^{13}C-labeled SPMs, we succeeded in determining the spin–spin coupling constants for six conformationally relevant bonds. SPM revealed diverse conformational changes upon interaction with ATP, ATP/Mg^{2+}, and tripolyphosphate. The greater increments of the *gauche* conformers in the SPM–ATP complex than those in the SPM–tripolyphosphate complex imply that SPM more preferentially recognizes the adenyl group of ATP rather than the polyphosphate moiety in the absence of Mg^{2+} (Scheme 2). On the other hand, SPM may chiefly interact with the tripolyphosphate of ATP in the presence of Mg^{2+}. The conformations reported here reflect the time-average images of an SPM–ATP complex. However, these conformational changes of SPM may provide insight into the functional structure responsible for the weak interactions under physiological conditions and could be important in other molecular interactions with biological polyanions, which may partly account for the physiologically differential roles among polyamines, spermine, spermidine, and putrescine.

Experimental Section

Preparation of labeled SPMs: Labeled SPMs **3–8** were prepared by three key steps: a) *erythro*-selective hydrogenation of a α,β-unsaturated lactone or lactam, b) Curtius rearrangement to provide a primary amino group, and c) *N*-alkylation by using the method of Fukuyama et al.[10] Details of the synthesis are provided in the Supporting Information and our previous report.[9]

Measurements of stoichiometry and association constants in the SPM–ATP complex: The Job's plot was performed in D_2O at 40 °C using a JEOL GSX-500 500 MHz spectrometer. The total concentration of SPM plus ATP was maintained at 10 mM. The pH value was adjusted to 7.3 with deuterium chloride and sodium deuteroxide. The chemical shifts were recorded from 3-(trimethysiliy)-1-propanesulfonic acid sodium salt (DSS). The $\Delta\delta \times n_{SPM}$ values ($\Delta\delta$: the change in the chemical shift of H7 of SPM induced by addition of ATP; n_{SPM}: molar fraction of SPM, [SPM]/([ATP]+[SPM])) were monitored as a function of n_{SPM}. The self-diffusion coefficient, *D*, was estimated by the pulsed-field-gradient spin echo (PFGSE) method[21] in D_2O at 40 °C using a Varian Unity Inova 750 MHz spectrometer. The concentrations of SPM and ATP were 25 and 100 mM, respectively. The pH value was adjusted to 7.3 with deuterium chloride and sodium deuteroxide. The titration experiments were performed in D_2O at 40 °C with constant SPM concentrations of 25 mM and ATP concentrations of approximately 6.25, 9.38, 12.5, 18.75, 25, 37.5, and 50 mM. The spectra were obtained using a JEOL GSX-500 500 MHz spectrometer. The pH value was adjusted to 7.3 with deuterium chloride and sodium deuteroxide. The chemical shifts were recorded from DSS. The downfield shift of the H7 signal of SPM was monitored as a function of the ATP concentration. The data were fitted to a theoretical titration curve to obtain the association constants by using Origin 6.1 software provided by OriginLab Corporation.

Method for determining spin–spin coupling constants: Samples of **3–8** were dissolved in D_2O. The concentrations of SPM tetrachloride salt, ATP dipotassium salt, $MgCl_2$, and TPP pentapotassium salt were 25, 100, 100, and 100 mM, respectively. The pH value was adjusted to 3.3, 5.6, or 7.3 with deuterium chloride and sodium deuteroxide. ^3J(H,H) values were extracted from 1D ^1H NMR spectra. NMR spectra were obtained using a JEOL GSX-500 500 MHz spectrometer and a JEOL ECA-500 500 MHz spectrometer. The chemical shifts were recorded from DSS. The digital resolution for the ^1H NMR spectrum was 0.076 Hz point^{-1}. Hence, measurement of coupling constants can be carried out with an accuracy of ±0.1 Hz. ^3J(C,H) values were measured with HMBC experiments[21] using a JEOL ECA-500 500 MHz spectrometer. The FID data were acquired with 80 scans per inclement of a 4096 (F2)×16 (F1) matrix. The temperature of the probe was kept at 40 °C. The intensities of HMBC cross peaks were monitored as a function HMBC interval from 0 to 400 ms in approximately 30 ms steps while keeping constant experimental time, and fitted to a theoretical curve to obtain the ^3J(C,H) value by using Origin 6.1 software. The each error of a measured ^3J(C,H) value was less than 0.1 Hz.

Determination of *anti*/*gauche* populations and conformation energy: The population ratio between the *anti* and *gauche* conformers were determined on the basis of ^3J(H,H) values with conformational fluctuation calculated as reported previously.[9] Determination of the *gauche* rotamers based on the ^3J(C,H) values was carried out in a similar manner by using the static *anti* and *gauche* values. Details of the analysis are provided in the Supporting Information and our previous report.[9]

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