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Conformational Change of Spermidine upon Interaction with Adenosine Triphosphate in Aqueous Solution

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Abstract: Endogenous polyamines, represented by putrescine, spermidine, and spermine, are known to exert their physiological functions by interacting with polyanionic biomolecules such as DNA, RNA, adenosine triphosphate (ATP), and phospholipids. Very few examples of conformation analysis have been reported for these highly flexible polymethylene compounds, mainly due to the lack of appropriate methodologies. To understand the molecular basis of the weak interaction between polyamines and polyanions that underlies their physiological functions, we aimed to elucidate the solution conformation of spermidine by using diastereospecifically deuterated and ^{13}C-labeled derivatives (**1**–**7**), which were designed to diagnose the orientation of seven conformationally relevant bonds in spermidine. ^{1}H–^{1}H and ^{13}C–^{1}H NMR coupling constants ($^{3}J_{H,H}$ and $^{3}J_{C,H}$) were successfully determined for a spermidine–ATP complex. The relevant coupling constants markedly decreased upon complexation. The results reveal that spermidine, when interacting with ATP, undergoes changes that make the conformation more bent and forms the complex with the triphosphate part of ATP in an orientation-sensitive manner.

Keywords: biomolecular interactions · conformation analysis · coupling constants · polyamines · polyanions

Introduction

The interaction between a bioorganic small molecule and its target biopolymer plays an important role in cell physiology. Highly specific bimolecular recognition, and thus high affinity, such as that between a ligand and its receptor, has been extensively studied; this has often resulted in the elucidation of receptor-bound conformations[1] of hormones, drugs, secondary messengers, and toxins. Besides these strict molecular recognitions with nanomolar or sub-nanomolar dissociation constants, weak interactions among biomolecules often play a crucial role. For example, neurotransmitters usually possess relatively low affinity for their intrinsic receptors, which facilitates the rapid on–off switching of nervous signal transmission.[2] The molecular basis responsible for weak interactions, however, remains mostly unknown because the compounds involved are largely flexible and rapidly reach an association–dissociation equilibrium, which makes the conformation analysis of a bound ligand extremely difficult. Thus, there are very few examples of conformation studies of highly flexible linear biomolecules.[3] For complexes between flexible ionic compounds, almost nothing has been reported on their 3D structures. As a first step toward understanding the structure requirements for these interactions, we aimed to establish a basic strategy for the conformation analysis of weakly interacting biomolecules in aqueous environments. For example, endogenous polyamines such as putrescine, spermidine, and spermine interact with polyanionic biomolecules and provide an ideal model for this purpose. Their interactions with adenosine triphosphate (ATP), DNA, RNA, and phospholipids are assumed to be involved in essential biological events such as cell growth, differentiation, and proliferation,[4–6] despite the fact that their affinity to polyanions is usually low, with dissociation constants in the millimolar range.[7] Among the polyamines, we focused on spermidine (SPD) because its affinity to biological polyanions is comparable with that of tetravalent spermine and its asymmetric structure allows us to deduce the functional difference between the tetramethylene (butanylene) and trimethylene (propanylene) parts. Batista de Carvalho's group has reported some conformation studies of polyamines[8] on

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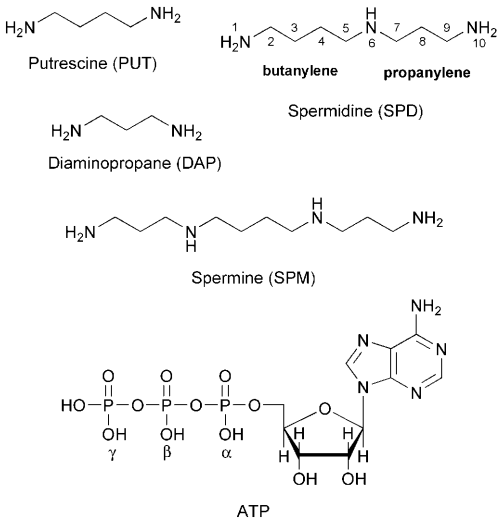


the basis of molecular orbital calculations and Raman spectroscopy, and these studies have partly provided the structure basis for polyamine interactions with biomolecules. Computer simulations cannot be applied for polyamine–polyanion complexes in aqueous environments because the coexistence of the multivalent cation and anion within a short distance causes hydration problems that make energy calculation and conformation analysis virtually impossible.

Among NMR methodologies,[9] NOEs often play an essential role in the conformation analysis of biomolecules. For highy flexible systems, however, NOEs are rather uninformative because minor conformers sometimes elicit disproportionally large NOE values. Instead, ^{1}H NMR spin–spin coupling constants are often utilized for conformation analysis of flexible compounds, including acyclic molecules. We needed to prepare isotope-labeled SPD derivatives in order to obtain the conformationally relevant coupling constants because of unresolved signals due to equivalent and/or overlapping signals of the methylene protons. In this study, we synthesized diastereoselectively ^{2}H/^{13}C-labeled SPDs **1**–**7** and determined their coupling constants in the presence of ATP. With these data in hand, we aimed to elucidate the conformational change in SPD upon complexation with ATP (Figure 1) in order to gain a better understanding of the interactions between polyionic biomolecules.

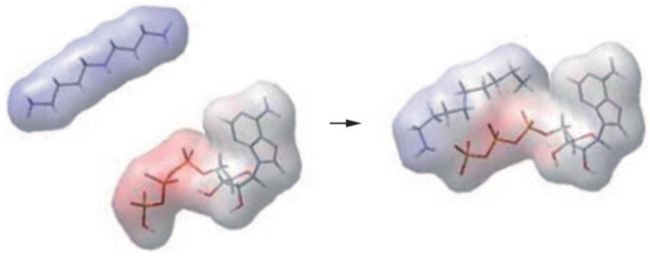
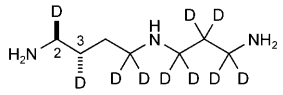
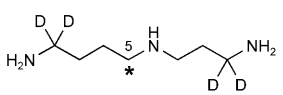


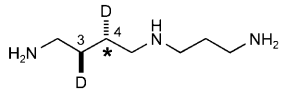
Figure 1. Hypothetical images of the SPD–ATP complex. The molecular surface is colored to indicate atom charges. The conformation of the complex on the right is that shown in Figure 4c.

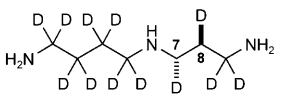
Results

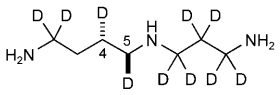
Molecular design of ^{2}H- and ^{13}C-labeled spermidines: We have previously reported that stereoselectively deuterated SPD may be used for conformation analysis by measurement of its ^{1}H–^{1}H coupling constants.[10] In this study, we extended this approach to all of the conformationally relevant bonds in SPD, and we designed molecular probes **1**–**7** for measurement of the spin–spin coupling constants $^{3}J_{H,H}$ and $^{3}J_{C,H}$. SPD **1**, deuterated in an *erythro* manner on the C2 and C3 atoms, was used for evaluating the populations of *gauche* and *anti* conformers around the C2−C3 bond on the basis of the $^{3}J_{H2,H3}$ value (carbon atom numbering follows the traditional pattern for polyamine derivatives).[10] An overlap of the signals for the methylene protons adjacent to the amino group, which hampered accurate measurement of the spin–spin coupling constants, was solved by introducing deuterium atoms at the C5, C7, C8, and C9 positions. In an analogous strategy, we carried out the conformation analysis for the C4−C5, C7−C8, and C8−C9 bonds by using labeled SPDs **3**, **6**, and **7**, respectively. However, this strategy was not applicable to the C5−N6 or N6−C7 bonds. To examine $^{3}J_{C,H}$ values instead of $^{3}J_{H,H}$ values, SPDs labeled with ^{13}C atoms at the C7 (**4**) and C5 (**5**) positions were synthesized; in these compounds, the methylene hydrogen atoms at the C2 and C9 positions were deuterated to avoid overlap with the methylene signals from the C5 and C7 positions, respectively. For the analysis of the remaining dihedral angle about the C3−C4 bond, labeled SPD **2**, in which the C3 and C4 positions were deuterated in the *erythro* configuration and C4 was ^{13}C-labeled, was designed. The close proximity of the chemical shifts of the H3 and H4 protons, which prevented measurement of the $^{3}J_{H3,H4}$ value, was resolved by introducing a ^{13}C atom at the C4 position to make the adja-

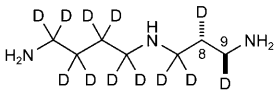

SPD-2,3-d_{10} (**1**)

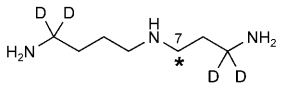

SPD-5-^{13}C (**5**)


SPD-4-^{13}C, 3,4-d_2 (**2**)


SPD-7,8-d_{12} (**6**)


SPD-4,5-d_{10} (**3**)

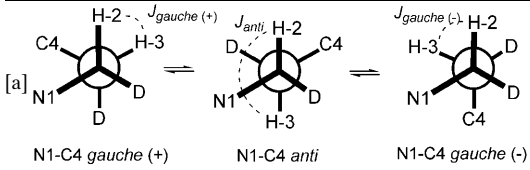

SPD-8,9-d_{12} (**7**)[10]


SPD-7-^{13}C (**4**)

cent methylene signals split by the $^1J_{C,H}$ value (see Figure 2). All of the *erythro*-labeled SPDs (**1**–**3**, **6**, and **7**) were racemic mixtures; this was better for determining the ratio of *anti*/*gauche* rotamers for the following reasons. ATP is optically pure so there are two ways for diastereomeric interactions to occur between ATP and racemic SPD. In the *anti* orientation, the $^3J_{H,H}$ values should be the same for both enantiomeric SPDs. In the *gauche* orientation, the populations of the two diastereomeric SPD–ATP complexes should be equal because the substitution effect of deuterium is negligible, as described later. The relevant $^3J_{H,H}$ value in this case is different between the enantiomers upon interaction with ATP because the $^3J_{H,H}$ value is dependent on the orientation of a nitrogen atom; the $^3J_{H2,H3}$ value of **1** for the *gauche* (+) rotamer is smaller than that for *gauche* (−) rotamer (as shown in Table 1). Even if there is a major rotamer with respect to one relevant bond upon interaction with ATP, the *gauche* (+) rotamer from one enantiomer and the *gauche* (−) rotamer from the other enantiomer equally contribute to the $^3J_{H,H}$ value. The same holds true with a minor rotamer. Thus, the total population of the *gauche* (+) and *gauche* (−) conformers can be determined from the average $^3J_{H,H}$ (or $^3J_{C,H}$) values (equal to the observed values) derived from two diastereomeric interactions.

Table 1. Calculated $^3J_{H,H}$ values for *anti* and *gauche* orientations in the SPD system.

Labeled SPD	*gauche* (+)[a] [Hz], near 60°	*anti*[a] [Hz], near 180°	*gauche* (−)[a] [Hz], near 300°
SPD-2,3-d_{10} (**1**)	1.2	11.5	2.8
SPD-4-^{13}C, 3,4-d_2 (**2**)	2.3	12.9	2.3
SPD-4,5-d_{10} (**3**)	2.8	11.5	1.2
SPD-7,8-d_{12} (**6**)	1.0	11.4	1.8
SPD-8,9-d_{12} (**7**)	1.7	11.4	1.0



Vicinal H−H orientations for *gauche* (+), *anti*, and *gauche* (−) are shown for **1**. Similar orientations occur for **2**, **3**, **6** and **7**, despite the fact that the interaction between the nitrogen and hydrogen atoms is inverted for **2**, **3**, and **7**. This leads to the reversed sizes of the $^3J_{H,H}$ values for *gauche* (+) and *gauche* (−).

Association constants of SPD–ATP, SPD–ATP–Mg, SPD–TPP, diaminopropane–ATP, and putrescine–ATP complexes: The association constant (K_a) and binding stoichiometry of the SPD–ATP complex were determined by NMR titration experiments, which were carried out with 25 mM SPD at pH 7.3 and various concentrations of ATP (see Figures S2–S5 in the Supporting Information for the experimental procedures and data).[11] We first examined the influence of deuterium substitution in SPD on the interaction with ATP. The association constant of decadeuterated SPD **1** was determined to be 540 M^{-1}, which is virtually equivalent with that of nondeuterated SPD (553 M^{-1}) and, thus, indicates that deuterium introduction hardly affects the affinity of SPD for ATP. Moreover, the K_a value is comparable with those reported by De Stefano et al, if the ionic strength is taken into account.[12] The stoichiometry of the SPD–ATP complex was determined by Job's method,[13] in which the induced shift ($\Delta\delta \times n_{SPD}$; see the Experimental Section), was plotted against the mole fraction of SPD to ATP (Figure S2 in the Supporting Information). One-to-one stoichiometric complexation was indicated by the maximum value at the host mole fraction of 0.5. As an alternative polyanion to ATP, we picked up tripolyphosphate (TPP) and determined its affinity with SPD, which turned out to be particularly high with a K_a value of 3960 M^{-1}. On the other hand, SPD showed much lower affinity for ATP–Mg^{2+} with a K_a value of 22.4 M^{-1}. Under the same conditions as those for the SPD experiments, the K_a values of the diaminopropane–ATP and putrescine–ATP complexes were determined to be 105.5 and 11.6 M^{-1}, respectively (Table S2 in the Supporting Information). Note the much higher affinity of diaminopropane to ATP than putrescine despite a difference of only one methylene moiety in their structures.

Determination of NMR coupling constants: The spin–spin coupling constants ($^3J_{H,H}$ and $^3J_{C,H}$) were measured for the labeled SPDs. The SPDs as HCl salts (Figure 2A) and in ATP complexes were subjected to NMR measurements at various pH values (Figure 2B–D). When **1** and **3** were used for determination of the $^3J_{H,H}$ values, the relevant ^{1}H NMR signals were simulated by the spectral-simulation software gNMR (Adept Scientific), because they were complicated by second-order couplings of the AB_2X type due to the close chemical shifts of the H3 and H4 signals. Upon measurement of **2**, the H4 signal was decoupled to a doublet by irradiation at the H5 protons. For the labeled SPDs **1**–**7**, $^3J_{H,H}$ and $^3J_{C,H}$ values could be determined with an accuracy of 0.1 Hz. The $^3J_{H,H}$ values of **1**–**3**, **6**, and **7** as 3 HCl salts of SPD ranged between 9.7 and 10.5 Hz, which revealed that the N1–C4, C2–C5, C3–N6, N6–C9, and C7–N10 sections predominantly take *anti* conformations (Table 2). With pH values of 3.3, 5.6, and 7.3, the $^3J_{H,H}$ values of the 3 HCl salts of the SPDs were virtually unchanged. Next, we carried out the same measurements for the SPD–ATP and SPD–TPP[10] complexes (see the Supporting Information for the SPD–TPP data). The association constant of the SPD–ATP complex indicated that 99% of SPD formed a complex with ATP under the conditions of the NMR measurements. The spin coupling constants in Table 2 should, therefore, be those of the SPD–ATP complex. Whereas SPD is a trivalent cation throughout the experiments, the net charge of ATP depends on the pH value, so ATP is present as divalent (−2), trivalent (−3), and tetravalent (−4) anions at pH 3.3, 5.6, and 7.3, respectively. At pH 3.3, the charges are −3 on the triphosphate and +1 on adenine; the charge is −3 on

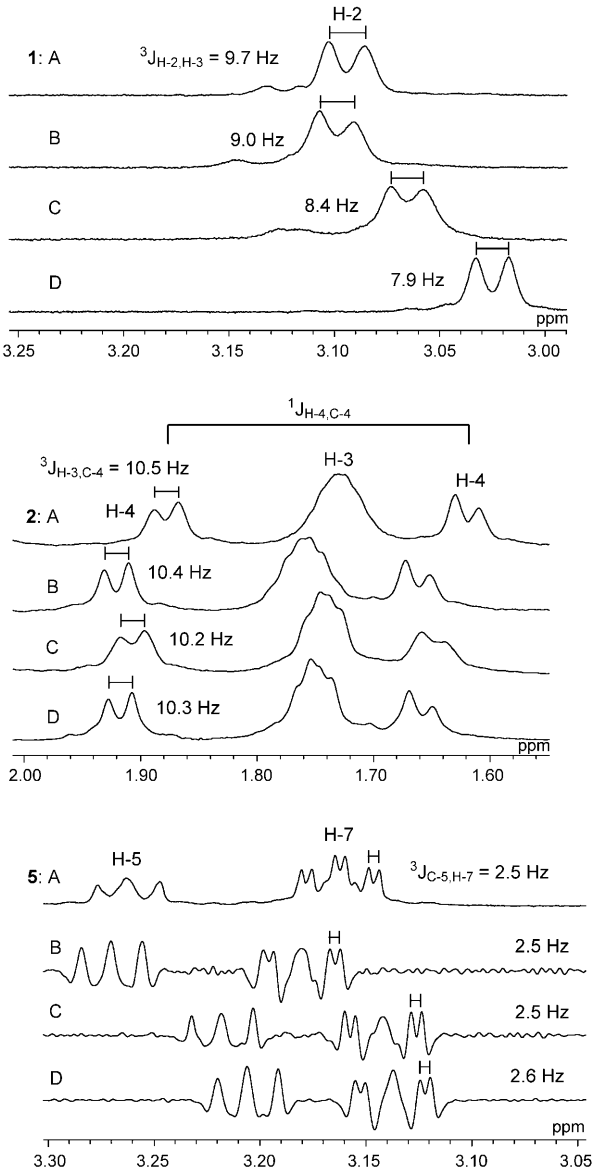


Figure 2. Partial ^{1}H NMR spectra (500 MHz, 40 °C) of the labeled SPDs **1**, **2**, and **5**. NMR spectra were measured with 25 mM labeled SPD as the 3 HCl salt in D_2O at pH 7.3 (A), and in the presence of 100 mM ATP at pH 3.3 (B), 100 mM ATP at pH 5.6 (C), and 100 mM ATP at pH 7.3 (D). The spectra of **1**–**7** as the 3 HCl salt at pH 3.3 and 5.6 were virtually identical with those shown in (A). Resolution enhancement was carried out for the spectra of compounds **4** and **5** in the presence of ATP. The spectra of other labeled SPDs are provided in the Supporting Information.

the triphosphate at pH 5.6 and it is −4 on the triphosphate at pH 7.3.[14] When compared with those of the HCl salt, the $^3J_{H,H}$ values of the ATP complex were notably lower for deuterated SPDs **1**, **3**, **6**, and **7**. The $^3J_{H,H}$ value was the smallest at a neutral pH value, at which the charge of ATP was −4; this clearly indicated that SPD undergoes conformational changes to increase the amount of the bent conformation (equivalent to *gauche* rich). On the other hand, only minimal changes in the $^3J_{H,H}$, $^3J_{C,H}$, and $^3J_{C,H}$ values were observed for **2**, **4**, and **5**, respectively, in the presence of ATP (Table 2). These results indicate that complexation with ATP does not significantly alter the average conformation with respect to the C3–C4, C5–N6, or N6–C7 bonds. In the presence of Mg^{2+} ions, which form a complex with ATP with a higher affinity than SPD, the conformational changes of SPD are much attenuated (Table 3).

Conformation analysis: In flexible or acyclic molecules, staggered conformers are predominant.[8,15] Ab initio calculations demonstrated that this holds true for alkyl amines[8] and further confirmed that the energy gap between the staggered and eclipsed rotamers in polyamines is comparable with that in alkane systems. The dihedral angles in the stable conformers are known to represent either the *anti* orientation at approximately 180° or the *gauche* orientation at approximately 60°, unless a bulky substituent is attached. Thus, we assumed that those conformations bearing intermediate angles or skew conformations hardly occur. When the $^3J_{H,H}$ value falls on an intermediate value, the H–H orientation is supposed to belong not to a nonstaggered rotamer but to interconverting *anti* and *gauche* rotamers. It is, therefore, possible to determine the population ratio between the *anti* and *gauche* conformers on the basis of spin–spin coupling constants,[10] provided that $^3J_{H,H}$ values typical of the *anti* and *gauche* conformers are in hand. The Karplus equation, which is used to determine dihedral angles from $^3J_{H,H}$ values, was originally derived from rigid cyclic compounds, so fluctuation from staggered rotamers should be taken into account for these acyclic compounds.[10] In this study, the modified Karplus equation[16] was applied for each rotamer in 1° steps. The weighted average of the $^3J_{H,H}$ values, which were obtained from the Boltzmann population and the coupling constant for each rotamer,[17,18] was calculated for the *gauche* (+), *anti*, and *gauche* (−) conformers around the C2–C3, C3–C4, C4–C5, C7–C8, and C8–C9 bonds (Table 1). The difference between hydrogen and deuterium is negligible for conformation potentials, so *gauche* (+) and *gauche* (−) should be equally populated, although their *J* values are different due to the hydrogen orientation with respect to a nitrogen atom (Table 1).

For the C5–N6 and N6–C7 bonds, however, the same strategy was not applicable due to the exchangeable protons of the ammonium groups. Instead, $^3J_{C,H}$ values were used for these bonds. The $^3J_{C,H}$ values of the *anti* and *gauche* orientations for a ^{13}C–N–C–H system were not available, so HMBC experiments were carried out for a conformationally restricted system, 1-deoxynojirimycin.[19] These values were converted into those for the flexible system by the same method as described above to give a $J_{CNCH,anti}$ value of 7.34 Hz and a $^3J_{CNCH,gauche}$ value of 1.59 Hz. The $^3J_{C,H}$ values for H5/^{13}C7 and H7/^{13}C5 can be used to determine the *anti*/*gauche* populations with respect to the C5–N6 and N6–C7 bonds, respectively.

The NMR spectra of labeled SPDs **1**–**7** (Figure 2 and Figure S1 in the Supporting Information) revealed that, for SPD as the HCl salt, the populations of the *anti* conformers around the C–C bonds were about 76–85% and those

Table 2. Populations of *anti*/*gauche* rotamers in the presence of ATP as obtained from ^{1}H NMR experiments.

Labeled SPD	Bond (torsion angle)	Observed J	Counterion	^3J [Hz]	*gauche*[a] [%]	Increment[b] [%]
SPD-2,3-d_{10} (**1**)	C2–C3 (N1/C4)	$^3J_{H,H}$	3 HCl	9.7	18	–
			pH 3.3 (ATP^{2-})	9.0	26	8
			pH 5.6 (ATP^{3-})	8.4	32	14
			pH 7.3 (ATP^{4-})	7.9	37	19
SPD-4-^{13}C, 3,4-d_2 (**2**)	C3–C4 (C2/C5)	$^3J_{H,H}$	3 HCl	10.5	24	–
			pH 3.3 (ATP^{2-})	10.4	24	0
			pH 5.6 (ATP^{3-})	10.2	25	1
			pH 7.3 (ATP^{4-})	10.3	25	1
SPD-4,5-d_{10} (**3**)	C4–C5 (C3/N6)	$^3J_{H,H}$	3 HCl	10.0	15	–
			pH 3.3 (ATP^{2-})	9.1	25	10
			pH 5.6 (ATP^{3-})	8.5	31	16
			pH 7.3 (ATP^{4-})	8.5	31	16
SPD-7-^{13}C (**4**)	C5–N6 (C4/C7)	$^3J_{C,H}$	3 HCl	2.7	39	–
			pH 3.3 (ATP^{2-})	2.6	35	−4
			pH 5.6 (ATP^{3-})	2.7	39	0
			pH 7.3 (ATP^{4-})	2.7	39	0
SPD-5-^{13}C (**5**)	N6–C7 (C5/C8)	$^3J_{C,H}$	3 HCl	2.5	32	–
			pH 3.3 (ATP^{2-})	2.5	32	0
			pH 5.6 (ATP^{3-})	2.5	32	0
			pH 7.3 (ATP^{4-})	2.6	35	3
SPD-7,8-d_{12} (**6**)	C7–C8 (N6/C9)	$^3J_{H,H}$	3 HCl	9.9	15	–
			pH 3.3 (ATP^{2-})	9.5	19	4
			pH 5.6 (ATP^{3-})	9.2	22	7
			pH 7.3 (ATP^{4-})	8.8	26	11
SPD-8,9-d_{12} (**7**)	C8–C9 (C7/N10)	$^3J_{H,H}$	3 HCl	9.8	16	–
			pH 3.3 (ATP^{2-})	9.0	24	8
			pH 5.6 (ATP^{3-})	9.0	24	8
			pH 7.3 (ATP^{4-})	8.2	32	16

[a] The percentage is a sum of *gauche* (+) and *gauche* (−) populations. [b] The increment of the *gauche* population relative to that of the 3 HCl salt of SPD.

around the C5–N6 and N6–C7 bonds were 61–68% (Table 2). When interacting with ATP, SPD significantly changes its conformation. For the C2–C3, C4–C5, C7–C8, and C8–C9 bonds, the *gauche* rotamer particularly increases by 11–19%, whereas the rest of the C–C and C–N bonds show insignificant changes of less than 4% (Table 2). These conformational alterations are dependent on the pH value and, thus, on the number of negative charges on ATP; **1**, **3**, **6**, and **7** showed higher *gauche* populations as the pH value was raised from 3.3, to 5.6, and then to 7.3. These data indicated that the conformation of SPD is significantly influenced by the number of charges on the counterions.

Discussion

Conformation of SPD as the HCl salt: To our knowledge, the present study provides the first experimental basis for the conformation of acyclic multivalent ions in solution. Before dealing with an SPD–ATP complex, we discuss the conformation of SPD as the HCl salt. The J values in Table 2 reveal that the population of the *anti* conformer about each C–C bond was 76–85%, whereas a significantly higher population of *gauche* conformer was observed for the central C5–N6 and N6–C7 bonds. With respect to a C2–C3 bond of butane, the population of the *anti* conformer is about 56% in the liquid phase.[20] The findings indicate that the *anti* conformer of SPD is more populated than that of alkanes, which can be accounted for by the electrostatic repulsion among the three ammonium groups of SPD. The energy difference between the N1–C4 *anti* (82%) and *gauche* (9%) conformers, despite the fact that the distance between N1 and N6 should be significantly shorter in the *gauche* conformer, is 1.3 kcal mol^{-1}, which implies that the counterions and hydration greatly attenuate the electrostatic repulsion. A similar tendency was observed for the other C–C bonds of SPD (Table 2). The highly populated *gauche* conformers for the C5–N6 and N6–C7 bonds are explainable by the notion that the distance between the neighboring ammonium groups (N1/N6 or N6/N10) is hardly affected by the conformation changes around these bonds. At acidic pH values of 3.3 and 5.6, the populations of rotamers around all the relevant bonds of the SPD HCl salt were essentially the same as those at pH 7.3 (data not shown), which indicates that SPD forms a trivalent

Table 3. Populations of *anti*/*gauche* rotamers of SPD in the presence of ATP and Mg^{2+}.

Labeled SPD	Bond (torsion angle)	Observed J	Counterion	^3J [Hz]	*gauche*[a] [%]	Increment[b] [%]
SPD-2,3-d_{10} (**1**)	C2–C3 (N1/C4)	$^3J_{H,H}$	pH 7.3 (ATP^{4-})	9.3	23	+5
SPD-4,5-d_{10} (**3**)	C3–C4 (C3/N6)	$^3J_{H,H}$	pH 7.3 (ATP^{4-})	9.9	16	+1
SPD-7-^{13}C (**4**)	C5–N6 (C4/C7)	$^3J_{C,H}$	pH 7.3 (ATP^{4-})	2.7	39	0
SPD-5-^{13}C (**5**)	N6–C7 (C5/C8)	$^3J_{C,H}$	pH 7.3 (ATP^{4-})	2.5	32	0
SPD-7,8-d_{12} (**6**)	C7–C8 (N6/C9)	$^3J_{H,H}$	pH 7.3 (ATP^{4-})	9.7	17	+2
SPD-8,9-d_{12} (**7**)	C8–C9 (C7/N10)	$^3J_{H,H}$	pH 7.3 (ATP^{4-})	9.6	18	+2

[a] The percentage is a sum of *gauche* (+) and *gauche* (−) populations. [b] The increment of the *gauche* population relative to that of the 3 HCl salt of SPD.

cation in this pH range. Under alkaline conditions (pH 11), in which case SPD was not ionized, the *gauche* conformer comprised 32% and 31% with respect to the C2–C3 and C4–C5 bonds, respectively, with increments of 14 and 10% from those of its trivalent cation at pH 7.3. These conformational changes support the conclusion that electrostatic repulsion between the ammonium groups moderately but significantly affects the conformation of SPD.

Conformation of SPD upon interaction with ATP: Upon interaction with ATP, the *gauche* orientation of SPD greatly increased (Table 2), which indicates that SPD tends to take a bent conformation in its ATP complex. The increments in the *gauche* rotamer are larger for the C2–C3, C4–C5, C7–C8, and C8–C9 bonds, for which the *gauche* populations are roughly doubled (Table 2). These conformational alterations are significant; the internal rotations with respect to the C2–C3, C3–C4, and C4–C5 bonds alter the distance between N1 and N6 in the butanylene part of SPD, in which the populations of *gauche*-containing conformers are estimated to comprise over 90%, whereas those for the 3 HCl salt of SPD comprise approximately 57%. For the propanylene part, the *gauche–anti* and *anti–gauche* (*GA* and *AG*) conformers of the C7–C8 and C8–C9 bonds, which affect the distance between N6 and N10, are estimated to comprise over 50% in the ATP complex as compared with approximately 30% in the SPD HCl salt. By contrast, the other bonds, C3–C4, C5–N6, and N6–C7, gave rise to only minimal changes upon complexation with ATP. These differences can be accounted for by the notion that a rotation around C5–N6 (or N6–C7) does not alter the distance between N1 and N6 (or N6 and N10). The rotational conformation around these bonds should, therefore, have little influence on the electrostatic repulsion among the ammonium groups in SPD. Similarly, the rotation with respect to the C3–C4 bond changes the distance between N1 and N6 to a smaller extent than that of the other C–C bonds. The interatomic distance between N1 and N6 was calculated to be 0.63 nm for the C3–C4 *anti* conformer (*AAA* for C2–C3/C3–C4/C4–C5) and 0.59 nm for the C3–C4 *gauche* one (*AGA*), whereas the distance was significantly reduced to 0.53 nm in the C2–C3 *gauche* (*GAA*) and C4–C5 *gauche* (*AAG*) conformers.

The same set of coupling constants were obtained for the complex with tripolyphosphate (Figure 3). The close similarity between the results for SPD–TPP and SPD–ATP demonstrates that SPD chiefly recognizes the triphosphate group of ATP, a conclusion that is further supported by the high affinity between SPD and TPP.

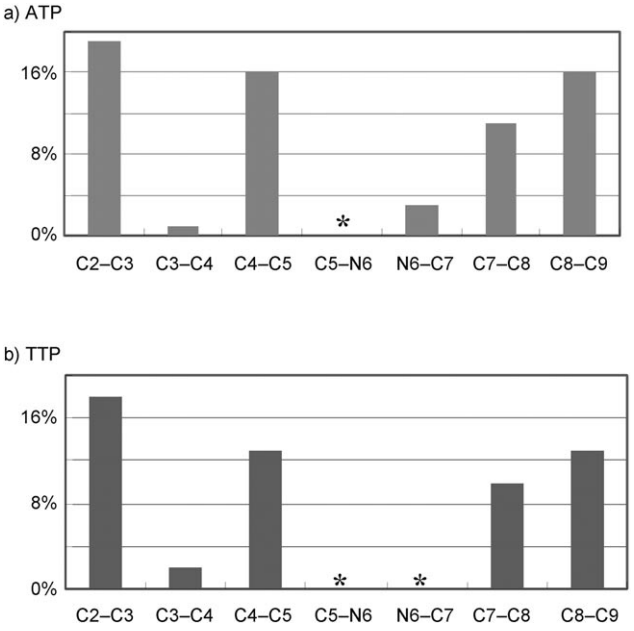


Figure 3. Increments of *gauche* conformers in SPD–ATP (a) and SPD–TPP (b) at pH 7.3 as compared with those in the 3 HCl salt of SPD. Detailed data for the SPD–TPP complex are provided in the Supporting Information. *: The increment percentage was less than the detection limit of 1.5% in measurements of C–H coupling constants.

The distances between the nitrogen atoms in the butanylene and propanylene parts (N1/N6 and N6/N10) are about 0.63 and 0.50 nm, respectively, in the extended conformation; these values are larger than the distance between neighboring oxygen atoms in the triphosphate moiety of ATP (0.41–0.43 nm). Therefore, SPD needs to change conformation so as to arrange each ammonium group close to an anionic point of ATP. The *gauche* conformer in the butanylene part was more populated than that in the propanylene section (Table 2), which indicates that the butanylene part needs to bend more upon interaction with the triphosphate group of ATP due to its longer alkyl chain.

The next question to be addressed is "what does the gross conformation of SPD in the ATP complex look like?" As

the first step, we estimated the distances of N1/N6 and N6/N10 for each conformer. In the propanylene part, the *gauche* C7−C8/*gauche* C8−C9 (*GG* or *GG′*) conformation is negligible because the distance between N6 and N10 in the *GG* conformer is too close (0.36 nm) to accommodate the negative charges of ATP. Therefore, the C7−C8/C8−C9 conformation relevant for the N6/N10 distance takes either the *AA* (42%), *AG* (32%), or *GA* (26%) orientation; the N6/N10 distance in these conformers is 0.50, 0.44, and 0.44 nm, respectively. For the butanylene part, the story is a little more complicated. There are three bonds relevant for the N1/N6 distance, namely C2−C3, C3−C4, and C4−C5. If these bonds take the *anti* or *gauche* conformation independently, the *AAA* conformer occurs with 33% population. The *GGG* conformation is very unlikely because of the short N1/N6 distance. The *GG′* conformers that have the 1,3-*syn* orientation are also negligible for the same reason. Therefore, the butanylene part can be represented by 8 conformers with relative stereochemistry: *AAA*, *AAG*, *AGA*, *GAA*, *AGG*, *GAG*, *GAG′*, and *GGA*. The N1/N6 distance of these conformers ranges from 0.47 (*GAG*) to 0.63 nm (*AAA*). Provided that each bond takes the *anti*/*gauche* rotation independently of the other bonds, the all-*anti* conformer occurs in 32.6%, single-*gauche* ones in 44.6%, and double-*gauche* ones in 19.9%. Since the double-*gauche* conformers are more unstable than the single-*gauche* ones due to electrostatic repulsion and steric hindrance, the single-*gauche* conformers may actually be more populated, probably in excess of 50%. In ATP, the distances of O_α–O_β and O_β–O_γ are 0.41 and 0.43 nm, respectively, which can be regarded as the distances between neighboring anionic sites. Therefore, in the propanylene part, the *AG* and *GA* conformers for the C7−C8/C8−C9 bonds are suitable to arrange the N6 and N10 close to the neighboring phosphate groups, and in the butanylene part, one (or two) *gauche* conformers for the C2−C3/C3−C4/C4−C5 bonds fit the distance between the phosphate anions. These notions may account for the increase in the *gauche* orientation in each C−C bond that was described earlier. The interaction between the butanylene and propanylene parts in the SPD–ATP complex remains unclear; the aforementioned accounts of the distances between N1 and N6 or N6 and N10 imply that these two parts change their conformations in a rather independent manner.

pH-Dependent conformation change of the SPD–ATP complex: We next examined the SPD–ATP interaction in different ionization states with respect to the *gauche* populations at various pH values. The optimized structures of ATP with net charges of −2, −3, and −4 have been deduced by a conformation search and the X-ray data for $Na^+_2ATP^{2-}$.[21,22] The conformational changes with a change from pH 3.3 to 5.6 indicate that the increment in *gauche* conformers is larger for C2−C3 (6%) and C4−C5 (6%) in the butanylene part than for C7−C8 (3%) and C8−C9 (0%) in the propanylene part (Table 2). At pH 3.3, at which point the average net charge of ATP is −2, the *anti* orientation of the adenine ring to the ribose is dominant (Figure 4a).[9] However, at

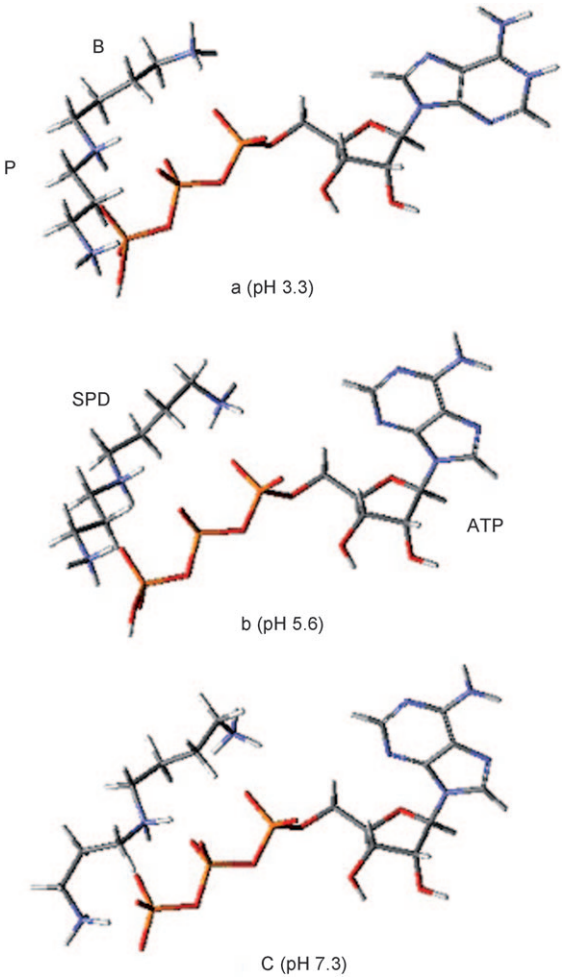


Figure 4. Hypothetical images of the SPD shapes accounting for the pH-dependent increase in *gauche* orientation. Three of the many possible conformations, a–c, are depicted. The orientations of the C2−C3, C3−C4, C4−N5, N5−C6, C6−C7, C7−C8, and C8−C9 bonds are *AAGAAAA* in (a), *GAGAAAA* in (b), and *GAGAG′AG* in (c). The conformation of ATP was obtained by calculations with the OPLS force field. When the pH value was raised from 3.3, the orientation of the adenine group of ATP switches to the *syn* conformation at pH 5.6, which leads to the bent butanylene part (B). The conformation of the propanylene part (P) is further altered by the second deprotonation of the γ-phosphate group at pH 7.3. The relative positioning of SPD and ATP derived from the simulation is not based on experimental data.

pH 5.6, at which point the ATP becomes trivalent, the adenine base takes the *syn* conformation (Figure 4b). This conformational alteration should give rise to a greater affect on a countercharge group with the α-phosphate group, so the butanylene part is assumed to interact preferentially with the α-phosphate side. On the other hand, with the conformation change from pH 5.6 to 7.3 in the presence of ATP, the *gauche* populations of the C7−C8 (4%) and C8−C9 (8%) bonds in the propanylene part increase more significantly (Table 2) than those of the C2−C3 (5%) and C4−C5 (0%) bonds. At pH 7.3, the γ-phosphate group becomes divalent and thus makes ATP tetravalent, so the propanylene part is assumed to interact with the γ-phosphate terminal. These findings imply that the interaction between ATP and

SPD is orientation sensitive and the propanylene part of SPD has a higher affinity to ATP at the neutral pH value. Furthermore, we obtained experimental results that show that the affinity of diaminopropane, corresponding to the propanylene portion, to ATP is significantly higher than that of putrescine, corresponding to the butanylene part (see the Supporting Information). A similar observation that polyamines with shorter N–N distances show higher affinity for ATP has been also reported by De Stefano et al.[12]

Conformation of SPD in SPD–ATP–Mg^{2+} ternary complex: Under physiological conditions, SPD is supposed to partly form a ternary complex with ATP–Mg^{2+}, which plays an important role in ATP-dependent reactions and in the physiological effects of endogenous polyamines.[7–9] We aimed to investigate the conformation of the SPD–ATP–Mg^{2+} ternary complex by using the same strategy. To evaluate the conformational change of SPD upon interaction with ATP–Mg^{2+}, the relevant J values were measured under conditions in which 60–80% of SPD was complexed with ATP–Mg^{2+} (Table 3). An increment in the *gauche* conformers was not apparent compared with those in the absence of Mg^{2+} (Table 2); respective 5, 2, and 2% increases were observed for the C2–C3, C7–C8, and C8–C9 bonds. These observations suggest that the ATP–Mg^{2+}–SPD ternary complex possesses a different SPD–ATP interaction. The ATP–Mg^{2+} complex is present as a divalent ion at the neutral pH value.[23] This small alteration in the SPD conformation may, therefore, be ascribable to the interaction with divalent anionic ATP–Mg^{2+}, an idea that is supported by the fact that SPD shows small conformational changes in the presence of the divalent phosphate ion HPO_4^{2-} (data not shown). The physiological significance of this weaker interaction of SPD with ATP–Mg^{2+} is currently unknown. Yet, its role in the dehydration of ATP–Mg^{2+} upon enzymatic hydrolysis may provide a plausible account because the SPD–ATP–Mg^{2+} complex is thought to occur in significant concentrations under cellular conditions.[7]

The conformations discussed in this study provide time-average images of an SPD–ATP complex. Combinations of these conformers may be able to explain the molecular basis for the weak biomolecular interactions. We think that these conformational changes of SPD are important for its interactions with biological polyanions, interactions that are plausibly implicated in the physiological functions of SPD, such as cell proliferation and differentiation. A similar conformation study for spermine to gain a better understanding of the role differentiation between SPD and spermine is now under way and will be reported in due course.

Conclusion

The present study proposed a method for the conformation analysis of flexible compounds in aqueous solutions. We synthesized the diastereoselectively ^{2}H-labeled and/or ^{13}C-labeled spermidines (SPDs **1**–**7**) and determined the spin–spin coupling constant for each C–C or C–N bond. SPD, comprising two alkyl parts, butanylene and propanylene, undergoes conformational change upon interaction with multivalent anions such as ATP and tripolyphosphate. With respect to the C–C bonds relevant to the distance between the neighboring pairs of ammonium groups, N1/N6 and N6/N10, the *gauche* rotamers were increased by 11–19% in the complexes as compared with those in the 3HCl salt of SPD. On the other hand, the rest of the bonds, the rotation of which does not greatly affect the distances between the ammonium groups, showed only minimal alterations. The pH-dependent conformation changes of SPD revealed that the interaction between SPD and ATP is orientation sensitive, in that the butanylene part of SPD tends to come to the ribose side and the propanylene resides near the γ-phosphate end. These results may provide a clue for a better understanding of weak and soft interactions between polyamines and anionic biomolecules such as DNA, RNA, and nucleotides.

Experimental Section

Preparation of labeled SPDs: Labeled SPDs **1**–**7** were prepared by three key steps: a) *erythro*-selective hydrogenation of a α,β-unsaturated lactone, b) Curtius rearrangement to provide a primary amino group, and c) *N*-alkylation by using the method of Fukuyama et al.[24] Details of the synthesis are provided in the Supporting Information. For the following experiments, including the Job's plot, NMR titrations, and measurements of coupling constants, spermidine trihydrochloride, ATP dipotassium salt, and tripolyphosphate tetrapotassium salt ($K_5P_3O_{10}$) were used.

Measurements of stoichiometry and association constants in the SPD–ATP complex: The Job's plot was performed in D_2O at 40°C. The total concentration of SPD plus ATP was maintained at 10 mM. The pH value was adjusted to 7.3 with deuterium chloride and sodium deuteroxide. The $\Delta\delta \times n_{SPD}$ values ($\Delta\delta$: the change in the chemical shift of H8 of SPD induced by addition of ATP; n_{SPD}: molar fraction of SPD, [SPD]/([ATP]+[SPD])) were monitored as a function of n_{SPD}. The titration experiments were performed in D_2O at 40°C with constant SPD concentrations of 25 mM and ATP concentrations of approximately 6.25, 9.38, 12.5, 18.75, 25, 37.5, and 50 mM. The pH value was adjusted to 7.3 with deuterium chloride and sodium deuteroxide. In these experiments, the downfield shift of the H8 signal of SPD was monitored as a function of the SPD–ATP ratio. The revised 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: $^{3}J_{H,H}$ and $^{3}J_{C,H}$ values were extracted from 1D ^{1}H NMR spectra. NMR spectra were obtained on a Jeol GSX-500 500 MHz spectrometer. The digital resolution for the ^{1}H NMR spectrum was 0.076 Hz/point. Hence, measurement of coupling constants can be carried out with an accuracy of ±0.1 Hz. The temperature of the probe was kept at 40°C. Samples of **1**–**7** were dissolved in D_2O. The concentrations of SPD trichloride salt, ATP dipotassium salt, and $MgCl_2$ were 25, 100, and 100 mM, respectively. The pH value was adjusted to 3.3, 5.6, or 7.3 with deuterium chloride and sodium deuteroxide. The chemical shifts were recorded from 3-(trimethylsilyl)-1-propanesulfonic acid sodium salt (DSS). The optimizations of the $^{3}J_{H,H}$ values containing second-order couplings were performed by the simulation application gNMR (Adept Scientific). The ^{1}H NMR spectra of **4** and **5** were processed with a shifted SinBell window to measure the $^{3}J_{C,H}$ value. HMBC measurements were performed at 25°C for a solution of 1-deoxynojirimycin (approximately 10 mg) in D_2O (0.2 mL) in a Shigemi sample tube on a Jeol ECA 500 MHz spectrometer. Details of the HMBC experiments will be published elsewhere.

Determination of *anti/gauche* populations and conformation energy: $^3J_{H,H}$ values with conformational fluctuation were calculated as reported previously.[10] Briefly, the energies (E_i) of 360 conformers (total number N) were obtained for each 1° in the dihedral angle along the C–C bonds by using the OPLSA[17] force field in the MacroModel software.[18] The calculations were performed with the solvent effect of water. These values were substituted into Equation (1) and the Boltzmann population (P_i) was obtained.

$$P_i = \frac{\exp(-E_i/\mathrm{RT})}{\sum_i^N \exp(-E_i/\mathrm{RT})} \quad (1)$$

A spin coupling constant ^3J_i in conformer i is given by substituting the dihedral angle between vicinal protons for the modified Karplus equation.[16] The averaged values for *gauche* (+) (60°), *anti* (180°), and *gauche* (−) (300°) in Table 1 were obtained from Equation (2) for the dihedral angles between 0 and 120°, between 120 and 240°, and between 240 and 360°, respectively. The energies of the SPD conformers were calculated by using the OPLS2001 force field in the MacroModel software. These calculations were performed with consideration of the solvent effect of water and the charges of the ammonium groups. To build each SPD conformer, constrained torsion angles were defined (*gauche* (+) 60°, *anti* 180°, and *gauche* (−) 300°). Determination of the *gauche* rotamers based on the $^3J_{C,H}$ values was carried out in a similar manner by using 7.34 and 1.59 Hz as the static *anti* and *gauche* values, respectively.

$$^3J_{H,H} = \sum_i^N P_i \times {}^3J_i \quad (2)$$

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