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3′-Fluoro-3′-deoxyribonucleoside 5′-triphosphates: synthesis and use as terminators of RNA biosynthesis

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3′-Fluoro-3′-deoxy-uridine, -cytidine, -adenosine and -guanosine have been synthesized by glycosylation of the corresponding silylated bases with 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-fluoro-3-deoxy-D-ribofuranose in the presence of Friedel-Crafts catalysts and were converted to the 5′- triphosphates, NTP(3′-F). It was shown that NTP(3′-F) are terminators of RNA synthesis catalyzed by DNA-dependent RNA polymerase from *E. coli* and may thus serve as tools for DNA sequencing.

Fluorodeoxyribonucleoside triphosphate; RNA synthesis, terminator; RNA polymerase, DNA-dependent; DNA sequencing

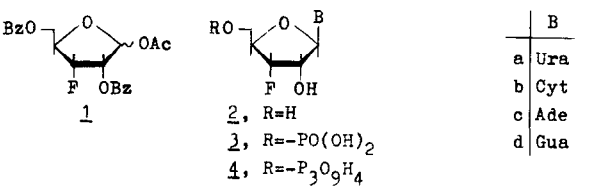
1. INTRODUCTION

Recently it has been shown that NTP analogues with NH_2-, N_3-, CH_3- and OCH_3- groups in 3′-position of sugar residues are effective terminators of RNA synthesis catalyzed by DNA-dependent RNA polymerase from *E. coli* [1–3].

Here we describe the synthesis of new NTP analogues with a fluorine atom in the 3′-position of the sugar residue – NTP (3′-F) with uracil (**4a**), cytosine, (**4b**), adenine (**4c**) and guanine (**4d**) bases (see scheme 1) and show that all these compounds are also effective terminators of RNA synthesis catalyzed by DNA-dependent RNA polymerase from *E. coli*.

2. MATERIALS AND METHODS

3′-Fluoro-3′-deoxyribofuranosides (**2a–d**) were prepared by using a universal sugar precursor, 1-*O*-acetyl-2,5-di-*O*-benzoyl-3-fluoro-3-deoxy-D-ribofuranose (**1**), the synthesis of which will be published elsewhere. Reaction of acetate **1** with persilylated derivatives of uracil, cytosine, N^6-benzoyladenine or N^2,N^9-diacetylguanine in the presence of tin(IV) chloride or trimethylsilyltriflate [4–7] after deblocking gives 3′-fluoro-3′-deoxyuridine (**2a**), -cytidine (**2b**), -adenosine (**2c**), or -guanosine (**2d**) in an isolated yield of 65, 61, 62 and 43%, respectively. For all nucleosides satisfactory C, H, N and F elemental analyses were obtained, and their structure was supported by UV- and ^{1}H-NMR data. The 5′-monophosphates (**3a–d**) were synthesized by reaction of the corresponding nucleosides with phosphorus oxychloride in trimethyl phosphate [9] according to [10] and/or with pyrophosphoryl chloride in acetonitrile [8] and/or by microbiological phosphorylation [11,12]; ^{1}H-NMR data for 5′-monophosphates are presented in table 1. The 5′-triphosphates (**4b–d**) were synthesized according to Hoard and Ott [13], isolated in a pure form by chromatography on DEAE-Sephadex A-25 (HCO_3^-) using a linear gradient of TEAB buffer (0.001–0.8 M) as eluent. NTP(3′-F) Na^+ salts were obtained as amorphous powders according to [16]. The purity of all synthesized NMP(3′-F) and NTP(3′-F) was checked by



Scheme 1.

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Table 1

^{1}H-NMR chemical shifts (δ_{TMS}, ppm) and coupling constants (J, Hz) of 5′-monophosphates **3a–d**[a]

Compound	H-8 (H-6)	H-2 (H-5)	H-1′	H-2′	H-3′	H-4′	H-5′	H-5″
AMP(3′-F)	8,51s	8,25s	6,19d $J_{2'}$ 7.8	4,92ddd $J_{2',3'}$ 4.2 $J_{2',F}$ 24.6	5,30ddd $J_{3',4'}$ 1.0 $J_{3',F}$ 54.0	[b]	4,15m	4,06m
GMP(3′-F)	8,12s		5,98d $J_{1',2'}$ 8.1	4,97ddd $J_{2',3'}$ 4.2 $J_{2',F}$ 25.2	5,29dd $J_{3',4'}$ 1.0 $J_{3',F}$ 54.4	[b]	4,10m	4,02m
UMP(3′-F)	7,93d	5,96d $J_{5,6}$ 7.8	6,15d $J_{1',2'}$ 8.1	4,47ddd $J_{2',3'}$ 4.2 $J_{2',F}$ 24.3	5,17dd $J_{3',4'}$ 1.0 $J_{3',F}$ 53.7	4,58m $J_{4',F}$ 27.3	4,09m	4,02m
CMP(3′-F)	7,92d	6,16d $J_{5,6}$ 7.5	6,20d $J_{1',2'}$ 7.8	4,47ddd $J_{2',3'}$ 4.5 $J_{2',F}$ 23.7	5,19dd $J_{3',4'}$ 1.0 $J_{3',F}$ 54.0	4,58m $J_{4',F}$ 27.0	4,10m	4,08m

[a] The ^{1}H NMR spectra were determined with Bruker WM 360 spectrometer in D_2O
[b] The signal is overlapped by HOD resonance

HPLC, UV spectroscopy and degradation to the corresponding fluorodeoxynucleosides under the action of alkaline phosphatase.

2.1. *RNA synthesis*

Reaction conditions were: 50 mM Tris-HCl (pH 8.0), 100 mM KCl, 10 mM $MgCl_2$, 0.1 mM EDTA, 0.1 mM DTT, 20 μg/ml gelatin, 100 μM CpA, 100 μg/ml ΔDIII T7 DNA and 1 μM of each NTP, including [α-^{32}P]ATP (2×10^5 cpm) in 10 μl total volume. The reaction was started by adding RNA polymerase (5 μg/ml) and proceeded for 6 min at 22°C, then up to 100 μM NTP(3′-F) was added and after 10 min at 22°C excess NTP (100 μM) and up to 0.7 M KCl were added. After another 10 min incubation the reaction was quenched by EDTA, and the transcripts electrophoresed as in [17].

3. RESULTS

The substrate properties of NTP(3′-F) were investigated in system of RNA synthesis catalyzed by *E. coli* RNA polymerase. The reaction was run on ΔDIII T7 DNA-template with CpA dinucleotide which provided RNA transcription initiation from A1 promoter. The sequence of newly synthesized RNA from the 20th to 80th nucleotide is [17]:

AUAGCCAUCCCAAUCCACACGUCCAACGG
30 40
GGCAACCGUAUGUACACCUGAUGGGUUCGCA
50 60 70 80

The resulting gel pattern of the sequence analysis of ΔDIII T7 DNA transcript from the 28th to 80th nucleotide, is shown in fig.1. The synthesis was carried out in the presence of all 4 substrates and one of NTP(3′-F) in each experiment under the described conditions (fig.1b). RNA synthesis in the presence of NTP(3′-NH_2) was run as a control for the experiment with NTP(3′-F) (fig.1a) [1]. It is clear that each track contains a discrete number of bands, which makes it possible to read fairly well the structure of newly synthesized RNA. The sequence of about 50 nucleotide residues was determined with the use of NTP(3′-F). This sequence corresponds to that determined with NTP(3′-NH_2).

This fact indicates that all NTP(3′-F) are terminators of RNA synthesis.

It should be noticed, however, that the bands 53A, 59A and 65A are almost invisible in the case of ATP(3′-F) (fig.1, track 5), in contrast to ATP(3′-NH_2) (fig.1, track 1). The same is true of ATP(OCH_3) (fig.1, track 9) or ATP(3′-N_3) [1]. We found that this phenomenon did not correlate with the analogue concentration in the range of 50–500 μM (at a naturally occurring NTP concentration of 1 μM). However, it was found to depend on the reaction time with the ATP analogue. Contrary to a 6-min incubation time all the bands are clearly seen after a 1-min incubation. Additional

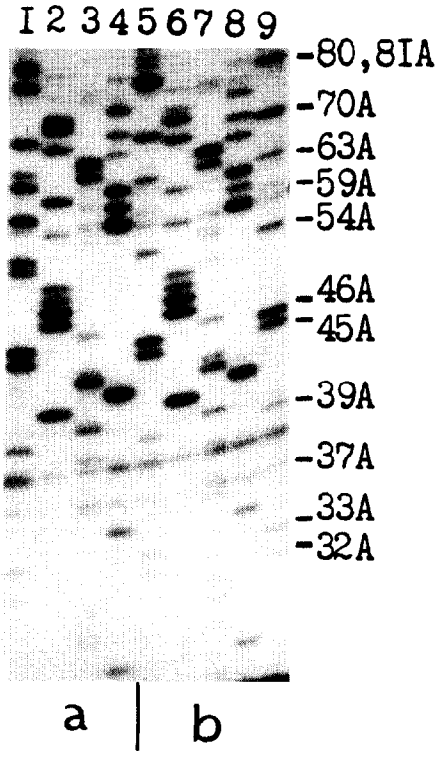

Fig.1. Gel pattern of the sequence analysis of ΔD111 T7 phage DNA transcripts, terminated (a) by NTP(3′-NH_2) and (b) by NTP(3′-F). Lanes: 1, ATP(3′-NH_2); 2, GTP(3′-NH_2); 3, CTP(3′-NH_2); 4, UTP(3′-NH_2); 5, ATP(3′-F); 6, GTP(3′-F); 7, CTP(3′-F); 8, UTP(3′-F); 9, ATP(3′-OCH_3).

experiments are needed to elucidate the nature of this phenomenon.

4. CONCLUSIONS

(i) The data presented here show that the NTP(3′-F) analogues are the true terminators for *E. coli* RNA polymerase and therefore these analogues can be used for DNA sequencing.

(ii) Specific incorporation of the nucleotide residue, containing F atom at the 3′-end of RNA provides new possibilities of investigating RNA polymerase-substrate and -nucleic acid interactions using NMR.

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