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NMR Lanthanoid-probe Analyses of Conformational Properties of 8,2′-*S*-Cycloadenosine 3′-Monophosphate in Aqueous Solution

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The advanced method of NMR lanthanoid-probe analyses was used for studying the molecular conformations of 8,2′-anhydro-8-thio-9-β-D-arabinofuranosyladenine (8,2′-*S*-cycloadenosine) 3′-monophosphate in aqueous solution. From the observed ratios of Pr(III)-induced shifts, Gd(III)-enhanced relaxation rates as well as vicinal spin-coupling constants, the most probable values (and standard deviations) of the fractional populations of the local conformations for the C5′–C4′ bond, sugar ring, C3′–O3′ bond, and O3′–P bond and the internal rotation angles of the two forms about the C3′–O3′ bond were determined by the program COFLEM. The internal rotation equilibrium about the C3′–O3′ bond was found to be interrelated with the puckering of the sugar ring. The advanced lanthanoid probe method is thus useful for the analyses of the conformational characteristics of cyclonucleotides as well as of unmodified nucleotides in aqueous solution.

The conformational properties of modified nucleotide units have been found to be related with the biological functions of transfer ribonucleic acids. For example, 2-thiouridine derivatives contribute to the correct codon recognition,[1] and to the thermostability[2] through the conformational characteristics that the C3′-endo form is much more stable than the C2′-endo form. Previously, NMR chemical shifts, spin-coupling constants, nuclear relaxation rates, and nuclear Overhauser effect have been used for elucidating local conformations.[3] However, these NMR data are not sufficient for studying overall conformations of nucleotide molecules. For such studies on flexible molecules, the lanthanoid probe analyses have been successfully utilized.[4] Recently, we have developed an advanced computer program (COFLEM) for the lanthanoid probe analysis;[5] the overall conformer mixture of flexible molecule is now treated as a combination of local conformational equilibria.

8,2′-Anhydro-8-thio-9-β-D-arabinofuranosyladenine (8,2′-*S*-cycloadenosine, to be abbreviated as A^s) (Fig. 1) is a cyclic nucleoside with the fixed internal rotation angle ($\chi \approx 120°$) about the glycosidic bond.[6] This χ value of A^s is in the high-anti region, as compared with the anti χ value ($\approx 0°$) of adenosine (A). By the analyses of the UV and CD spectra, the molecules of dinucleoside monophosphate (A^spA^s) and oligonucleotides $(pA^s)_n$ [n=2—10] have been found to take left-handed helical conformations with stacked bases.[6] For A^spA^s in aqueous solution, the proton NMR spectra have been analyzed.[7] However, as an aid for studying the conformation of A^spA^s in solution, it is important to analyze the conformational feature of the modified nucleoside A^s, which will be different from that of A. Accordingly, in the present study, the advanced lanthanoid probe analysis has been made for elucidating the conformational characteristics of the sugar-phosphate moiety of 8,2′-*S*-cycloadenosine 3′-monophosphate (A^sp) in aqueous solution.

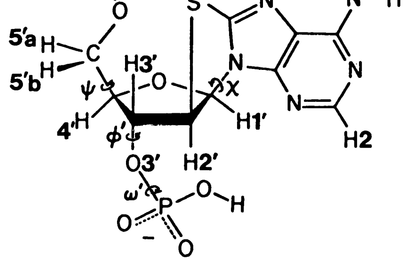

Fig. 1. Structure of A^sp.

Experimental

Materials. The sample of A^sp was synthesized as reported previously.[8] The nitrates of Pr(III) and Gd(III) of higher than 99.9% purity were obtained from Nakarai Chemical Co. Lanthanum oxide of higher than 99.99% purity was purchased from Nakarai Chemical Co. and was dissolved in nitric acid to obtain lanthanum nitrate.

Sample Solutions. For the measurements of lanthanoid-induced proton shift-ratios of A^sp, the successive dilution method[9,10] was used. The initial concentrations of A^sp and Pr(III) ion were 50 mM and 45 mM, respectively, in ^2H_2O solution at pH 1.7 (direct pH meter reading). Diamagnetic La(III) ion was used for the correction for the complex formation shifts. The initial solution was diluted stepwise with ^2H_2O, so that the sample solution was lowered from 50 mM to 5 mM without changing the molar ratio of A^sp/Pr(III) or A^sp/La(III). For the measurements of Gd(III)-enhanced proton relaxation rates of A^sp in ^2H_2O solution (A^sp 60 mM, pH 1.7), the concentration of Gd(III) ion was raised stepwise, by 5 μM, from 0 to 20 μM. The ^{13}C NMR spectrum of A^sp was measured in ^2H_2O solution (85 mM) at pH 2.0.

NMR Measurements. NMR spectra were measured with a Bruker WH270 spectrometer operating at 270 MHz for ^1H and 67.9 MHz for ^{13}C. The probe temperature was kept at 23 °C. Spin-lattice relaxation rates were obtained by a standard 180°-τ-90° pulse sequence.

Data Analyses. The computer program COFLEM developed previously[5] was used on the M-200H system at the Computer Center of the University of Tokyo.

Conformational Analysis

Observed NMR Data. The observed ratios of Pr(III)-induced shifts and Gd(III)-enhanced relaxation

Table 1. Ratios of Pr(III)-induced shifts and Gd(III)-enhanced relaxation rates, and vicinal coupling constants of A^sp

	Shift ratio		Relax. ratio	
	Obsd	Calcd	Obsd	Calcd
H1′	0.39	0.44	0.30	0.21
H2′	1.20	1.29	1.58	1.60
H3′	2.17	1.82	2.50	2.56
H4′	1.00	1.00	1.00	1.00
H5′a	0.28	0.38	0.60	0.59
H5′b	0.35	0.42	0.70	0.70
H2	0.04	0.08	0.10	0.01

	Spin-coupling constant/Hz	
	Obsd	Calcd
H1′–H2′	7.0	9.2
H2′–H3′	2.6	4.2
H3′–H4′	4.0	3.8
H4′–H5′a	3.7	3.7
H4′–H5′b	5.5	5.5
H3′–P	7.4	7.7
C2′–P	3.0	2.5
C4′–P	6.7	6.6

rates of A^sp are listed in Table 1. The ^{1}H resonances of A^sp in $^{2}H_2O$ solution (pH 2) are broadened on addition of a large excess of $LaCl_3$ but the ^{1}H–^{1}H and ^{1}H–^{31}P vicinal coupling constants are unaffected within the experimental uncertainty of 0.3 Hz. Therefore, the ^{1}H–^{1}H, ^{1}H–^{31}P, and ^{13}C–^{31}P coupling constants of free A^sp (Table 1) may be used for conformation analyses.

Local Conformations. The ribose ring and deoxyribose ring of unmodified nucleosides have been known to take two puckering forms, namely the N (C3′-endo) form or the S (C2′-endo) form. However, for 8,2′-*S*-cyclonucleosides, the N and S forms of the sugar ring are probably distorted from those of unmodified nucleosides, because the C2′ atom of 8,2′-*S*-cyclonucleosides is covalently linked, through a sulfur atom, to the C8 atom of the purine base. In fact, in the crystal of 8,2′-*S*-cycloinosinyl-(3′,5′)-8,2′-*S*-cycloadenosine (I^spA^s) hexahydrate,[11] the sugar ring of I^s nucleoside is slightly distorted from the C2′-endo form to the C4′-endo form. On the other hand, the sugar-ring conformation of A^s nucleoside in I^spA^s is nearly the same as the C3′-endo form. Accordingly, in the present conformation analysis of A^sp in aqueous solution, the C3′-endo (N) form and C4′-endo (S) form were used as the two types of sugar-ring conformations of A^sp. For this sugar ring, there is only one population parameter since the sum of the fractional populations of N and S forms is equal to unity. As for the internal rotation about the C3′–O3′ bond of A^sp, the two rotamers (G^+ and G^-)[5] were used (Fig. 2), and accordingly there is only one population parameter for this flexible site. The ϕ' [C4′–C3′–O3′–P] angles of these two rotamers of A^sp were taken as adjustable structure parameters in the present study. The three-rotamer equilibria were adopted for the internal rotation about the C5′–C4′ bond (gg, gt, and tg) and the O3′–P bond (g^+, t, and g^-) (see Fig. 2 of Ref. 5).

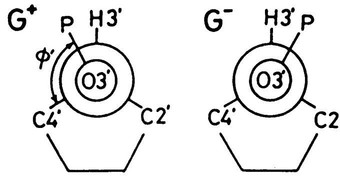

Fig. 2. Two rotamers about the C3′–O3′ bond of A^sp.

For each of these bonds, there are two population parameters since the sum of the fractional populations of three rotamers is equal to unity. The lanthanoid ion was set on the bisector of monoanionic OPO angle, 0.3 nm away from the phosphorus atom as in the case of uridine 3′-monophosphate.[5]

Interrelations among Local Conformational Equilibria. As a prominent feature of the advanced lanthanoid probe method of conformation analyses,[5] the interrelations among local conformational equilibria of flexible sites may be elucidated quantitatively. For the example of uridine 3′-monophosphate, the fractional populations of the G^+ and G^- forms have been determined for each of the C3′-endo form and C2′-endo form.[5]

In the present conformation analysis on A^sp in aqueous solution, four schemes of interdependency were examined among local conformational equilibria of three flexible sites, namely C5′–C4′ bond, sugar ring, and C3′–O3′ bond. The rotamer equilibrium about the O3′–P bond was set to be independent of the conformational equilibria of the other three flexible sites, as found previously for uridine 3′-monophosphate.[5]

Conformation of A^sp in Solution. By the use of the program COFLEM,[5] the most probable values (and standard deviations) of population parameters and structure parameters were obtained, so that the calculated values of the ratios of lanthanoid-induced shifts and relaxation rates as well as spin-coupling constants agreed best with the observed data (Table 1). There are six shift ratios, six relaxation-rate ratios and eight coupling constants and thus are a total of twenty observed data for the conformation analysis of A^sp in aqueous solution. The quality of the fit between the calculated values and observed data was judged by the agreement factor (R), as described previously.[5]

In Scheme 1, the local conformation equilibria are all independent of one another, and there are eight adjustable parameters, including six population parameters and two structure parameters. Even with this small number of parameters, the total of twenty observed data were reproduced with the agreement factor of $R=9.2\%$. On the introduction of the interdependency between the puckering of sugar ring and the internal rotation about the C5′–C4′ bond (Scheme 2), the number of adjustable parameters was increased from eight to ten and the agreement factor was slightly improved to 8.6%. On the other hand, the introduction of the interdependency between the ring-puckering and the internal rotation about the C3′–O3′ bond (Scheme 3) increased the number of parameters only by one but significantly improved the agreement factor to 8.3%. With this scheme, the calculated values of shifts ratios, relaxation-rate ratios and coupling constants agreed quite well with the observed data (Table 1). However, when the internal-rotation equilibria about the C5′–C4′ bond and the C3′–O3′ bond were both assumed to depend upon the ring puckering (Scheme 4 with eleven parameters), the agreement factor (R=8.2%) was nearly the same as that of Scheme 3 and the standard deviations of adjustable parameters were much larger than those for Scheme 2. This indicates that, in Scheme 4, there are actually too many parameters as compared with the available set of observed data even though the number of parameters is much smaller than the nominal number of observed data. Thus, in the present study, Scheme 3 was adopted as the most reliable model and the most probable values (and standard deviations) of the population parameters and structure parameters are shown in Fig. 3.

It may be remarked here that the structure of the C1′–C2′–C3′ moiety of $A^{s}p$ is different from that of ribonucleotides. Because of 8,2′-*S*-cyclization, the N–C1′–C2′ bond angle of $A^{s}p$ is decreased by about 10° and the spin-coupling constants of the C1′–C2′–C3′ moiety are possibly affected. In fact, for the H1′–H2′ and H2′–H3′ coupling constants, the deviations of the calculated values from the experimental ones are larger than those for other coupling constants (Table 1). Nevertheless, the conformational analysis of the $A^{s}p$ has been successfully carried out by the use of lanthanoid-induced shifts and relaxations as well as various spin coupling constants.

Discussion

Recently, the crystal structure of $A^{s}p$ was analyzed, and crystallographically independent two molecules of $A^{s}p$ were both found to take the S (C4′-endo) form of the sugar ring.[12] However, in the present study, the conformational equilibria of $A^{s}p$ in aqueous solution were quantitatively analyzed by the advanced lanthanoid probe method.[5] Thus, the fractional populations of the S (C4′-endo) form and N (C3′-endo) form of the sugar ring were determined to be $81\pm6\%$ and $19\pm6\%$, respectively, in solution (Fig. 3). This is consistent with the observation that the molecules of 8,2′-cyclonucleotides mostly take the S form in crystal.[12]

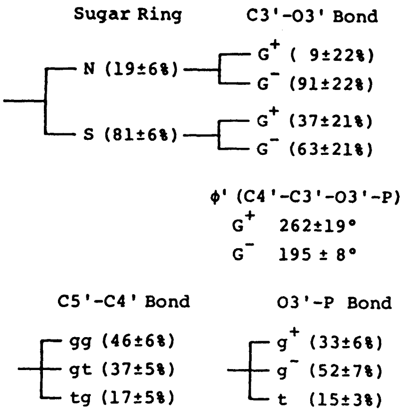

Fig. 3. Molecular conformation and conformational equilibrium of $A^{s}p$ in aqueous solution (Scheme 3).

The interdependency of the sugar-ring puckering and the internal rotation about the C3′–O3′ bond of $A^{s}p$ was experimentally established, in the present study, by the lanthanoid probe method. For the sugar ring in the N (C3′-endo) form, the fractional populations of the G^{+} and G^{-} forms are found to be $9\pm22\%$ and $91\pm22\%$, respectively (Fig. 3); in other words, the sugar ring of the N form exclusively takes the G^{-} form. This is probably because of strong steric repulsion between the 5′-CH_2OH group and the 3′-phosphate group in the N-G^{+} form of the sugar-phosphate moiety. The molecular conformation of the gg-N-G^{-}-g^{-} form is shown in Fig. 4.

For the sugar ring in the S (C4′-endo) form, the fractional populations of the G^{+} and G^{-} forms are found to be $37\pm21\%$ and $63\pm21\%$, respectively (Fig. 3). In this case, the 5′-CH_2OH group is nearly oriented to the axial direction and there is no serious steric repulsion between the 5′-CH_2OH group and the 3′-phosphate group even in the S-G^{+} form. By the combination of local conformational equilibria of the sugar ring and C3′–O3′ bond, the fractional populations of the S-G^{+} form and S-G^{-} form of $A^{s}p$ are obtained to be about 30% and 50%, respectively, in aqueous solution. This again is consistent with the result of the X-ray

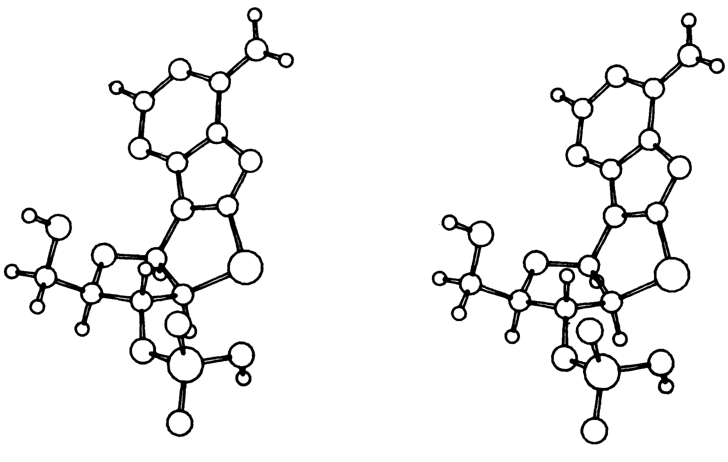

Fig. 4. The stereoscopic view of the gg-N-G^{-}-g^{-} form drawn by the ORTEP program (Ref. 13).

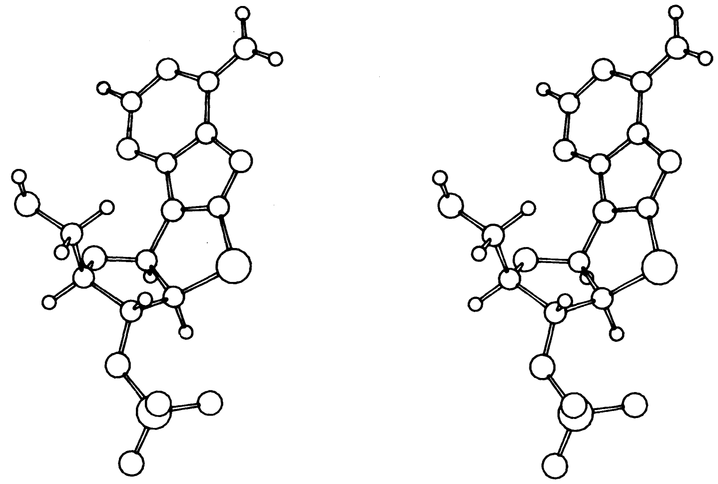

Fig. 5. The stereoscopic view of the gt-S-G$^-$-g$^-$ form drawn by the ORTEP program (Ref. 13).

analysis[12] that, in the crystal of A^{s}p, one molecule takes the S-G$^+$ form and the other takes the S-G$^-$ form. Furthermore, in the present study, the internal rotation angles (ϕ') about the C3′–O3′ bond were determined to be 262±19° and 195±8° for the G$^+$ and G$^-$ forms, respectively, in aqueous solution. Surprisingly, these values for A^{s}p in solution closely agree with the ϕ' values found in the crystal (G$^+$: 255° and G$^-$: 192°),[12] indicating the potentiality of the advanced lanthanoid probe method of conformation analyses.[5]

As for the local conformations about the C5′–C4′ bond, both of the two A^{s}p molecules in the crystal were found to take the gt form. However, in aqueous solution, the gt and gg forms are both the major conformations about the C5′–C4′ bond (Fig. 3). Previously, for the molecule of uridine 3′-monophosphate, the local conformational equilibrium about the C5′–C4′ bond was found to be interrelated with the ribose ring puckering.[5] However, in the present study on the 8,2′-cycloadenosine moiety, the conformational equilibrium about the C5′–C4′ bond was not found to depend significantly on the puckering of sugar ring. The molecular conformation of a major form (gt-S-G$^-$-g$^-$) of A^{s}p in solution is shown in Fig. 5.

Thus, the advanced lanthanoid-probe method[5] is further found to be useful for the quantitative elucidation of the molecular conformations and conformational equilibria of modified cyclonucleotides as well as of unmodified nucleotides (such as uridine 3′-monophosphate[5]). The conformational characteristics of the A^{s}p molecule in aqueous solution, as elucidated in the present study, may be utilized in the model-building analyses on the left-handed helical conformations of A^{s}p containing polynucleotides.

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