Conformational Aspect of Indazole Adenine Dinucleotides in Aqueous Solution

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Conformational properties of various title compounds in aqueous solution were examined with the aid of 500 MHz ^{1}H-NMR spectroscopy. In particular, the signals of aromatic protons on both the indazole and adenine rings were shifted to higher fields without exception, compared with the corresponding signals in indazole and adenine mononucleotides. These observations, which can be explained as resulting from diamagnetic shielding effect of the indazole and adenine rings, indicate that the base pairs in the dinucleotides are closely stacked in parallel planes. On the other hand, the average conformation of riboses were deduced from $J_{1',2'}$-values as a proportion of $N \rightleftharpoons S$ equilibrium composition. Indazole riboses showed a roughly 50/50 equilibrium, whereas adenine riboses showed a small preference for the *N*-type conformer. Such a conformational state may be indispensable for taking up the strongly folded conformation between the base pairs in the dinucleotides, as judged by a somewhat favored *S*-type conformation of both riboses in the indazole- and adenine mononucleotides.

In studies of nucleoside and nucleotide compounds, the conformational analysis of the furanose rings is an important subject, because the difference in conformation of the compounds influences the biological activity. For that reason, nucleic acid-ribonucleosides components (adenosine, cytidine, uridine, *etc.*)[1,2] and some related compounds (alkylated, halogenated, aza, deaza analogs, *etc.*)[3—5] have been examined for molecular conformation with the aid of NMR spectroscopy. On the other hand, the stereochemistry in aqueous solution of pyridine dinucleotides has so far been studied in connection with coenzyme function. The conformational properties of two riboses, as well as the relative conformations of both the pyridine and adenine bases, were investigated with NAD and its alkylated analogs or the like.[6—9] Recently we have reported the synthesis and biochemical activity of some novel 2*H*-indazole dinucleotides.[10] The indazole dinucleotides, which contain a bicyclic base instead of pyridine one, are expected to adopt a different conformation in comparison with that of pyridine dinucleotides. This paper describes the conformational aspects of the indazole nucleotides in aqueous solution, comparing them with those of pyridine nucleotides, on the basis of the information obtained by high-frequency ^{1}H-NMR spectroscopy.

Experimental

Materials. The indazole adenine dinucleotides, indazole mononucleotides, and adenine mononucleotide (AMP) used for this study were prepared by an enzymic method in our laboratory.[10] Deuterium oxide (D_2O, 99.8%) and sodium 3-trimethylsilyl-1-propanesulfonate (DSS) were obtained from E. Merck, Darmstadt.

NMR Spectroscopy. ^{1}H-NMR measurements were performed in D_2O (pH 7) at a *ca.* 0.01 M (1 M=1 mol dm^{-3}) concentration of each sample, which was once lyophilized beforehand from D_2O, on a 500 MHz JEOL FX-500 spectrometer operating in a pulse Fourier-transform mode with quadrature detection. All spectra were obtained from accumulation of 64—128 free induction decays after each 45° pulse (5 μs) repeated every 4 s and were observed over a range of 5×10^3 Hz, corresponding to 32 K data points. Chemical shifts (δ-values) were measured in parts per million (ppm) downfield from DSS as an internal standard and estimated with an accuracy within $\pm$0.001 ppm. Coupling constants were determined as an average value based on two measurements under the same conditions, and the precision was $\pm$0.2 Hz. The residual HDO signal was suppressed by the gated decoupling method.[11]

Results and Discussion

Conformation between Indazole and Adenine Bases. NMR studies by Sarma *et al.*[7,8] have strongly indicated that both the oxidized and reduced pyridine dinucleotides exist in a folded conformation, in which the pyridine and adenine rings are stacked in parallel planes, rather than in an unfolded conformation. This conclusion has been derived from interpretation based on the following NMR spectral observations: All aromatic protons on both the pyridine and adenine rings, and anomeric protons are shifted to higher fields as compared with those in the NAD-constituting nicotinamide mononucleotide (NMN) and adenine

Table 1. Chemical shift difference ($\Delta\delta$) of adenine protons between indazole adenine dinucleotide and AMP[a]

Entry	R^1/R^2	$\Delta\delta$/ppm[b] Adenine		
		H_2	H_8	$C_1'H$
1	H/H	0.267	0.264	0.133
2	NH_2/H	0.291	0.407	0.188
3	H/NH_2	0.262	0.344	0.216
4	NHAc/H	0.335	0.390	0.150
5	H/NHAc	0.341	0.444	0.148
6	OH/H	0.277	0.431	0.179
7	H/OH	0.259	0.380	0.201
8	OCH_3/H	0.294	0.387	0.136
9	H/OCH_3	0.248	0.380	0.175
10	Cl/H	0.257	0.369	0.122
11	H/Cl	0.252	0.414	0.123

a) All spectra were measured in D_2O (pH 7) at a 0.01 M concn of each sample at 25 °C. b) The precision was within $\pm$0.001 ppm.

TABLE 2. CHEMICAL SHIFT DIFFERENCE ($\Delta\delta$) OF INDAZOLE PROTONS BETWEEN INDAZOLE DINUCLEOTIDE AND INDAZOLE MONONUCLEOTIDE[a)]

Entry	$\Delta\delta$/ppm[b)] Indazole					
	H_3	H_4	H_5	H_6	H_7	$C_1'H$
1	0.350	0.292	0.240	0.248	0.283	0.250
2	0.219	n.o.[c)]	—	0.175	0.253	0.174
3	0.261	0.244	0.171	—	n.o.[c)]	0.130
4	0.399	0.315	—	0.336	0.369	0.288
5	0.361	0.401	0.420	—	0.284	0.298
6	0.324	0.368	—	0.342	0.353	0.222
7	0.369	0.356	0.323	—	n.o.[c)]	0.195
8	0.296	0.376	—	0.360	0.320	0.213
9	0.275	0.323	0.354	—	0.373	0.193
10	0.381	0.450	—	0.381	0.361	0.253
11	0.306	0.330	0.329	—	0.420	0.261

a,b) See the footnote in Table 1. c) The signals were not observed.[10)]

mononucleotide (AMP). The upfield resonances in the dinucleotide could be explained as resulting from diamagnetic shielding due to the mutual "ring-current effect" of the pyridine and adenine bases. Thus, the base pairs were found to be stacked in parallel planes. In connection with these studies based on the difference in chemical shifts, Freyne *et al.*[9)] have also investigated 3-acetyl-5-alkylpyridine analogs of NAD and shown the occurrence of significant intramolecular interaction between the base pairs.

Various 2*H*-indazole adenine dinucleotides (**1**—**11**) which we have recently prepared[10)] were examined for their chemical shifts of aromatic protons at pH 7 by 500 MHz ^{1}H-NMR spectroscopy. The chemical shift difference ($\Delta\delta$) of adenine and anomeric protons between each indazole dinucleotide and AMP are summarized in Table 1. All the resonances of H_2 ($\Delta\delta$ 0.25—0.34), H_8 ($\Delta\delta$ 0.26—0.44), and $C_1'H$ ($\Delta\delta$ 0.12—0.22) were shifted to higher fields compared with those in AMP. As for NAD, $\Delta\delta$-values of H_2, H_8, and $C_1'H$ in the adenine moiety have been estimated to be 0.15, 0.14, and 0.13 ppm, respectively.[6)] These values are less than in indazole dinucleotides. On the other hand, the $\Delta\delta$-values of indazole protons between each indazole dinucleotide and the corresponding mononucleotide were also evaluated by a third order approximation (Table 2). It is apparent that, in the dinucleotide, all the aromatic signals are shifted remarkably to higher fields ($\Delta\delta$ 0.17—0.45) as observed for adenine protons. Furthermore, the substituent *O*-methyl and *N*-acetyl protons showed a slight upfield shift ($\approx$0.10 and $\approx$ 0.02 ppm, respectively). Previously Jardetzky and Wade-Jardetzky[6)] have reported the $\Delta\delta$-values of pyridine signals between NAD and NMN (Table 3). One can see from Tables 2 and 3 that $\Delta\delta$-values between the indazole nucleotides are, on the whole, greater than those between the pyridine nucleotides. Thus, it was found that, in the indazole dinucleotides, the indazole and adenine rings suffer more diamagnetic shielding effect of each other than the pyridine and adenine base pairs in NAD. The enhancement of the anisotropic effect

TABLE 3. CHEMICAL SHIFT DIFFERENCE ($\Delta\delta$) OF PYRIDINE PROTONS BETWEEN NAD AND NMN

Compd	$\Delta\delta$/ppm[a)] Pyridine				
	H_2	H_4	H_5	H_6	$C_1'H$
NAD	0.286	0.198	0.105	0.213	0.070

a) Calculated from the chemical shifts previously reported by Jardetzky and Wade-Jardetzky.[6)]

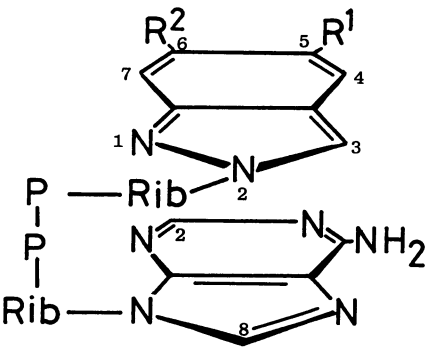

Fig. 1. Closely folded conformation for indazole dinucleotides.

seems to arise from the two-aromatic-ring system of indazole base, which may provide a wide shielding zone in the direction perpendicular to the ring plane.

These observations indicate that the base pairs in the indazole dinucleotide are more closely stacked in parallel planes as compared to those in the pyridine dinucleotide (Fig. 1).

Ribose Conformations. Altona and Sundaralingam[12)] have introduced a comprehensible notation of furanose ring conformations based on the concept of pseudorotation: *N*-type conformers include the classical C(2′)-*exo* and C(3′)-*endo* conformations; *S*-type conformers include the classical C(2′)-*endo* and C(3′)-*exo* forms (Fig. 2). They have also shown that both conformers in dinucleotides as well as in mononucleotides in solution are present in a dynamic equilibrium. An equilibrium shift should bring about a change of average torsion angle between vicinal C–H bonds, and a coupling con-

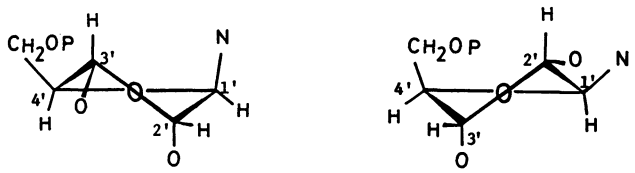

Fig. 2. Perspective views of the *N*- and *S*-type ribose rings.[12]

Table 4. The $J_{1',2'}$-values of indazole dinucleotides and mononucleotides

Entry	$J_{1',2'}$/Hz[a]		
	Dinucleotide		Mononucleotide[b]
	Adenine	Indazole	Indazole
1	3.9	5.4	5.8
2	4.4	4.9	5.4
3	4.4	4.9	
4	4.4	4.4	
5	4.0	4.2	5.2
6	4.2	4.9	5.4
7	4.2	4.9	
8	4.2	5.1	
9	4.3	4.9	
10	4.3	4.9	5.3
11	4.4	4.9	

a) Average value of two measurements: the precision was ±0.2 Hz. b) Obtained from the dinucleotide of the corresponding entry number.

stant for the vicinal protons can be expressed in terms of the dihedral torsion angle.[13] From a Dreiding model, one can see that the adoption of either of *N*- and *S*-type conformers induces no significant change of the vicinal torsion angle between C_2'–H and C_3'–H bonds. Thus, a practical and simple equation was proposed to deduce the conformational aspect of a ribose ring from the vicinal coupling constant, $J_{1',2'}$ or $J_{3',4'}$: $S(\%)=10\times J_{1',2'}$ or $10\times(10.1-J_{3',4'})$.[12]

Even in the 500 MHz NMR spectra of indazole dinucleotides, the signals of an indazole C_3'H and adenine C_3'H were overlapped each other to such an extent that a reasonable estimate of each $J_{3',4'}$ is impossible. As to the anomeric protons, which appeared as two clearly separate doublets, the two $J_{1',2'}$'s could be accurately determined. We, therefore, use the value of $J_{1',2'}$ to estimate equilibrium compositions of *N*- and *S*-type conformers. $J_{1',2'}$-values observed with indazole nucleotides are summarized in Table 4. In the dinucleotides, all indazole riboses show a greater $J_{1',2'}$-value, compared with adenine riboses, by 0.3—1.5 Hz. Upon conversion of the dinucleotides into mononucleotides, the coupling constant came to increase by 0.3—1.0 Hz in the indazole ribose and by 1.3—2.0 Hz in the adenine ribose, indicating a small change in conformation of respective ribose rings. Concerning pyridine dinucleotides, it has previously been reported[9] that pyridine $J_{1',2'}$-values were less than adenine ones by ≈0.5 Hz.

Table 5. Equilibrium composition of *N*-type conformers in indazole nucleotides[a]

Entry	*N*-type conformation/%[b]		
	Dinucleotide		Mononucleotide[c]
	Adenine	Indazole	Indazole
1	61	47	42
2	56	51	46
3	56	51	
4	56	56	
5	60	58	48
6	58	51	46
7	58	51	
8	58	49	
9	57	51	
10	57	51	47
11	56	51	

a) Calculated from the equation of Altona and Sundaralingam:[12] $S(\%)=10\times J_{1',2'}$. b) $N(\%)=100-S(\%)$. c) See the footnote b in Table 4.

The percentages of *N*-type conformer (100—*S*%) in the indazole nucleotides were calculated by taking the aforementioned equation (Table 5). In the dinucleotides, the adenine ribose showed a small preference for the *N*-type conformer, whereas the indazole ribose was observed to exist in a roughly 50/50 equilibrium between both conformers with a few exceptions. In the indazole mononucleotides, a slightly increased proportion of *S*-type form occurs in all cases. In the AMP ribose ($J_{1',2'}$=6.0 Hz), the percentage of *N*-type form (40%) decreased in favor of *S*-type form (60%). Such difference in ribose conformation of the dinucleotides compared with the mononucleotides may be closely correlated with the folded conformation between the indazole and adenine base pairs. The conformational deviation of ribose rings are probably necessary for the occurrence of enhanced intramolecular interaction between the base pairs, which would contribute to the stable conformation with a lower energy level for the whole molecule.

In a part of the previous paper of Freyne *et al.*[9] regarding the ribose conformation in pyridine dinucleotides, the percentage of *S*-type conformers has been based on $J_{3',4'}$-value. However, the method using $J_{3',4'}$ should be restricted to the case where $J_{1',2'}+J_{3',4'}=10.1$.[12] In their report, $J_{1',2'}$'s and $J_{3',4'}$'s for the pyridine ribose have been estimated to be 5.2—5.4 and 2.4—2.8 Hz, respectively. The sum of the two terms (*ca.* 8 Hz) may be too little to fit the equation. It can, therefore, be said that the *S* percentages (more than 70%) they have estimated are much greater than those actually to be expected.

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