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NMR ANALYSES OF CONFORMATION OF RIBOSYL ADENOSINE 5',5"-BIS(PHOSPHATE) IN AQUEOUS SOLUTION

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SUMMARY NMR analyses of spin coupling constants, nuclear Overhauser effects and Gd(III)-induced relaxation rates were made of ribosyl adenosine 5',5"-bis(phosphate), [Ado(P)-Rib-P], in aqueous solution. The α-configuration of the C1" atom of the Rib-P moiety was confirmed. The Ado(P) moiety takes the *anti* form about the glycosidic bond, the *gg* form about the exocyclic bond, and the 2'-*endo* and 3'-*endo* forms of the ribose ring with nearly equal fractional populations. The ribose ring of the Rib-P moiety preferentially takes the *gt/tg*-2"-*endo* form. The Ado(P)-Rib-P molecule has a fairly extended overall molecular conformation.

Poly(ADP-Rib) synthesis, from β-NAD^+ in eukaryotic cell nuclei, is possibly involved in the regulation of DNA synthesis, DNA repair, cultured cell density, tissue differentiation, transformation and chromatin structure (1-3). Rapid turnover of NAD^+ in D98/AH2 human cells may also be explained by rapid turnover of poly(ADP-Rib) (4). Furthermore, antibody against poly(ADP-Rib) is found in the serum of patients with systemic lupus erythematosus (5). The primary structure of ribose-ribose bonds in poly(ADP-Rib) has recently been elucidated as α-(1"→2') (6,7). Because poly(ADP-Rib) has a unique α-anomeric linkage, unlike that in other naturally occurring compounds with ribose-ribose bonds, and a unique biological significance, it is of considerable importance to study the molecular conformations of poly(ADP-Rib) and monomer units.

This paper reports studies on the conformation of Ado(P)-Rib-P, a monomer unit of poly(ADP-Rib), by measurements of spin-coupling constants, nuclear Overhauser effects and Gd(III)-induced proton relaxation enhancements.

Abbreviations: Ado(P)-Rib-P, ribosyl adenosine 5',5"-bis(phosphate); poly(ADP-Rib), poly(adenosine diphosphate ribose); NMR, nuclear magnetic resonance; NOE, nuclear Overhauser effect.

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MATERIALS AND METHODS

The sample of Ado(P)-Rib-P was prepared as described previously (6). Preparations of the nitrates of Eu(III) and Gd(III) of more than 99.9% purity were obtained from Nakarai Chemical Co. The nitrate of La(III) was prepared by dissolving lanthanum oxide (99.99% from Nakarai Chemical Co.) in nitric acid.

270 MHz proton NMR spectra were measured with a Bruker WH270 spectrometer. Chemical shifts were measured with sodium 2,2-dimethyl-2-silapentane-5-sulfonate as an internal standard. Spin-lattice relaxation times (T_1) were measured by the inversion recovery method. The nuclear Overhauser effect (NOE) was measured by the selective gated decoupling method. In these measurements, the paramagnetic contamination was removed with a solution of chelating agent in toluene. For measurements of Gd(III)-induced relaxation enhancements, small amounts of Gd(III) nitrate solution were added successively to a ^2H_2O solution of Ado(P)-Rib-P (18 mM), containing La(III) nitrate (65 mM) and Eu(III) nitrate (60 mM), to give concentrations of Gd(III) of zero to 81 μM.

RESULTS AND DISCUSSION

The 270 MHz proton NMR spectrum of Ado(P)-Rib-P in ^2H_2O solution at pH 2 is shown in Fig. 1A. Overlapping signals of A5', R2" and R3" protons were separated by the addition of lanthanide nitrates, as shown in Fig. 1B.

Confirmation of the Anomeric Configuration of the Rib-P Moiety

The spin lattice relaxation rate, measured by the nonselective inversion method, of the anomeric proton R1" (Fig. 1) of Rib-P moiety ($T_1^{-1} = 1.68\ s^{-1}$) was found to be much faster than that of the A1' proton of Ado(P) moiety ($0.72\ s^{-1}$). This significant difference in T_1^{-1} is clearly due to the difference in the anomeric configurations (8), indicating that the Rib-P moiety has an α-anomeric configuration (Fig. 2), in contrast with the β-configuration of the Ado(P) moiety. This configuration is also consistent with the observed NOE (Table I). On irradiation of the R2" proton, NOE enhancement of as much as 10% was observed for the R1" proton, whereas on irradiation of the A2' proton NOE enhancement of the A1' proton signal was as little as 5%.

Conformation of Ado(P)-Rib-P

pH dependences of proton chemical shifts. On the basis of the pH titration curves of the chemical shifts of A2, A8, A1' and R1" protons, the pK_a values were obtained as 6.68 and 4.22, corresponding to the ionizations of the phosphate group and adenine ring, respectively. The former is significant for only the chemical shift of the A8 proton, indicating that this proton is close to the phosphate group. Accordingly, in the predominant conformers, the internal rotations about the exocyclic bond and glycosidic bond of the

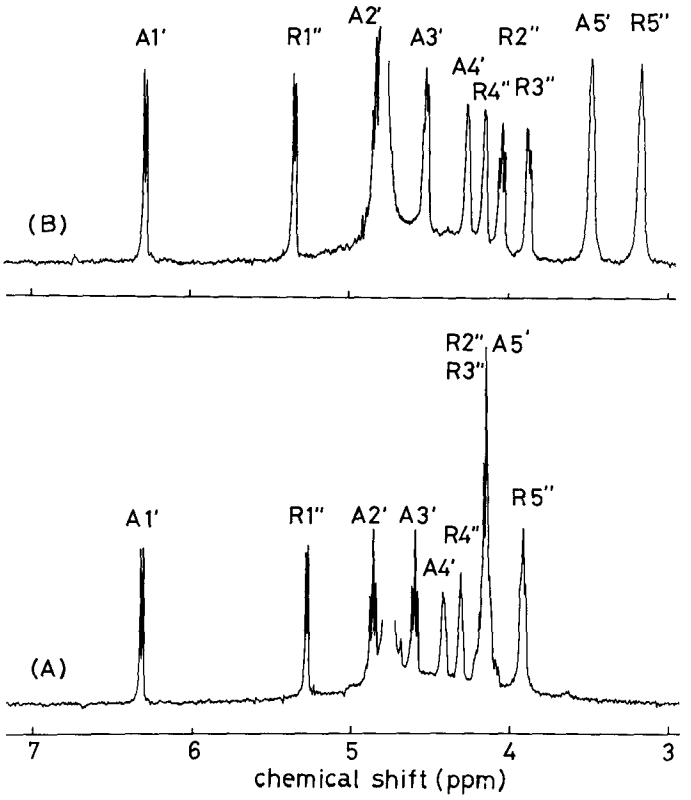

Fig. 1. 270 MHz NMR spectra of ribose protons of Ado(P)-Rib-P in ^2H_2O solution (18 mM), (A) at pH 2.0 and 23°C and (B) with the addition of La(III) nitrate (75 mM) and Eu(III) nitrate (40 mM) at pH 2.0 and 23°C.

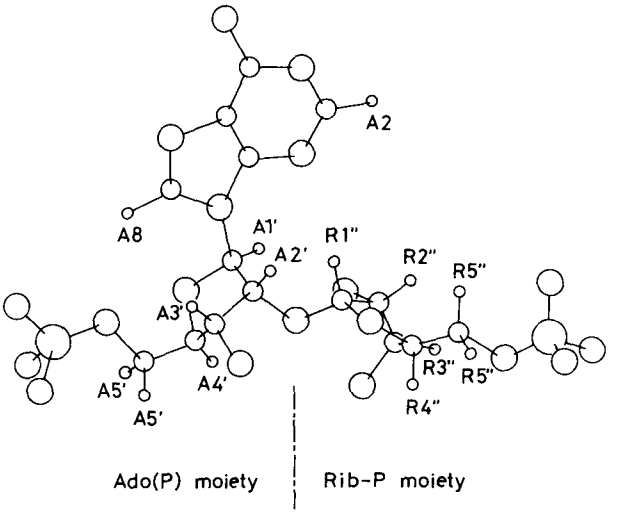

Fig. 2. Conformer of Ado(P)-Rib-P, with the 3'-*endo-gg-anti* form of the Ado(P) moiety and the 2"-*endo-tg-anti* form of the Rib-P moiety.

Table I. NOE Enhancements (%) of Ado(P)-Rib-P*

Irradiated proton	Observed proton signal A8	A1'	A2'	R1"
A8	*	4 (4)	5	1 (1)
A1'	5 (5)	*	5	3 (3)
A2'	7 (9)	5 (4)	*	10 (9)
A3'	2 (2)	1 (1)		-1 (-1)
R1"	0 (0)	7 (6)	11	*
R2"**				10 (10)

* In ^2H_2O solution (18 mM) at pH 8.0. NOE enhancements in the presence of La(III) nitrate (65 mM) at pH 2.0 are listed in parentheses.
**The R3" proton was also irradiated since the R3" proton signal overlaps the R2" proton signal.

Ado(P) moiety are in the *gg-anti* form in aqueous solution (Fig. 2). Further, the chemical shift of the R1" proton (but not of the A1' proton) is affected by the ionization of the phosphate group. This is again consistent with an α-anomeric configuration for the Rib-P moiety. The effect of protonation of the adenine ring is observed in the chemical shift of the A1' proton, but more significantly in that of the R1" proton. Therefore, the R1" proton is not far from the adenine ring in the predominant conformers of Ado(P)-Rib-P (Fig. 2).

Spin coupling constants. For elucidating the conformation of ribose rings, the vicinal spin coupling constants were measured as shown in Table II. The fractional populations of the 2'-*endo* and 3'-*endo* forms of the Ado(P) moiety were calculated from the $J_{1'2'}$ values, by the method of Davies and Danyluk (9)(Table II). For the Rib-P moiety, the R2" and R3" proton signals were separated by the addition of lanthanide nitrates. In this case, the fractional populations of the 2"-*endo* and 3"-*endo* forms were calculated from the $J_{3"4"}$ value, since the configuration of the C1" atom of the Rib-P moiety is different from that of normal nucleosides. For the Ado(P) moiety, the fractional populations of the 2'-*endo* and 3'-*endo* forms are nearly equal, exactly as in 5'-AMP (9). In contrast, the 2"-*endo* form is predominant for the Rib-P moiety in aqueous solution (Fig. 2). This is consistent with the predominance of the 2"-*endo* form in dimethylsulfoxide (7).

NOE enhancements. The signal intensity of the R1" proton is enhanced almost 10% by irradiation of the A2' proton but is enhanced only about 3% by irradiation of the A1' proton (Table I). Similarly, on irradiation of the

Table II. Spin Coupling Constants (Hz)* and Fractional Conformer Populations of Ado(P)-Rib-P in ^2H_2O solution (18 mM)

	Ado(P)			Rib-P		
	pH 8	pH 2	pH 2**	pH 8	pH 2	pH 2**
$J_{1'2'}$	5.6	5.0	5.2	3.6	3.9	4.3
$J_{2'3'}$	5.1	5.0	5.2			5.8
$J_{3'4'}$	3.7	4.6	4.3			2.5
2'-*endo*	60%	54%	56%			73%
3'-*endo*	40%	46%	44%			27%

*Accuracy, 0.25 Hz.
**Eu(III) nitrate (40 mM) and La(III) nitrate (75 mM) were added for separating the A5', R2" and R3" proton signals.

R1" proton, the A2' proton signal is enhanced as much as 11% while the A1' proton signal is enhanced only 7%. These observations indicate that the R1" proton is closer to the A2' proton than to the A1' proton (Fig. 2). Furthermore, on irradiation of the A3' proton, the signal intensity of the R1" proton is decreased slightly but distinctly (by 1%). These NOE values of the R1" proton signal indicate that the R1" proton of the Rib-P moiety and the A2' and A3' protons of the Ado(P) moiety are arranged nearly on a straight line in the predominant conformers of Ado(P)-Rib-P in solution (Fig. 2). Thus the overall molecular conformation of Ado(P)-Rib-P appears to be fairly extended.

Gd(III)-induced relaxation enhancements. It should be emphasized here that the spin coupling constants (Table II) and NOE values (Table I) of Ado(P)-Rib-P do not change appreciably on changing the pH from 8.0 to 2.0 or on addition of La(III) and Eu(III) ions at pH 2.0. Accordingly, the lanthanide ion probe method is useful for studying the molecular conformation of Ado(P)-Rib-P in aqueous solution. The Gd(III)-induced relaxation rates are proportional to the average values of the inverse sixth power of the distances from the Gd(III) ion to the observed nuclei.

The Gd(III)-induced relaxation rates of all the proton resonances of Ado(P)-Rib-P were measured in ^2H_2O solution (18 mM) at pH 2.0 and 23°C, where the overlapping signals of A5', R2" and R3" protons were separated by the addition of Eu(III) nitrate (60 mM) and La(III) nitrate (65 mM). For the Ado(P) moiety the relaxation rate (relative to A5' proton) of the A8 proton (0.40) is much faster than that of the A2 proton (0.08), and so the local conformation about the glycosidic bond is predominantly *anti*. This

is in agreement with the effect of the second ionization of the phosphate group on the chemical shifts of the A2 and A8 protons. The relaxation ratios of the A3' proton (0.44) and A4' proton (0.19) suggest that the internal rotation about the glycosidic bond is predominantly in the *gg* form (Fig. 2), as in 5'-UMP (10).

For the Rib-P moiety, the relaxation rate (relative to the R5" proton) of the R4" proton (0.30) is faster than that of the R3" proton (0.25). These ratios are similar to those for the R4' proton (0.32) and R3' proton (0.30) of 5'-TMP, where the fractional population of the *tg/gt* form is as high as 60% (F. Inagaki, T. Hayase, M. Tasumi and T, Miyazawa, unpublished). Accordingly, for the Rib-P moiety of Ado(P)-Rib-P (Fig. 2), the internal rotation about the exocyclic bond is predominantly in the *tg/gt* form, which is known to favor the 2'-*endo* form of the ribose ring (11,12). In fact, for Ado(P)-Rib-P, the fractional population of the 2"-*endo* form is calculated to be as high as 73%, from the $J_{3"4"}$ value. Furthermore, the relaxation rate of the R1" proton (0.38) of the Rib-P moiety is much faster than that of the A1' proton (0.08) of the Ado(P) moiety, providing further strong support for the α-anomeric configuration of the R1" carbon of the Rib-P moiety.

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