MECHANISM OF THE DIMROTH REARRANGEMENT IN ADENOSINE[1]

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SUMMARY: An unambiguous mechanism for the Dimroth rearrangement has been established for adenosine by observation of isotopic [^{15}N] nitrogen exchange between the exocyclic (N^6) and endocyclic (1-) positions of the adenine ring which occurs simultaneously with the well documented methyl migration (from positions 1- to N^6). The elucidation of this rearrangement by NMR techniques indicates that ring cleavage between the N_1-nitrogen and C_2-carbon atoms followed by 180° internal rotation, is the correct pathway.

It is well known that the majority of biologically active nucleic acids (e.g. tRNA, rRNA and DNA) are methylated *in vivo*, but the functional significance of such methylation has only been elucidated for the modification-restriction enzyme reactions with DNA. Possibly the first recorded incidence of the artifact of the Dimroth rearrangement interfering with investigators was when tRNA chromatograms (which were used to develop alkaline tRNA hydrolysates) showed N^6-methyladenosine as a major modified base constituent (2). It was shown (3) that some portion of these residues may have been produced by chemical rearrangement during isolation of the mononucleotides, thus necessitating reinvestigation of the original results and control experiments. Although investigators are now aware of the problem and take special precautions to not introduce alkali into modified base isolations (4), we perceived that the utilization of this rearrangement might have special advantages for investigators of polynucleotide structure.

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We recently completed studies designed to examine the conformational consequences of amino-group methylation of the nucleic acid bases at the mononucleotide level (5), and in attempting to extend these studies, we are preparing polydeoxyribonucleotides which are monomethylated at the 6-amino position of adenine. By treating these polymers with $(CH_3)_2SO_4$, which first methylates adenine residues at the 1-position (6,7), we reasoned that we could then promote the Dimroth rearrangement (8) (by addition of base) to finally form a random copolymer of adenine (A) and 6-methylamino-adenine (m^6A) residues. Since the mechanism of this rearrangement has not been rigorously established for adenosine, the experiments outlined here were performed to define the reaction pathway.

MATERIALS AND METHODS

Isotopically labeled [^{15}N] amino adenosine (Ado) was synthesized (9) by reaction of 0.5 g of 6-chloropurine-9-β-d-ribofuranoside (Cyclo) with 500. ml of [^{15}N] ammonia (BioRad) in 5 ml of methanol in a sealed tube at 100°C for 8 hours. The product was filtered and recrystallized two times from H_2O. Low resolution mass spectral parent ion peak appears at m/e^- = 268. (Expected from $C_{10}H_{13}O_4$ [^{14}N]$_4$ [^{15}N].) Tlc results from ascending cellulose chromatograms (Eastman Kodak) yielded one spot in three solvent systems (Table I) which comigrated with an authentic sample of Ado. 100 MHz nmr spectra (taken on a Varian XL100-15) were recorded in the frequency sweep mode locking on an internal deuterium reference. The labeled Ado reacted with CH_3I in $CH_3CON(CH_3)_2$ (10) to form a single product (Table I) which rearranged in the presence of hydroxide ion to form N^6-methyadenosine (m^6Ado).

RESULTS AND DISCUSSION

Nucleophilic attack on adenosine by certain alkylating reagents [e.g., $(CH_3)_2SO_4$, CH_3I] is directed almost exclusively to the 1-position of the heterocyclic base ring of Ado in both mononucleosides and -tides (11) and in polynucleotides which contain Ado but lack guanosine (6,7,11). Several

TABLE I

Compound	R_f in Solvent		
	0.5 M LiCl	1-butanol: water: acetic acid (5:1:4)	ethanol:water (7.3)
adenosine	.56	.65	.65
[^{15}N]-adenosine	.59	.61	.62
1-methyl-[^{15}N]-adenosine	.96	.50	streak: .0 to .42
N^6-methyladenosine	.70	.90	.88
N^6-methyl-[^{15}N]-adenosine	.70	.90	.77

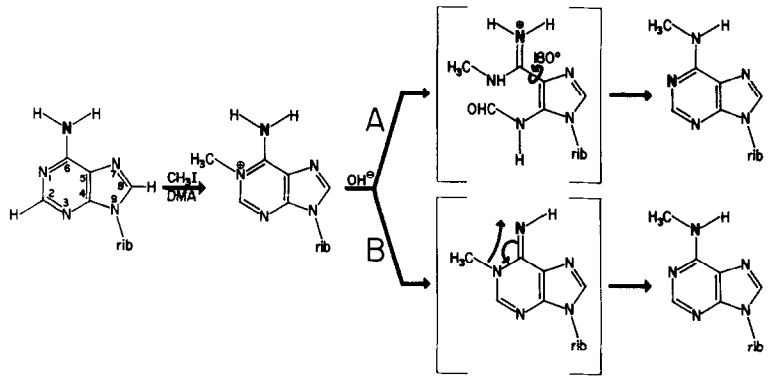

Figure 1. Proposed pathways for the mechanism of the Dimroth rearrangement in adenosine.

studies have shown that subsequent to methylation, the methyl group migrates to the exocyclic (N^6) amino group (in the presence of base) to form m^6Ado. Detailed kinetic studies (12,13) assumed a mechanism of hydroxide attack at the C_5-C_6 bond (Figure 1A). Upon ring closure, the methyl group is attached

at the 6-amino position whereas the exocyclic amino nitrogen is now reformed as the endocyclic (N_1) imino nitrogen. Precedent for this type of "ring-opened" mechanism was established from analogous studies on pyrimidines where Brown (14) showed that such a pathway was correct by analysis of 2-amino-pyrimidine methylation products by mass spectrometry. However, one can

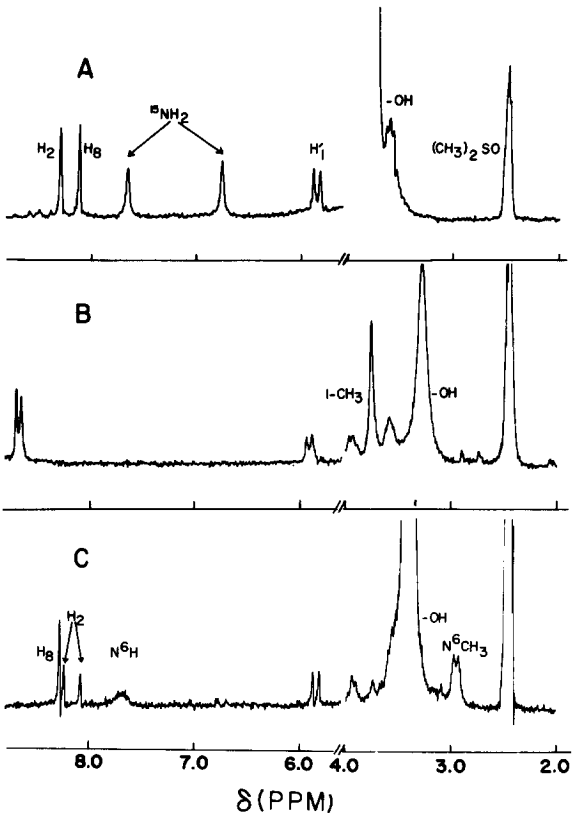

Figure 2. Nmr spectra of: A, ([^{15}N]-amino)-adenosine; B, 1-methyl-[^{15}N]-adenosine; C, N^6-methyl-[^{15}N]-adenosine were recorded in $(CH_3)_2SO$-d_6. Chemical shifts are reported as ppm downfield from HMD (K and K). Abbreviations used in spectral assignments are: S = singlet, D = doublet, Bd = broad peak. The amino protons in A were identified by exchange with D_2O; the peaks at 6.86 and 7.77 ppm disappear. Spectral assignments are: A, H_2 (8.30, S, A1), H_8(8.10, S, A1), [^{15}N]-amino (8.68 and 7.77, D, A2); B, H_2 and H_8 (8.70 and 8.66, both S, both A1), and 1-CH_3 (3.75, S, A3); C, H_8 (8.29, S, A1), H_2 (8.26 and 8.09, D, A1), C_6-NH (7.70, BdS, A1) and C_6-NCH_3 (2.96, BdD, A3). The doublet at 2.96 in spectrum C could be collapsed by homonuclear irradiation at 7.70 ppm or by deuterium replacement. The impurity in the solvent is centered at 2.445 ppm.

easily envision an alternate pathway in purines involving removal of a proton from the exocyclic amino group (Figure 1B). In this communication is is shown that the ring-opening mechanism is correct by use of Ado specifically labeled with [^{15}N] nitrogen at the exocyclic amino position. The advantage of using the [^{15}N] substituted Ado is that groups or atoms attached to a dipolar nucleus (instead of the normal [^{14}N] quadrupolar nucleus) will exhibit quite characteristic nmr spectra (15). If the reaction pathway followed were that outlined in 1A, one would expect the nmr spectrum of the final product (m^6Ado) to display: (i) a broad methyl doublet (area 3.), since the methyl group resonance would be split by a proton through a [^{14}N] nucleus at ~3. ppm; (ii) a broad resonance (area 1.) due to the amino proton split by the methyl group at ~7.5 ppm; and (iii) a C_2-proton peak split into a sharp doublet with a large coupling constant (each area, 0.5) due to its proximity to the (dipolar) endocyclic nitrogen. On the other hand, a mechanism involving methyl transfer as outlined by the pathway in Figure 1B would dictate that: (i) the methyl group at ~3. ppm would be comprised of two sets of sharp doublets with a large coupling constant between them (each area, 1.5); (ii) the single proton at ~7.5 ppm would be split (area 0.5 each) with a coupling constant of ~90 Hz (15) (since it is bonded to the [^{15}N] dipole); and (iii) the C_2- and C_8-protons should be undisturbed from a normal Ado spectrum. As can be seen in the nmr spectra presented (Figure 2), only pathway A is consistent with the experimental data; i.e., the pathway must involve ring opening and 180° internal rotation and finally ring reformation.

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