

## TAUTOMERISM OF NUCLEIC BASES AND THE TEMPLATE SYNTHESIS OF POLYNUCLEOTIDES

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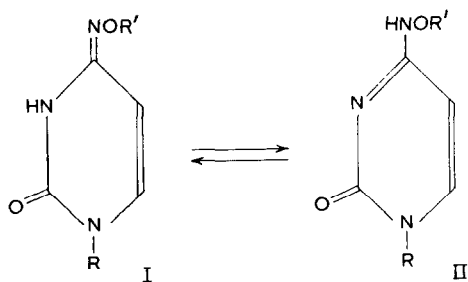
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### 1. Introduction

Tautomerism of nucleic bases is believed to be one of the possible reasons for spontaneous mutations of the transition type. This possibility is suggested by the increased frequency of spontaneous mutations on substitution of 5-bromouracil for thymine in DNA [1–3] since the tautomeric equilibrium constant of this base is smaller than those of natural bases [4–6].

Hydroxylamine and *O*-methylhydroxylamine are powerful mutagens that induce transitions of the C → T type. It was proposed [7–9] that the transitions are due to the ability of modified cytidine residues (*N*<sub>(4)</sub>-hydroxy- and *N*<sub>(4)</sub>-methoxycytidine) to act as uridine ones.

Compounds of this type are known to exist as mixtures of tautomers:



Scheme 1. R = CH<sub>3</sub> or β-D-ribofuranosyl, R' = H or CH<sub>3</sub>

and their tautomerism equilibrium constants,  $K_T = [I]/[II]$ , unlike those of natural bases are about 10–25 [10, 11].

It seemed interesting to find out whether modified cytidine residues of this type may act due to tautomerism as both uridine and cytidine residues, and to compare the efficiencies of their substitution for the two natural bases.

### 2. Materials and methods

*N*<sub>(4)</sub>-hydroxy- and *N*<sub>(4)</sub>-methoxycytidine triphosphates were obtained by reaction of hydroxylamine and *O*-methylhydroxylamine (0.85 M) with cytidine triphosphate (pH 5.0, 54°, 6–7 hr) and purified by chromatography on DEAE-Sephadex A-25 (HCO<sub>3</sub><sup>−</sup>) at pH 8.6 followed by chromatography on AG 1 × 8 at pH 5.0 (the detailed procedure will be published elsewhere).

Radioactivity was counted in toluene–PPO–POPOP scintillation mixture using an Intertechnique SL-30 scintillation spectrometer.

The synthesis of RNA was assayed by direct filtration on nitrocellulose filters [12]. Composition of the incubation mixture (0.5 ml): 25 μmole of Tris-acetate pH 7.9; 2.5 μmole of MgCl<sub>2</sub>; 0.5 μmole of MnCl<sub>2</sub>; 5 μmole βmercaptoethanol; 25 μmole of each nucleoside triphosphate; 3.0 nmole of <sup>3</sup>H-GTP (2.5 Ci/mole); 10 μg DNA of T2 phage; 11 μg of RNA polymerase isolated according to [13] after chromatography on DEAE-cellulose. After 35 min of incubation at 37° the reaction was stopped by addition of 50 μl 0.5 M EDTA, pH 7.6. The mixture was filtered through a nitrocellulose filter. The filter was dried and its radioactivity counted. The results obtained are shown in table 1.

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Table 1  
Synthesis of RNA in an RNA polymerase system after substitution of natural pyrimidine nucleoside triphosphates by  $N_{(4)}$ -hydroxy- or  $N_{(4)}$ -methoxycytidine triphosphates ( $C^*$ ).

Nucleoside triphosphates	Average incorporation of $^3H$ -GTP into RNA (cpm)	Increase of incorporation compared with incomplete system	Reliability of the observed deviation
A + G + U + C	1600		
A + G + U	231	—	—
A + G + U + $C^*$ ( $R' = H$ )	518	282	99%
A + G + U + $C^*$ ( $R' = CH_3$ )	399	168	90%
A + G + C	154	—	—
A + G + C + $C^*$ ( $R' = H$ )	389	235	95%
A + G + C + $C^*$ ( $R' = CH_3$ )	355	201	95%

Table 2  
Incorporation of  $N_{(4)}$ -methoxycytidine ( $C^*$ ) in an RNA polymerase system.

Nucleoside triphosphates	Radioactivity (counts per 100 sec) incorporated into polymer fraction		nmoles of $C^*$ incorporated into polymer fraction
	zero time	60 min of incubation	
A + G + C + $C^*$	550	4500	0.7
A + G + $C^*$ + U	530	3980	0.6
A + $C^*$ + C + U	500	500	0
$C^*$ + G + C + U	500	680	0.03

In another series of experiments, incorporation of  $^{14}C$ - $N_{(4)}$ -methoxycytidine triphosphate ( $3.4 \times 10^6$  cpm/ $\mu$ mole, label at methyl group) into newly synthesized RNA was measured. The reaction mixture, 0.25 ml, contained: 10  $\mu$ mole of Tris-HCl, pH 7.9; 1.25  $\mu$ mole of  $MgCl_2$ ; 0.25  $\mu$ mole of  $MnCl_2$ ; 2.5  $\mu$ mole of  $\beta$ -mercaptoethanol; 37  $\mu$ g of thymus DNA; 430  $\mu$ g of RNA polymerase, isolated according to [13] (fraction IV obtained by ammonium sulphate precipitation); 100 nmole of each natural nucleoside triphosphate and 80 nmole of  $^{14}C$ - $N_{(4)}$ -methoxycytidine triphosphate. In 30 min of incubation at 30° 0.25 ml of 0.1 M EDTA, pH 7.0, was added

followed by 0.5 ml of cold water and 2.1 ml of 50% trichloroacetic acid; the mixture was chilled in ice water for 10 min and filtered through a nitrocellulose filter, the precipitate was washed with 20 ml cold 3% trichloroacetic acid, filter dried and its radioactivity counted. The results obtained are presented in table 2.

### 3. Results and discussion

It is seen in tables 1 and 2 that  $N_{(4)}$ -hydroxycytidine and  $N_{(4)}$ -methoxycytidine triphosphates are functionally active though their activity is about one order of magnitude lower as compared with the natural nucleoside triphosphates (cf. [14, 15]). It is also seen that they exhibit dual (amphoteric) specificity and substitute both CTP and UTP. Hence, it appears in fact that the shift of the tautomerism equilibrium constant of the base towards unity leads to ambiguity of transcription.

Non-classic complementary base pairing, particularly that involving one of the bases in a rare tautomeric form, has been discussed by many authors as a source of misreading in template biosynthesis [16–19]. However, evidence is accumulating in favour of the important role of polymerases in selecting appropriate nucleoside residues during template biosynthesis [22–27]. Hence, the results obtained

in the present studies must be considered not only from the viewpoint of the possibility of complementary base pairs formation, but also from the viewpoint of nucleoside moiety recognition by the enzyme. In this connection it does not seem surprising that the efficiency of the substitution of UTP and CTP by modified cytidine triphosphates is approximately the same, although the tautomeric equilibrium constant is equal to 10–25 (see above).

However, the most important result of the present studies seems to be the biochemical demonstration of the fact that analogs of natural nucleosides with comparable concentrations of both tautomers exhibit well-defined dual specificity in template biosynthesis.

In conclusion it will be mentioned that the presence of only one precursor with dual specificity in the triphosphate pool can obviously lead to transition of *all* the four types. Hence, the present results provide a reasonable explanation of the data of Tessman et al. [28] who observed four types of transitions when S 13 phage was treated with hydroxylamine *in vivo*.

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