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On the Chemical Structure and Some Labile Hydrogens of 7-Methylguanosine

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Synopsis. Proton magnetic resonance and infrared absorption studies indicate that 7-methylguanosine has 1-NH, 2-NH_2, and 8-CH groups in dimethylsulfoxide solution, while in an aqueous system of pH 6.0 these groups are subjected to rather rapid hydrogen exchange reactions with the solvent molecules, so that their life-times are respectively $\ll 10^{-2}$ s, $=0.025$ s and $=180$ s at 25 °C.

7-Methylguanosine has recently been found as a modified structure of the 5′-end of the messenger RNA in eukaryote system[1–4]. Its chemical structure may be strange and unique among nucleosides, because of the positively charged nitrogen atom at position 7. Thus, the following questions, for example, are to be answered: Is the hydrogen atom located on 1-N or 6-O? and how labile are the protons of the 1-NH, 2-NH_2, and 8-CH groups?

7-Methylguanosine was prepared by a modification of the procedure of Jones and Robins.[5] A solid sample obtained by lyophilization from a pH 4.0 aqueous solution was carefully dried in vacuum and dissolved in dry dimethylsulfoxide-d_6. The proton magnetic resonance spectrum of this solution is shown in the Figure. The 1-NH, 8-CH, and 2-NH_2 signals are observed at 11.9, 9.6, and 7.6 ppm, respectively (from tetramethylsilane). Thus, the structure shown in the Figure is now supported. The 7-NCH_3 signal is found at 4.2 ppm; this is lower than those of other usual N-CH_3, probably because of the positive charge on 7-N.

In aqueous solutions, 7-methylguanosine is found to be stable only in the pH range lower than its pK (=7.1[6]) at 25 °C). In a H_2O solution of pH 4.0, as well as in that of pH 6.0, the 1-NH signal is not observed. This fact is attributable to a rapid hydrogen exchange reaction of this groups with the solvent molecule. The NH_2 signal (at 6.9 ppm) is broad and from its line width the rate constant of its hydrogen exchange with solvent is estimated to be 40 s^{-1} at 25 °C, pH 6. The hydrogen exchange reaction of 8-CH (at 9.2 ppm) is known to be faster than those of adenosine and guanosine.[7,8] We have followed the reaction not only by means of the proton magnetic resonance measurement but also by means of the infrared absorption measurement of the 8-CH stretching bands (two, at 3100 and 3170 cm^{-1}) in D_2O solutions. The reaction was found to be of the first-order. The rate constant k_e has been determined to be $6.4\times10^{-5}\,s^{-1}$ at pH 4.0 (25 °C) and 5.5×10^{-3} s^{-1} at pH 6.0 (25 °C). Thus, k_e is proportional to the OH^- concentration. On the basis of the relation,

$$k_e = k_{OH}[OH^-] = \{k_d\, 10^{14-pK}/(10^{14-pK}+1)\}[OH^-],$$

(where k_d is the diffusion constant and was assumed to

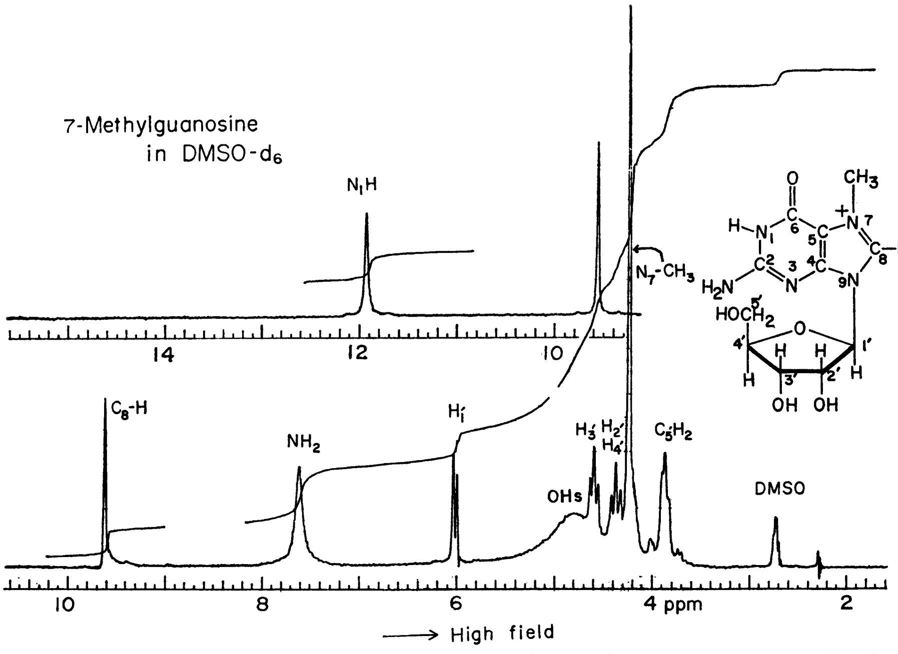

Fig. 100 MHz ^1H NMR spectrum of 7-methylguanosine in dimethylsulfoxide-d_6. The abscissa scale is the chemical shift in ppm from the tetramethylsilane resonance.

be equal to 10^{10} $M^{-1}\,s^{-1}$) the pK value of the 8-CH group has been estimated to be $18._3$ at 25 °C. The k_e value just determined would be useful in estimating the "fluctuation amplitude"[9] of the 7-methylguanine residue involved in a secondary structure of a messenger RNA or a transfer RNA.

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