Biochemistry (Moscow), Vol. 70, No. 8, 2005, pp. 890-896. Translated from Biokhimiya, Vol. 70, No. 8, 2005, pp. 1078-1085.

Structural–Functional Relationships between Terminal Deoxynucleotidyltransferase and 5′-Triphosphates of Nucleoside Analogs

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Received June 24, 2004
Revision received August 26, 2004

Abstract—Substrate properties of nucleoside 5′-triphosphate (NTP) analogs, namely, 5′-triphosphates of L- and D-arabinonucleosides (D-FIAUTP, D-FMAUTP, and L-FMAUTP), D- and L-enantiomers of ddCTP analogs (D-ddCTP, L-ddCTP, D-FOddCTP, L-OddCTP, and L-SddCTP), and acyclic guanosine analogs (acyclovir and penciclovir) towards terminal deoxynucleotidyltransferase (TdT, EC 2.7.7.31) were studied. TdT can polymerize 5′-triphosphates of arabinonucleoside analogs (D-FIAUTP and D-FMAUTP). In contrast, L-FMAUTP is not recognized by TdT as a substrate. Kinetic parameters of D- and L-enantiomers of ddCTP analogs and 5′-triphosphates of acyclic nucleosides were evaluated. It is shown that stereospecificity of dNTP analogs and structure of the furanose residue play crucial roles in the interaction with TdT: L-enantiomers are much less potent as substrates compared to their D-counterparts. 5′-Triphosphates of acyclovir (ACVTP) and penciclovir (PCVTP) are about two orders of magnitude less effective as substrates than nucleosides bearing furanose residues, with PCVTP being a better substrate than ACVTP. It can be assumed that the hydroxyl group of PCVTP mimics the 3′-hydroxyl group of the ribose residue and plays an important role in the interaction with TdT.

Key words: nucleoside 5′-triphosphate analogs, terminal deoxynucleotidyltransferase, kinetic parameters

Terminal deoxynucleotidyltransferase (TdT, EC 2.7.7.31) is a unique template-independent DNA polymerase expressed exclusively in pre-lymphocytes (in thymus and bone marrow) and in their neoplastic counterparts (lymphoblastic leukemic cells) [1, 2]. The biological role of the enzyme has not been elucidated completely. It has been postulated that TdT plays an important role in the diversification of the immunoglobulin and T cell receptor repertoires by adding random nucleotides to the junctions of gene segments during V(D)J recombination [3-5]. TdT has been classified in a subclass of an ancient nucleotidyltransferases [6-9], whose members share a common signature in the active site and catalyze the same chemical reaction but have diverse biological roles [10, 11]. Multiple alignments of the catalytic domain sequences of all known TdT reveals several stretches of highly conservative amino acids residues, the functional importance of which was shown by the three-dimensional structure analysis of murine TdT published recently [12, 13]. The two longest motifs of conserved residues (446-454 and 331-337) form the incoming dNTP-binding site. Three strictly conserved aspartate residues are essential for the catalytic activity and for metal ion binding. TdT has attracted great interest because of its significant role in the formation of immunoglobulins, unique catalytic capability, and numerous applications in molecular biology. Unlike any other DNA polymerases, TdT can incorporate both ribo- and deoxyribonucleotides *in vitro* with the same efficacy [14] as well as several unnatural nucleoside triphosphates [15-18]. Hence, a detailed investigation of the molecular mechanism of TdT action,

Abbreviations: TdT) calf thymus terminal deoxynucleotidyltransferase; D-ddCMP and D-ddCTP) 2′,3′-dideoxycytidine 5′-mono- and 5′-triphosphates, respectively; 5′-mono- and 5′-triphosphates of all other nucleoside analogs have the same abbreviations throughout the paper; araNTP) 5′-triphosphates of arabinonucleosides; ACVTP) 5′-triphosphate of acyclovir (9-[(2-hydroxyethoxy)methyl]guanine); PCVTP) 5′-triphosphate of penciclovir (9-[4-hydroxy-3-(hydroxy-methyl)but-1-yl]guanine). Abbreviations of other nucleoside analogs are given in Fig. 1.

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0006-2979/05/7008-0890

as well as the interaction of 5′-triphosphates of nucleoside analogs, are of interest, both for basic molecular biology and potential clinical applications. The structure–function aspects of the interaction of TdT with acyclic dNTP analogs, such as ACVTP and PCVTP, have not been investigated so far. There is also no information about L-stereoisomers of ddNTP analogs as substrates of TdT. Kinetic parameters for the TdT-catalyzed incorporation of nucleotide analogs into oligonucleotide 3′-ends have been reported for only a few compounds [14-16].

Herein, substrate properties of dNTP analogs of three types, which differ in the structure of the furanose residue and the stereochemistry, towards TdT were investigated. These included 5′-triphosphates of L- and D-arabino nucleosides (D-FIAUTP, D-FMAUTP, and L-FMAUTP), D- and L-enantiomers of ddCTP analogs (D-ddCTP, L-ddCTP, D-FOddCTP, L-OddCTP, and L-SddCTP), and acyclic guanosine analogs (ACVTP and PCVTP). Kinetic parameters of the reaction of the ddCTP analogs and ACVTP and PCVTP interactions with TdT were evaluated. We show that both stereochemistry and furanose residue structure of nucleoside analogs have a major impact on the properties of dNTP analogs as substrates. Some of the analogs used are of special interest because they are either in clinical use or in clinical trials as antiviral or anticancer agents. The data can be explained by the crystal structure of TdT [12].

MATERIALS AND METHODS

L- and D-stereoisomers of ddC and L-OddC and their 5-fluoro-derivatives were synthesized as described previously [19, 20]; D-FIAU, D-FMAU, and L-FMAU were a kind gift from Dr. C. K. Chu (University of Georgia, USA). Penciclovir was obtained from GlaxoSmithKline (UK). Nucleoside triphosphate analogs were synthesized from the corresponding nucleosides according to the previously published method [21]. Unlabeled dNTP and ddNTP were purchased from Boehringer Mannheim (Germany). [γ-^{32}P]ATP (6000 Ci/mmol) was obtained from Izotop (Russia). T4 polynucleotide kinase was obtained from Amersham Pharmacia Biotech (UK). The oligonucleotide primer CCGTCAATTCCTGTAGTC was synthesized by Lytech (Russia). The primer was labeled at the 5′-end with T4 polynucleotide kinase using [γ-^{32}P]ATP [22] and purified on a Sephadex-G25 column. Calf thymus TdT was purchased from Gibco Corp (USA). Primer extension reactions were performed at 37°C as described in [16]. The reaction mixture (10 μl) contained 100 mM cacodylate buffer, pH 7.2, 2 mM Co^{2+}, 2 mM dithiothreitol, 15 nM primer, and dNTP analogs at concentrations given in the legends to the figures. The reactions were initiated by the addition of TdT at concentrations shown in the legends to the figures. The reactions were terminated by the addition of formamide and EDTA, and reaction products were separated by denaturing 15% polyacrylamide gel electrophoresis with the gel being subsequently exposed to Kodak X-ray film (USA).

The bands on the X-ray film resulting from the incorporation of dNMP analogs into the primer were scanned with the aid of a computer densitometer (Molecular Dynamics, USA) as described previously [23, 24]. The results are presented as double reciprocal values. The K_m and relative $k_{cat\ rel}$ values were calculated from the Lineweaver–Burk double reciprocal plot.

RESULTS

The structures of the nucleoside analogs used in the present study are shown in Fig. 1. Substrate properties of 5′-triphosphates of the nucleoside analogs towards TdT were evaluated by the ability of TdT to elongate 5′-^{32}P-primer in their presence.

Figure 2 shows the dose-dependent incorporation of D-FIAUTP, D-FMAUTP, and L-FMAUTP into the primer 3′-end by TdT. The natural dTTP was used as a standard. As one can see, TdT can elongate the primer many times in the presence of D-FIAUTP (lanes *4-6*) and D-FMAUTP (lanes *7-9*). If D-FMAUTP was used as a substrate, the oligonucleotide obtained turned out to be longer compared to the oligonucleotide generated in the presence of dTTP (lanes *1-3*). This shows that the replacement of dNTP ribose residue with 2′-fluoro arabinoside insignificantly affect their substrate properties. It is worth noting that the L-enantiomer of FMAUTP (L-FMAUTP) could not serve as a substrate of TdT (lanes *10-12*). This means that the position of ribose residue towards the nucleic base has a major impact on the interaction between dNTP analogs and TdT. The multiple incorporation of D-FMAUTP or D-FIAUTP into the primer could be confirmed by the different electrophoretic mobility of oligonucleotides containing D-FMAUMP (lanes *4-6*), D-FIAUTP (lanes *7-9*), or dTTP (lanes *1-3*) residues (Fig. 2).

Figure 3 shows an autoradiograph of the dose-dependent incorporation of D- and L-enantiomers of ddCTP analogs into the primer 3′-end by TdT. In contrast to the arabinonucleotides, all these compounds are obligate terminators since they lack a 3′-hydroxyl group, and only one of their residues could be incorporated into the primer. As one can see from Fig. 3, TdT catalyzes the incorporation of all the ddCTP analogs, albeit with different efficacy. Equal intensity of bands on the autoradiograph could be achieved at various concentrations of the substrates. We evaluated kinetic parameters of the reaction of the incorporation of ddNTP analogs into the 3′-end of the primers (table). The K_m and relative k_{cat} of nucleoside triphosphate analogs are presented in the table. While measuring kinetic parameters of the reac-

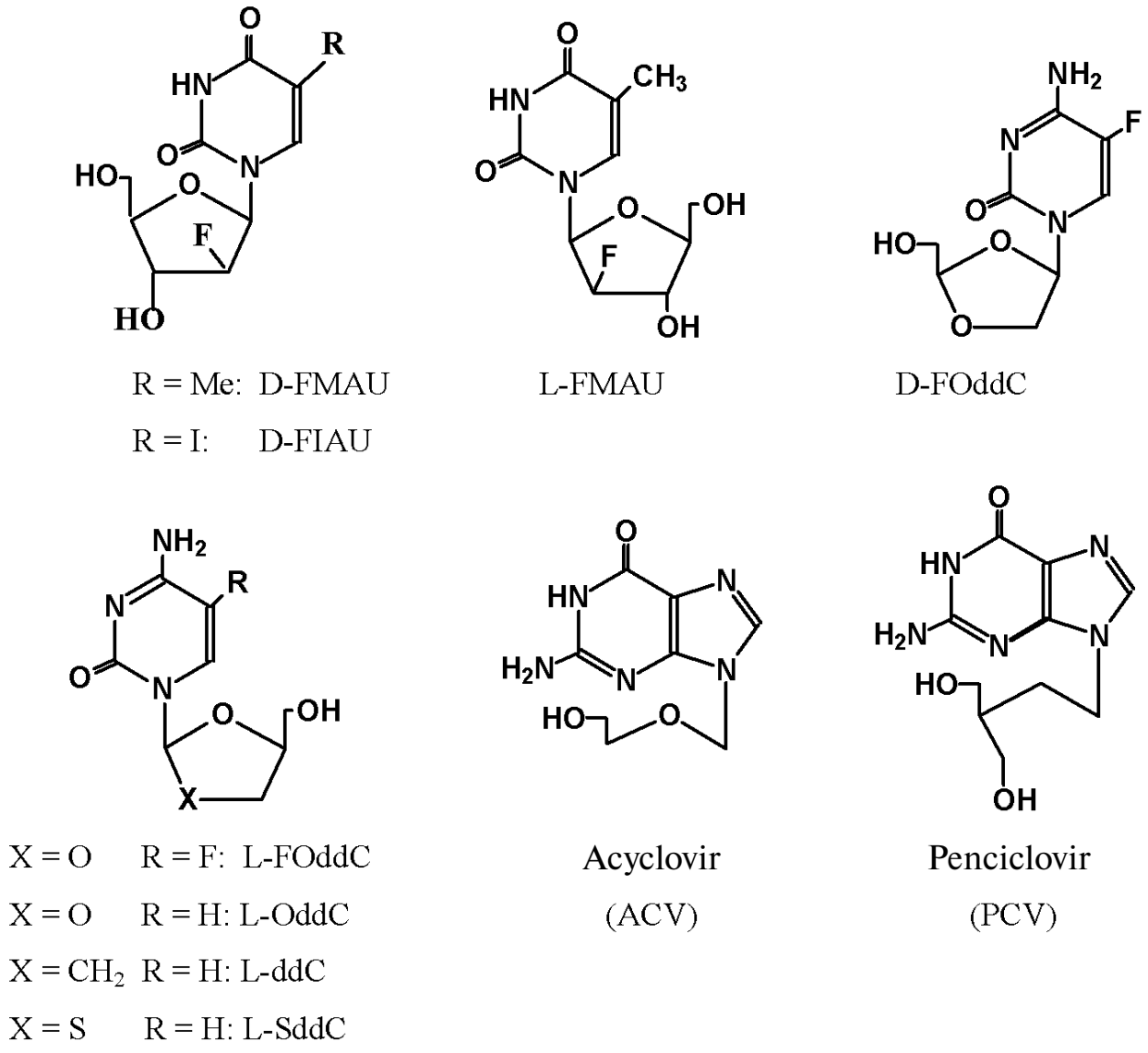

Fig. 1. Structures of the nucleoside analogs used in this study.

tion, the product yield had a linear dependence on time. The k_{cat} value of the reaction of TdT with D-ddCTP was taken as 1, and k_{cat} of the incorporation reaction of other derivatives were evaluated relatively to D-ddCTP. As is seen in the table, the K_m and k_{cat} of the reaction of TdT with D-ddCTP and D-ddGTP were similar to each other. This confirms the previously published data that the nucleotide base affects neither the affinity of substrates to TdT nor the reaction rate [12]. The K_m value of D-FOddCTP did not differ much from K_m of D-ddCTP, although k_{cat} decreased by 2.5-fold. L-Nucleoside 5′-triphosphate derivatives appeared to be much less effective as substrates for TdT compared to the corresponding D-counterparts. The K_m values for L-enantiomers (L-OddCTP, L-FOddCTP, L-SddCTP) were 3-7-fold higher and the $k_{cat\ rel}$ were 3-5-fold lower compared to that for D-ddCTP. Thus, the total efficacy of incorporation of L-nucleoside 5′-triphosphate analogs into the primer 3′-end was 12-30-fold lower than that for D-ddCTP. These results reveal that similarly to arabinonucleotide, stereospecificity of ddNTP analogs has a major impact on the properties of ddNTP as substrates for TdT. The introduction of a fluorine atom into the position 5 of L-OddCTP (L-FOddCTP) did not affect the substrate properties towards TdT.

Figure 4 presents dose dependence (a) and time course (b) of incorporation of ACVTP and PCVTP into the primer 3′-end. Both compounds are acyclic derivatives of guanosine but, in contrast to ACVTP, PCVTP is not an obligate terminator due to the presence of a

hydroxyl group that could mimic the 3′-OH group of the ribose ring and enables the chain elongation after the PCVMP residue incorporation into the primer 3′-end. Evidently, both compounds could be incorporated into the primer by TdT. It is noteworthy that only one residue of PCVMP was attached to the primer 3′-end under the conditions used. We could see a weak band on the radioautograph corresponding to the incorporation of the second residue PCVMP only at PCVTP concentrations exceeding 100 μM and a large excess of TdT. Primer terminated with PCVMP could not be elongated by dGTP either (data not shown). The estimated kinetic parameters of ACVTP and PCVTP incorporation are given in the table. The comparison of the efficacy of the incorporation reaction of PCVTP to that of ddGTP shows that substrate properties of PCVTP are at least 500-fold weaker than those of ddGTP, and ACVTP is about 10-fold weaker as a substrate as compared to PCVTP.

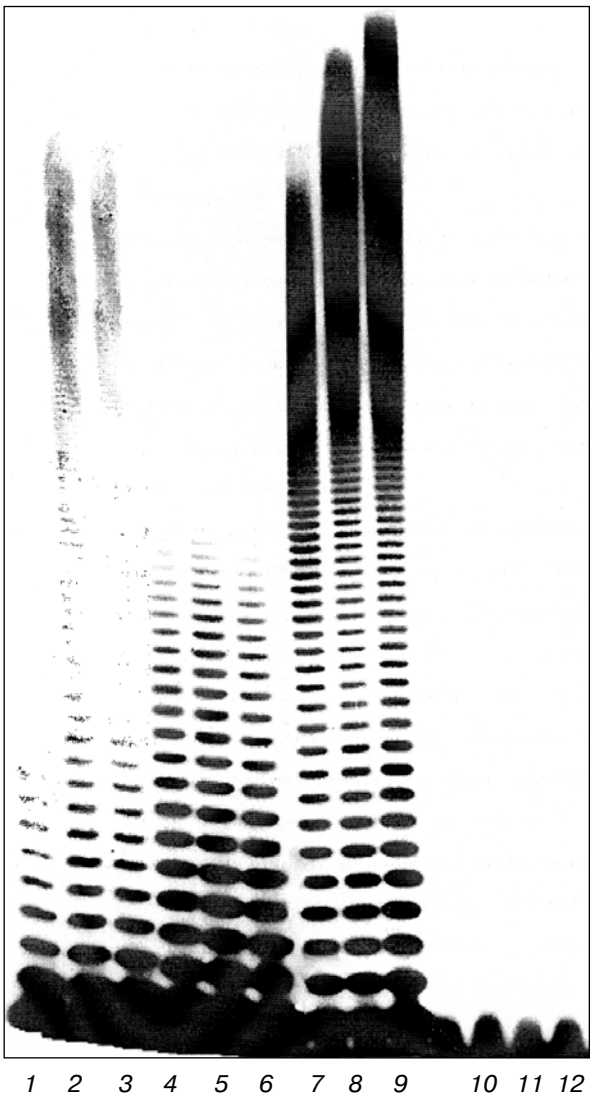

Fig. 2. Autoradiograph of the products of 5′-^{32}P-primer elongation reaction catalyzed by TdT with dTTP at concentrations 10, 30, and 60 μM (lanes *1-3*, respectively), D-FIAUTP at concentrations 10, 30, and 60 μM (lanes *4-6*, respectively), D-FMAUTP at concentrations 30, 60, and 90 μM (lanes *7-9*, respectively), and L-FMAUTP at concentrations 30, 60, and 90 μM (lanes *10-12*, respectively). The reactions were started by the addition of 0.2 U TdT. The reaction time was 10 min at 37°C. Other reaction conditions are described in "Materials and Methods".

Kinetic parameters of the 5′-^{32}P-primer elongation reaction catalyzed by TdT in the presence of ddNTP analogs

Substrate	K_m, μM	$k_{cat\ rel}$	k_{cat}/K_m
D-ddCTP	0.05 ± 0.01	1.0**	20
D-ddGTP	0.04 ± 0.01	1.1 ± 0.2	27.5
D-FOddCTP	0.06 ± 0.01	0.40 ± 0.15	6.6
L-ddCTP	>25*	n.d.	
L-FOddCTP	0.38 ± 0.12	0.60 ± 0.09	0.6
L-OddCTP	0.37 ± 0.09	0.3 ± 0.1	0.8
L-SddCTP	0.12 ± 0.08	0.20 ± 0.08	1.6
PCVTP	2.5 ± 0.8	0.10 ± 0.05	0.04
ACVTP	8 ± 2	0.025 ± 0.010	0.003

Note: The K_m mean values ± S.D. were assessed as described in "Materials and Methods" and in the legend to Fig. 2. Each value is the average of at least three separate experiments.
* The K_m was not estimated due to very poor incorporation.
** k_{cat} for D-ddCTP was taken as 1 and the k_{cat} of other compounds were compared to this value.

DISCUSSION

TdT is an unique template-independent DNA polymerase that contributes to the diversification of antigen receptors by adding nucleotides to the gene segment junctions during V(D)J recombination [1, 3, 4]. The full-length TdT is a single polypeptide chain of 55 kD with highly conserved amino acid sequence across species [4, 8]. Although TdT purified from calf thymus glands is the most abundantly available and widely used enzyme for functional and structural study, its crystal structure has not been solved yet. Recently, the three-dimensional structures of catalytic subunit of murine TdT and its two binary complexes, one with an oligonucleotide primer and the other with an incoming ddATP–Co^{2+} complex, were reported [12]. Available crystal structure of the enzyme allows understanding of the structure–function relationships between nucleoside triphosphate analogs and TdT. For example, the ability to incorporate both deoxy- and ribonucleotides with the similar efficacy [14]

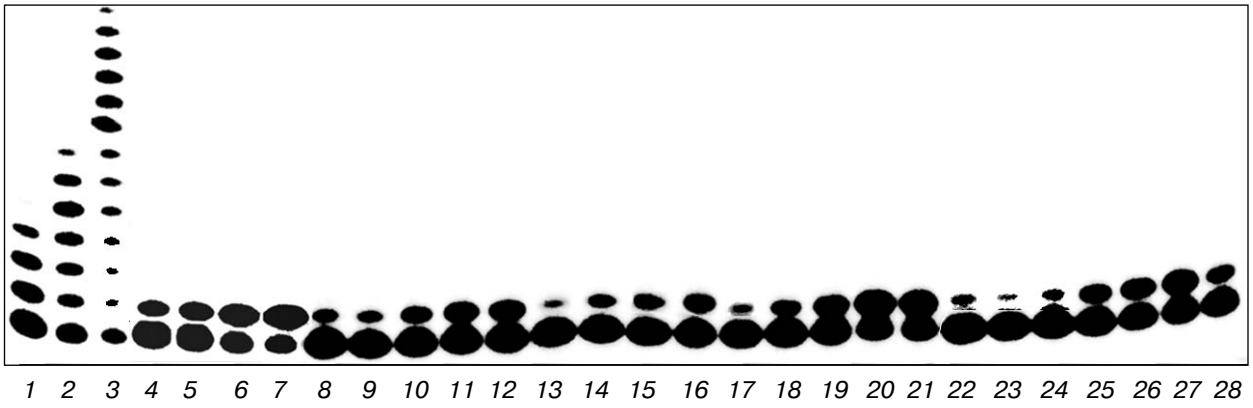

Fig. 3. Autoradiograph of the products of 5′-^{32}P-primer elongation reaction with ddCTP analogs shown in Fig. 1. Concentration of the compounds are: dCTP (lanes *1-3*), 0.5, 2.0, and 5.0 μM, respectively; ddCTP (lanes *4-7*), 0.01, 0.02, 0.1, and 0.5 μM, respectively; D-OddCTP (lanes *8-12*), 0.02, 0.05, 0.1, 0.5, and 1.25 μM, respectively; L-FOddCTP (lanes *13-16*), 0.1, 0.5, 1.0, and 1.5 μM, respectively; L-OddCTP (lanes *17-21*), 0.05, 0.1, 0.5, 1.0, and 2.0 μM, respectively; L-ddCTP (lanes *22-24*), 2.0, 5.0, and 10 μM, respectively; L-SddCTP (lanes *25-28*), 0.02, 0.05, 1.0, and 1.5 μM, respectively. Conditions: 0.1 U of TdT, 5-min incubation. Other reaction conditions are described in "Materials and Methods".

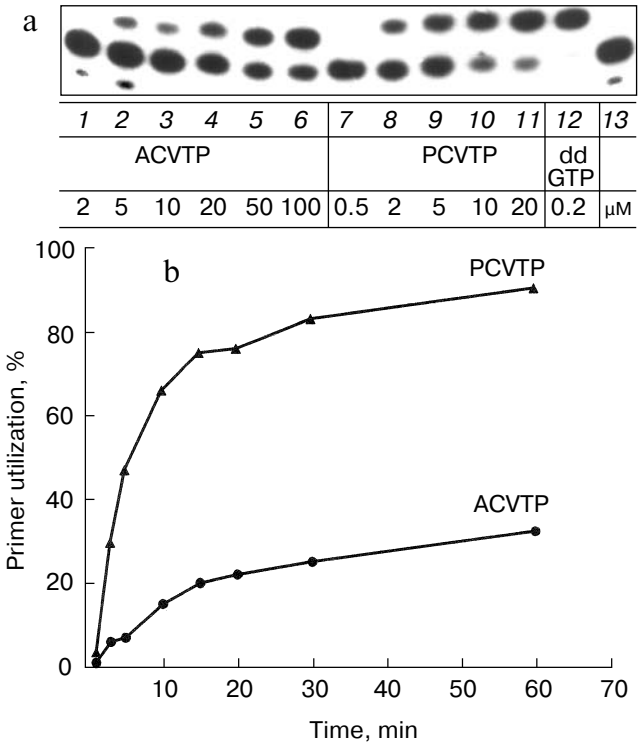

Fig. 4. a) Autoradiograph of the products of 5′-^{32}P-primer elongation reaction with ACVTP (lanes *1-6*) and PCVTP (lanes *7-11*), respectively; D-ddGTP (0.2 μM) (control) (lane *12*). Concentrations of the compounds are shown in the figure. Conditions: 0.2 U of TdT and 5 min incubation were used for the reactions. Lane *13* corresponds to the primer position. b) Time dependence of the incorporation of ACVTP and PCVTP. The data were obtained by scanning the autoradiograph of the products of 5′-^{32}P-primer elongation reaction. Conditions: 0.2 U of TdT and 50 μM concentration of the compounds were used for the reactions.

could be accounted for the fact that none of the enzyme atoms is close to the 2′- or 3′-positions of the furanose moiety [11].

Herein, we studied three different types of 5′-triphosphates of nucleoside analogs, namely, L- and D-arabinonucleosides (D-FIAUTP, D-FMAUTP, and L-FMAUTP), D- and L-enantiomers of ddCTP analogs (D-ddCTP, L-ddCTP, D-FOddCTP, L-OddCTP, and L-SddCTP), and acyclic guanosine analogs (ACVTP and PCVTP) as TdT substrates. Previously it was reported that TdT could incorporate a maximum of three araNMP residues, and then DNA cannot be further elongated [25]. We showed that TdT could incorporate araNTP analogs bearing a 2′-fluorine atom (D-FIAUTP and D-FMAUTP) into the primer 3′-end many times with efficacy close to or even exceeding that of dTTP. Although the conformation of furanose residues of araNTP and its 2′-fluoro-derivative has the same 2′-*endo*-geometry, the bulky 2′-OH-group leads to alteration of the torsion angles around the glycoside bond. Besides, the solvation sphere around the OH-group differs from that of a fluorine atom [26, 27]. These properties of arabinonucleotides remain the same in polyarabinonucleotides. The introduction of arabinonucleosides into an oligomer decreases the thermostability of the duplex between polyarabinonucleotide and polyribonucleotide. The destabilization of the complex can result in changing of the conformation around N-glycoside bond, since the 2′ group in arabinonucleosides is located in *cis*-position relative to the heterocyclic base, which results in local alterations of the coupling of bases [27, 28]. At the same time, oligomers bearing 2′-fluoro-arabinonucleotides have structural similarity with natural oligomers [26, 28]. These data are in accordance with our results showing

that TdT can polymerize 2′-fluoro-arabinonucleotides as a result of the structural similarity of the synthesized oligonucleotides and natural oligomers.

The different ability of TdT to polymerize araNTP and its fluoro-analogs (D-FIAUTP or FMAUTP) can be attributed to their different furanose conformations. CD spectroscopy of oligomers with incorporated 2′-fluoro-arabinonucleotides shows their structural similarity to the natural oligomers in contrast to the oligonucleotides containing 2′-arabinonucleosides [26]. According to the crystal structure of the binary complex TdT with primer [12] and previously published data [2], TdT has an absolute requirement for a primer containing at least three nucleotides. So, after incorporation of three araNTPs, the conformation of the synthesized primer does not allow its further elongation due to the incorrect position of the 3′-OH group of the resulting oligomer in contrast to the primer structure formed after incorporation of fluoro-analogs of araNTP. These data are in agreement with our results, showing the ability of TdT to polymerize 2′-fluoro-arabinonucleotides, since the structure of the oligonucleotide obtained is similar to that for the natural oligomers.

As was shown in this work, stereospecificity of dNTP analogs has a major impact on their substrate properties. In contrast to D-FMAUTP, TdT did not utilize its L-stereoisomer (L-FMAUTP) as a substrate. L-FMAU is the only L-2′-deoxynucleoside bearing an OH-group in 3′-position that effectively inhibits reproduction of hepatitis B and Epstein–Barr viruses. All known to date nucleosides that inhibit hepatitis B virus do not bear the 3′-OH group, being terminators of the DNA synthesis. The mechanism of action of L-FMAU is not clear. L-FMAU is phosphorylated in cells to give the corresponding triphosphate; however, L-FMAUTP displays neither substrate nor inhibitor properties towards template-dependent cellular DNA polymerases and Epstein–Barr virus DNA polymerase [29]. We hoped that the wide spectrum of the substrate specificity of the template-independent TdT would allows it to utilize L-FMAUTP as a substrate. However, in this case L-FMAUTP also was not a substrate of TdT. Based on published results and the results reported here, it could be assumed that the 3′-hydroxyl group in L-dNTPs prevents the formation of the productive complexes [DNA polymerase–primer–L-dNTP], creating a steric barrier.

We also reported here that similar to araNTP, the stereochemistry of ddNTP analogs has a major impact on their interaction with TdT. The L-ddCTP analogs displayed much weaker substrate properties towards TdT as compared to that of the D-enantiomers. Stereospecificity of ddCTP analogs affects both their affinity towards the enzyme (K_m) and k_{cat} of the reaction.

Triphosphates of acyclic guanosine analogs (ACVTP and PCVTP) were much less effective as substrates for TdT than the substrates bearing a furanose residue, PCVTP being a more effective substrate compared to ACVTP. Favorable substrate properties of PCVTP over ACVTP can be attributed to the 4-hydroxy-3-hydroxymethylbutyl fragment of PCVTP that can mimic the 2′-deoxyribose residue of dGTP and play an important role in increasing the affinity of PCVTP to TdT. As seen from the crystal structure of TdT, the sugar moiety of the incoming dNTP is bound by Trp450 and is close to the peptide bond between Gly452 and Ser453 [12]. It seems likely that only the sugar-phosphate side chain of substrates is important for their binding to TdT.

We interpreted our results on the basis of the crystal structure of murine TdT, while in the present paper we used calf thymus TdT, the structure of which has not yet been reported. However, the primary structure of the cDNA of the calf thymus TdT has 86 and 90% of the homology with the human and murine TdT, correspondingly. Moreover, the amino acid sequence of the nucleotide-binding domain is highly conservative in TdT from various sources. On the basis of these speculations we assume that general regularity of the substrate–function relationships between TdT and dNTP analogs could be also applied to the TdT from other sources. The information about the interaction of nucleoside 5′-triphosphate analogs with TdT could be significant for the prediction of the toxicity of nucleoside analogs, being in various stages of the clinical trials, relative to lymphocytes.

These studies were supported by the Russian Foundation for Basic Research (grants 05-04-49500 and 04-04-49621) and by the program of the Presidium of the Russian Academy of Sciences “Molecular and Cellular Biology”.

REFERENCES

1. Bollum, F. J. (1974) in *The Enzymes* (Boyer, P. D., ed.) Vol. 10, Academic Press, New York, pp. 145-171.
2. Kato, K. I., Goncalves, J. M., Houts, G. E., and Bollum, F. J. (1967) *J. Biol. Chem.*, **242**, 2780-2789.
3. Alt, F., and Baltimor, D. (1982) *Proc. Natl. Acad. Sci. USA*, **79**, 4118-4121.
4. Gilfillan, S., Dierich, A., Lemeur, M., Benoist, C., and Mathis, D. (1993) *Science*, **261**, 1175-1178.
5. MacGaffrey, R. T., Harrison, T. A., Parkman, R., and Baltimor, D. (1975) *New Engl. J. Med.*, **292**, 775-780.
6. Hansen, J. D. (1997) *Immunogenesis*, **46**, 367-375.
7. Lee, A., and Hsu, E. (1994) *J. Immunol.*, **152**, 4500-4507.
8. Yang, B., Gathy, K. N., and Coleman, M. S. (1995) *Nucleic Acids Res.*, **234**, 2041-2048.
9. Ito, J., and Braithwaite, D. K. (1991) *Nucleic Acids Res.*, **19**, 4045-4057.
10. Avarind, L., and Koonin, E. V. (1999) *Nucleic Acids Res.*, **27**, 1609-1618.
11. Boule, J. B., Rougem, F., and Papanicolaous, C. (2000) *J. Biol. Chem.*, **275**, 28984-28988.

12. Delarue, M., Boule, J. B., Lescar, J., Expert-Bezancon, N., Jourdan, N., Sukumar, N., Rougeon, F., and Papanicolaous, C. (2002) *EMBO J.*, **21**, 427-439.
13. Sukumar, N., Boule, J. B., Expert-Benzancon, N., Jourdan, N., Lescar, J., Rougeon, F., Papanicolaous, C., and Delarue, M. (2000) *Acta Crystallogr.*, **D56**, 1662-1664.
14. Boulle, J. B., Rougeon, F., and Papanicolaous, C. (2001) *J. Biol. Chem.*, **276**, 31388-31393.
15. Dyatkina, N., Semizarov, D., Victorova, L., Krayevsky, A., Theil, F., and von Janta-Lipinski, M. (1995) *Nucleosides Nucleotides*, **14**, 723-726.
16. Krayevsky, A. A., Victorova, L. S., Arzumanov, A. A., and Jasko, M. V. (2000) *Pharmacol. Therap.*, **85**, 165-173.
17. Semizarov, D. G., Arzumanov, A. A., Dyatkina, N. B., Meyer, A., Vichier-Guerre, S., Gosselin, G., Rayner, B., Imbach, J. P., and Krayevsky, A. A. (1997) *J. Biol. Chem.*, **14**, 9556-9560.
18. Semizarov, D. G., Victorova, L. S., Dyatkina, N. B., von Janta-Lipinski, M., and Krayevsky, A. A. (1994) *FEBS Lett.*, **354**, 187-190.
19. Kim, H. O., Shanmuganathan, K., Alves, A. J., Jeong, L. S., Beach, J. W., Schnizi, R. F., Chang, N. C., and Cheng, Y. C. (1992) *Tetrahedron Lett.*, **33**, 6899-6902.
20. Lin, T. S., Luo, M. Z., Liu, M. C., Pai, B., Dutschman, G. E., and Cheng, Y. C. (1994) *J. Med. Chem.*, **37**, 798-803.
21. Chang, C. N., Doong, C. L., Zhou, J. H., Beach, J. W., Jeong, L. S., Chu, C. K., Tsay, C. H., and Cheng, Y. C. (1992) *J. Biol. Chem.*, **267**, 13938-13942.
22. Sambrook, G., Fritch, E. F., and Manniatis, T. (1989) *Molecular Cloning*: *A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York.
23. Kukhanova, M. K., Liu, S. H., Mozzherin, D. Yu., Lin, T. C., Chu, C. K., and Cheng, Y. C. (1996) *J. Biol. Chem.*, **270**, 23055-23059.
24. Mendelman, L. V., Petrushka, J., and Goodman, M. F. (1990) *J. Biol. Chem.*, **265**, 2338-2346.
25. Chidgeavadze, Z. G., Beabealashvilli, R. Sh., Krayevsky, A. A., and Kukhanova, M. K. (1986) *Biochim. Biophys. Acta*, **868**, 145-152.
26. Noronha, A. M., Wilds, C. G., Lok, C. N., Viazovkina, K., Arion, M. D., Parniak, M. A., and Damha, M. J. (2000) *Biochemistry*, **39**, 7050-7062.
27. Ma, T., Pai, S. B., Zhu, Y. L., Jon, J. S., Shanmuganathan, K., Du, J., Wang, C., Kim, H., Newton, M. G., Cheng, Y. C., and Chu, C. K. (1996) *J. Med. Chem.*, **39**, 2835-2843.
28. Denisov, A. Yu., Noronha, A. M., Wilds, C. J., Trempe, J. F., Pon, R. T., Gehring, K., and Damha, M. J. (2001) *Nucleic Acids Res.*, **29**, 4284-4293.
29. Kukhanova, M. K., Lin, Z. Y., Yasco, M. V., and Cheng, Y. C. (1998) *Biochem. Pharmacol.*, **85**, 165-173.