In Vivo Reshaping the Catalytic Site of Nucleoside 2′-Deoxyribosyltransferase for Dideoxy- and Didehydroribonucleosides via a Single Amino Acid Substitution*

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Nucleoside 2′-deoxyribosyltransferases catalyze the transfer of 2′-deoxyribose between bases and have been widely used as biocatalysts to synthesize a variety of nucleoside analogs. The genes encoding nucleoside 2′-deoxyribosyltransferase (ndt) from Lactobacillus leichmannii and Lactobacillus fermentum underwent random mutagenesis to select variants specialized for the synthesis of 2′,3′-dideoxyribonucleosides. An Escherichia coli strain, auxotrophic for uracil and unable to use 2′,3′-dideoxyuridine, cytosine, and 2′,3′-dideoxyctydine as a source of uracil was constructed. Randomly mutated lactobacilli ndt libraries from two species, L. leichmannii and L. fermentum, were screened for the production of uracil with 2′,3′-dideoxyuridine as a source of uracil. Several mutants suitable for the synthesis of 2′,3′-dideoxyribonucleosides were isolated. The nucleotide sequence of the corresponding genes revealed a single mutation (G → A transition) leading to the substitution of a small aliphatic amino acid by a nucleophilic one, A15T (L. fermentum) or G9S (L. leichmannii), respectively. We concluded that the “adaptation” of the nucleoside 2′-deoxyribosyltransferase activity to 2,3-dideoxyribofuranosyl transfer requires an additional hydroxyl group on a key amino acid side chain of the protein to overcome the absence of such a group in the corresponding substrate. The evolved proteins also display significantly improved nucleoside 2′,3′-didehydro-2′,3′-dideoxyribosyltransferase activity.

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2 The abbreviations used are: NDT, nucleoside 2′-deoxyribosyltransferase; Lf-NDT, L. fermentum wild-type NDT; Lf-NDT A15T, modified variant of Lf-NDT in which Thr substitutes for Ala-15; LI-NDT, L. leichmannii wild-type NDT; LI-NDT G9S, modified variant of LI-NDT in which Ser substitutes for Gly-9; Hx, hypoxanthine; dX or dY, 2′-deoxyribosyltransferase; dR, 2′-deoxyribosyl transferase; dR, 2′,3′-dideoxyribosyl; dR, 2′,3′-dideoxyribosyl; MES, 2-(N-morpholino)ethanesulfonic acid; d4U, 2′,3′-dideoxy-2′,3′-dideoxyuridine; d4T, 2′,3′-dideoxy-2′,3′-dideoxythymidine.

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| TABLE 1 | Oligonucleotides used in the different polymerase chain reactions |
|---------|---------------------------------------------------------------|
| ycEL    | 5'-NNCCCACGGGGCGACCTGGCTGGCCACACTTGACCGG-3' |
| dinR    | 5'-GATTTCTAATTAAGAAGTCAATGAAATTTTAGTG-3' |
| yceR    | 5'-GATTTCTAATTAAGAAGTCAATGAAATTTTAGTG-3' |
| dinL    | 5'-GATTTCTAATTAAGAAGTCAATGAAATTTTAGTG-3' |
| codBR   | 5'-GATTTCTAATTAAGAAGTCAATGAAATTTTAGTG-3' |
| codYL   | 5'-GATTTCTAATTAAGAAGTCAATGAAATTTTAGTG-3' |
| codYL   | 5'-GATTTCTAATTAAGAAGTCAATGAAATTTTAGTG-3' |
| codYL   | 5'-GATTTCTAATTAAGAAGTCAATGAAATTTTAGTG-3' |

the nucleoside 2’-deoxyribosyltransferase activity to a nucleoside 2’,3’-dideoxy- or 2’,3’-didehydro,2’,3’-dideoxyribosyltransferase activity.

EXPERIMENTAL PROCEDURES

Chemicals

2’,3’-Dideoxyuridine (ddU), 2’,3’-dideoxyxytidyne (ddC), 2’,3’-dideoxythyridyne (ddT), 2’,3’-dideoxyinosynosine (ddI), 2’,3’-didehydro-2’,3’-dideoxytymidine (d4T), 2’,3’-dideoxynucleosides and bases were from Sigma-Aldrich. 2’,3’-Dideoxynuanosine (ddG), 2’,3’-dideoxyadenosine, and 2’,3’-dideoxy-2’,3’-dideoxyuridine (d4U) were from Berry and Associates (Ann Arbor, MI).

Growth of Bacterial Strains and DNA Manipulation

Bacteria were routinely grown in MS minimal medium (24) or LB (25). When necessary, ribo- and deoxyribonucleosides were added at a final concentration of 0.3 mM. Antibiotics were added at the following concentrations: gentamycin and tetracycline (10 μg/ml), ampicillin (100 μg/ml), chloramphenicol and kanamycin (30 μg/ml). All DNA manipulations were performed according to Sambrook et al. (25). Preparation of L. fermentum CIP 102980T DNA, construction of the genomic DNA bank, and screening for NDT activity were as previously described (7).

General Procedure for Gene Deletion

A DNA fragment containing 1-kilobase upstream and downstream of the gene to be deleted was first amplified from the E. coli MG1655 genome by PCR with a high fidelity polymerase and cloned into pCR-XL-TOPO (Invitrogen). The gene to be deleted was then replaced by a DNA fragment containing an antibiotic resistance gene using restriction enzymes and T4 DNA ligase. Finally, a PCR-generated fragment containing the antibiotic resistance gene flanked by the DNA region surrounding the gene to be deleted was used to recombine into the E. coli chromosome as described by Chaveroche et al. (26). Oligonucleotides ycEL, dinR, yceR, and dinL (Table 1) and a 1.1-kilobase EcoRI fragment from pUC1318Ap/gm (gift of D. Mazel) conferring resistance to gentamycin were used to construct the MG1655 ΔpyrC::gm strain. Oligonucleotides codBL, cyanTR, codBR, and codYL (Table 1) and a 1.2-kilobase DNA fragment from pUC4K (GE Healthcare) conferring resistance to kanamycin were used to construct strain MG1655 Δcoda::km. Deletions were confirmed by PCR and by testing the auxotrophy for uracil for MG1655 ΔpyrC::gm or by testing the sensitivity to 5-fluorouracil (10 μg/ml) and the resistance to 5-fluorocytosine (10 μg/ml) for the MG1655 Δcoda::km. The coda::km deletion was introduced into strain MG1655 ΔpyrC::gm by P1 transduction. Transductants were selected on LB medium supplemented with kanamycin, gentamycin, and uracil. The resulting strain PAK15 was transduced with a P1 stock phage from strain S05110 that has a Tn10 transposon insertion in the cdd gene (27) to yield strain PAK9 (MG1655 ΔpyrC::gm, Δcoda::km, cdd::Tn10).

Construction of ndt Mutant Libraries and Selection of the Nucleosome 2’,3’-Dideoxyribosyltransferase

L. fermentum—Oligonucleotides T7prom and T7term (Table 1) were used to amplify the ndt gene from plasmid pLF6 (pBam3 carrying the L. fermentum ndt gene, GenBank™ accession number AY064168) using the GeneMorPh PCR mutagenesis kit (Stratagene). The cycle parameters were: 1 cycle of 5 min at 95 °C, 30 cycles with 3 steps: 30 s at 95 °C, 30 s at 51.5 °C, 3 min at 72 °C; 1 cycle of 10 min at 72 °C. Two concentrations of DNA template were used, 10 ng and 10 pg.

The purified PCR products were digested for 2 h at 37 °C with BamHI and Ndel restriction enzymes and purified after separation on an agarose gel with the QIAquick gel extraction kit (QIAGen). The purified digested PCR products were ligated to pSU19N (a derivative of the general purpose cloning vector pSU19 (28) that contains an Ndel site, CATATG, ATG being the start codon of the β-galactosidase α peptide) digested with the same restriction enzymes and purified by the same procedures. The ligation products were dialyzed against water on Millipore filters before transformation of strain PAK9 by electroporation using the electroporation system (25).
troporation. Electroporated cells were incubated in LB medium supplemented with uracil for 1 h at 37 °C. The cells were washed twice with MS medium before plating on solid MS agar medium supplemented with chloramphenicol, ddU, and C. Plates were incubated 4 days at 37 °C. Colonies were reisolated on the same medium before preparation of the plasmid DNA.

L. leichmannii—The GeneMorph libraries were constructed as described above for L. fermentum except that plasmid pH1 (29) was used as DNA template. Three other libraries were constructed (i) by adding various concentrations of manganese in the PCR reaction, (ii) by adding various concentrations of manganese and by altering the nucleotides ratio in the PCR reaction (30), and (iii) by introducing the purine analog 1-(2-deoxy-β-D-ribofuranosyl)-imidazole-4-carboxamide 5'-triphosphate in the PCR reaction (31). Purification of the PCR products, digestion, subcloning in the PSU19N plasmid, transformation, and selection were as described above.

Overexpression of Wild-type and Engineered Lf-NDT and LI-NDT and Recombination of Recombinant Proteins

L. fermentum—Oligonucleotides PAK 5 and PAK 6 (Table 1) were used to amplify the L. fermentum ndt gene using plasmid pLF6 as DNA template in a standard reaction.

Oligonucleotides + (A15S+, A15C+, or A15V+) and T7 term, oligonucleotides − (A15S−, A15C−, or A15V−) and T7 prom (Table 1) were used in two separate PCR reactions. An aliquot of each PCR was used as a template for a third PCR reaction using T7 prom and T7 term, oligonucleotides as DNA template in a standard reaction. Each PCR product was cleaved to ddR and U by uridine or thymidine phosphorylase (see “Experimental Procedures”) were used to generate libraries having an almost unconstrained spectrum of mutations with a variable frequency (between 1 to 10 nucleotide changes per sequence).

A functional screen allowing the selection of variants was established in E. coli for the reaction ddU + C ⇄ ddC + U. This reaction was chosen because we hypothesize that the determinants of the base specificity are different from those for sugar recognition and neither ddU nor ddC interfered with the growth of E. coli. Consequently, variants with improved dideoxyribosyl transfer between U and C should also have an enhanced transferase activity between any bases.

The engineered strain should be auxotrophic for uracil (U) and unable to grow on minimal medium supplemented with cytosine (C), ddC, or ddU as a source of uracil. This strain (PAK9) was obtained by deleting the pyrC gene, which codes for dihydroorotase along with the genes coding for cytosine deaminase (codA) and cytidine deaminase (cdd) (Fig. 1). PAK9 will grow if one of the following conditions is fulfilled; (i) ddU is oxidized to uric acid by xanthine oxidase (0.2 unit). The specific activity was calculated using an e value for uric acid of 12 ± 10^3 mol cm⁻¹ at 290 nm. One unit corresponds to the formation of 1 µmol of product/min at 37 °C in 50 mM MES buffer (pH 6.0).

Mass Spectrometry—Ion spray mass spectra were recorded on an API 365 mass spectrometer (Perkin-Elmer-Sciex Thornhill, Canada). Samples dissolved in water/methanol/formic acid (50/50/5) were introduced at 5 µl/min with a syringe pump (Harvard Apparatus, South Natick, MA). The mass spectra were acquired from 1100 to 1700 with a scan step of 0.1 and a dwell time per step of 2.0 ms, resulting in a scan duration of 16.0 s. The molecular mass of native Lf-NDT and LI-NDT (18895.58 and 18080.45) and of the Lf-A15T and LI-G9S NDT mutants (18925.6 and 18110.48) were in agreement with that calculated from the sequences, the methionine being deleted in the case of LI-NDT and LI-NDT G9S.

RESULTS

Genetic Selection for a Nucleoside 2',3′-Dideoxyribosyltransferase Activity—Random mutant libraries of ndt genes from L. leichmannii and L. fermentum were constructed. The L. leichmannii ndt gene was used because the x-ray structure of LI-NDT is known and the function of several amino acids involved in substrate binding and catalysis are defined by site-directed mutagenesis experiments (21, 33). L. fermentum ndt gene was also mutagenized, because Lf-NDT is quite distant from LI-NDT (33% identity, data not shown), whereas the amino acids important for the transfer of the 2′-deoxyribose are conserved. Different mutagenesis protocols (see “Experimental Procedures”) were used to generate libraries having an almost unconstrained spectrum of mutations with a variable frequency (between 1 to 10 nucleotide changes per sequence).

The engineered strain should be auxotrophic for uracil (U) and unable to grow on minimal medium supplemented with cytosine (C), ddC, or ddU as a source of uracil. This strain (PAK9) was obtained by deleting the pyrC gene, which codes for dihydroorotase along with the genes coding for cytosine deaminase (codA) and cytidine deaminase (cdd) (Fig. 1). PAK9 will grow if one of the following conditions is fulfilled; (i) ddU is cleaved to ddR and U by uridine or thymidine phosphorylase (upd or deoA) or (ii) an NDT exchanges ddR between two nucleobases, U and X, according to the reaction ddU + X ⇌ ddX + U.

The random mutant libraries were screened. Ten clones from L. fermentum out of 1.2 × 10^6 grew on MS supplemented with ddU and C. Fig. 2 illustrates the growth differences of the PAK9 strain expressing the L. fermentum ndt (Fig. 2B, c) or the mutated ndt gene (Fig. 2B, a). Because lactobacilli NDT
enzymes can marginally transfer dDR between bases (22), a residual growth of strain PAK9 with ddU as the uracil source was observed (Fig. 2B, c). However, variants of NDT with improved dideoxyribosyl transfer activity have been isolated as having a growth advantage over the wild type. Nucleotide sequences of these clones showed the same G → A mutation at position 43 of their coding region which results in the substitution of Ala-15 by a Thr in the corresponding protein.

_L. leichmannii ndt_ variants were isolated using the same procedures. All variants carried the same mutation changing the ninth codon, GGT (Gly) to an AGT (Ser).

**Activity of the wild-type and engineered NDTs**—The enzyme activities were determined for both wild-type _L. leichmannii_ and _L. fermentum_ NDTs with different purines and pyrimidines as donor and acceptor substrates (Table 2). The highest specific activity was obtained with the couple dC/A, as previously observed with _L. helveticus_ and _L. leichmannii_ NDT (7, 34, 35). Pyrimidines appear to be better donors of dideoxyribose, and purines are often better acceptors. Deoxynosine is a poor substrate as for the _L. helveticus_ NDT (7). The transfer of dDR by either LI-NDT or LF-NDT is between 2 and 4 orders of magnitude lower than the transfer of 2-deoxyribose depending on the donors and acceptors (Table 2). With 2′-dideoxyribose donors, the specific activity of the purified LI-NDT G9S and LF-NDT A15T mutants was between 2 and 30% that of the wild-type activity, depending on the dideoxyribose donor and acceptor pairs. In contrast, the transfer of dDR between two bases is enhanced by a factor of 10–250 (Table 2). Remarkably, the transfer of dDR is enhanced by a factor of at least 100 with three different pairs (indicated in bold in Table 2). These results confirm that growth differences on minimal medium with ddU as the uracil source between strains carrying wild-type or mutated NDTs are due to improved activity of the mutated enzyme (Fig. 2). It should also be mentioned that the NDT mutants display an improved activity for the transfer of 2,3-didehydro-2,3-dideoxyribose as illustrated by the d4T + A exchange reaction, the specific activity of LF-NDT A15T (9.28 units/mg of protein) being enhanced by a factor of 250 compared with wild-type LF-NDT (0.036 unit/mg of protein) (highlighted in bold in Table 2).

**Kinetic Characterization of the Wild-type and Engineered NDTs**—Considering the measured specific activity (Table 2), the kinetic constants of _L. leichmannii_ and _L. fermentum_ NDTs were then determined with the best substrates, adenine as glycosyl acceptor and dC or ddC, respectively, as glycosyl donor. The _K_m for adenine was between 40 and 80 μM when measured using constant and saturating concentrations of dC or ddC irrespective of the enzyme tested (data not shown). Similarly, the _K_m for dC or ddC when measured with constant and saturating concentrations of adenine varied by a factor of two (Table 3). However, _k_cat and consequently the_ k_cat/K_m were greatly affected. Indeed, when dC was used as a donor, the catalytic
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TABLE 2
Specific activity (units/mg of protein) of nucleoside 2'-deoxyribosyltransferase from *L. leichmannii* and *L. fermentum* with adenine (A), cytosine (C), and thymine (T) as acceptors and various 2'-deoxy- and 2',3'-dideoxynucleosides as donors

Transfer of ddR is enhanced by a factor of at least 100 with three different pairs (indicated in bold). WT, wild type.

| Substrates | *L. leichmannii* | *L. fermentum* |
|------------|------------------|----------------|
|            | WT | G9S | G9S/WT | WT | A15T | A15T/WT |
| ddC + A    | 173 | 27.1 | 0.15 | 83.7 | 9.3 | 0.11 |
| ddC + Hx   | <0.01 | 1.25 | >125 | 0.06 | 7.1 | 0.18 |
| ddD + A    | 29  | 6   | 0.2  | 6.9  | 1.29 | 0.18 |
| ddD + Hx   | 0.04 | 0.8  | 20   | 0.05 | 6.8 | 0.18 |
| ddT + A    | 110 | 1.25 | 0.2  | 0.03 | 0.5 | 0.18 |
| ddT + Hx   | 0.017 | 0.8 | 20 | 0.0036 | 9.28 | 0.18 |
| ddU + T    | <0.01 | 0.8 | 20 | 0.03 | 0.12 | 0.18 |
| ddG + T    | 42.5 | 3.1 | 0.07 | 40.2 | 12.7 | 0.31 |
| ddG + Hx   | 0.056 | 1.08 | 0.008 | 0.19 | 1.6 | 0.09 |
| ddG + A    | 5.2 | 1.1 | 0.2 | 4.95 | 0.57 | 0.11 |
| dl + T     | <0.01 | 0.8 | 20 | 0.03 | 0.12 | 0.18 |
| dl + A     | 3.6 | 0.47 | 0.13 | 1.08 | 0.02 | 0.02 |

TABLE 3
Kinetic constants of *L. fermentum* and *L. leichmannii* NDT with adenine (A) as glycosyl acceptor and 2'-deoxyadenosine (ddC) and 2',3'-dideoxycytidine (ddC) as glycosyl donor

The \( k_{cat} \) (s\(^{-1}\)) was calculated assuming a molecular mass of 18.9 kDa for Lf-NDT and 18.1 kDa for Li-NDT. The mass difference due to amino acid substitution was negligible and was not taken into consideration. ND, not detectable.

| Organism | Enzyme | \( k_{cat} \) as variable substrate | \( k_{cat} \) as variable substrate |
|----------|--------|-----------------------------------|-----------------------------------|
|          |        | \( k_{cat} \) | \( k_{m} \) | \( k_{cat}/k_{m} \) | \( k_{cat} \) | \( k_{m} \) | \( k_{cat}/k_{m} \) |
|          |        | s\(^{-1}\) | ms | s\(^{-1}\) | ms | s\(^{-1}\) | ms | s\(^{-1}\) | ms |
| *L. fermentum* | Wild type | 45.1 | 0.3 | 1.5 \times 10\(^5\) | 0.047 | 0.38 | 1.2 \times 10\(^2\) |
|          | A15T   | 5.36 | 0.39 | 1.4 \times 10\(^3\) | 10.4 | 0.88 | 1.3 \times 10\(^4\) |
| *L. leichmannii* | Wild-type | 62.2 | 0.25 | 2.5 \times 10\(^4\) | 2.54 | 0.92 | 9.1 \times 10\(^3\) |
|          | G9S    | 12.4 | 0.30 | 4.1 \times 10\(^3\) | ND | ND | ND |

TABLE 4
Effects of other amino acid substitutions on NDT activity with 2'-deoxyadenosine (ddC) and 2',3'-dideoxycytidine (ddC) as donors and adenine (A) as acceptor

Efficiency (expressed by the \( k_{cat}/k_{m} \) ratio) of the Lf-NDT A15T and the Li-NDT G9S was 1 order of magnitude lower than the corresponding wild-type enzymes. Conversely, when ddC was used as the sugar donor, a 2 orders of magnitude increase of catalytic efficiency was observed in the mutant Li-NDT A15T in comparison to the wild-type Li-NDT. We also analyzed the 2,3-didehydro-2,3-dideoxyribose exchange reaction between U and C. The \( k_{m} \) for ddU was similar for both the wild-type and Lf-NDT A15T mutant, whereas the \( k_{cat} \) was 75-fold higher with the A15T mutant (38.9 s\(^{-1}\)) as compared with the wild-type enzyme (0.51 s\(^{-1}\)).

**Effect of Other Amino Acid Substitutions on NDT Activity**

To determine whether in vivo selected mutations expressed the highest transfer activity within 2',3'-dideoxynucleosides and nucleobases, the Gly-9 of Li-NDT and the Ala-15 of Lf-NDT were substituted by site-directed mutagenesis by other polar or hydrophilic amino acids. The activity of the new variants is reported in Table 4. Ser appears as a good alternative for Thr in the case of Li-NDT as the rate of transfer of ddR from ddC to A of the two mutants (i.e., A15T and A15S) is practically identical. Moreover, the specific activity of Li-NDT A15S with ddC and A as substrates is closer to that of the wild-type enzyme than that of the Lf-NDT A15T mutant. In the case of Li-NDT, Ser remains a better substituent for Gly-9 than Thr, probably due to their size difference, namely Ser being closer to Gly than Thr. As expected, neither Cys, nor the other non-polar amino acids were capable of improving 2',3'-dideoxyribose transfer reaction within any explored substrate pairs.

**DISCUSSION**

Crude extracts from *L. helveticus* or *L. leichmannii* have been used to synthesize a large number of deoxyribonucleosides with modified acceptor bases or sugar residues. Some of these analogs have been shown to have antiviral, antimicrobial, or antitumor activity (11, 23, 36–38). However, other enzymes such as deaminases present in the crude extracts can interfere with the synthesis (39). The activity of NDT is also generally low with nucleoside analogs as compared with natural 2'-deoxyribonucleosides (22, 37). Thus, an evolved NDT with better activity on analogs could be very advantageous. To initially demonstrate the evolvability of the NDT enzyme, 2',3'-dideoxyribonucleosides were chosen because they are poor substrates for NDT (22, 40), and some, ddI and ddC, are of therapeutic interest. Although the three-dimensional structure of *L. leichmannii* NDT is known, mutation(s) required for an efficient transfer of ddR between bases is not easily predictable (21). Thus, a genetic selection was established in *E. coli*. The difficulty in the establishment of a screen with unnatural substrates resides in the substrate/product toxicity or in the marginal activity of some metabolic enzymes. The selection was based on the res-
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NDT Lactobacillus acidophilus
NDT Lactobacillus helveticus
NDT Lactobacillus leichmannii
NDT L. LfG98
PTD Lactobacillus helveticus
Lactobacillus gasseri
NDT Lactobacillus fermentum
NDT LfA15T
Oenococcus oeni MCW
Leuconostoc mesenteroides ATCC 8293
Lactobacillus plantarum WCFS1
Lactococcus lactis IL1403

FIGURE 3. Shown is the partial alignment of nucleoside 2'-dideoxyribosyltransferases from different lactobacilli and of related sequences. The amino acid substitutions in Ll-NDT G9S and Lf-NDT A15T are indicated in red characters.

A single amino acid change is enough to gain activity with dideoxynucleosides as substrate. The specificity for the base is unchanged, and only the sugar recognition is modified. In the native NDT, the deoxyribose binding site is lined by three acidic residues; that is, the catalytic Glu-98, which interacts with the side chain of Ser-9 (or of Thr-15 in L. fermentum NDT) and with the catalytic Glu-98 (Fig. 4), which consequently slow down the catalysis. Another additional explanation is a weak steric clash due to the presence of the OH group of Ser-9.

As mentioned earlier, the 3'-OH of the sugar that interacts with the catalytic Glu-98 is important for the proper orientation of the sugar for optimal catalysis. When absent, the reaction is much slower. The transfer of 2',3'-dideoxyribose is improved significantly if the missing 3'-OH group is brought by the side chain of Ser-9 (or of Thr-15 in L. fermentum NDT) and to a lesser extent by Thr-9 or Ser-15, respectively.

Differences in catalytic activities or in affinities due to the presence of a single hydroxyl group have been reported for mutants of human nucleoside diphosphate kinase (41), for transition state analogs of adenosine deaminase (42), and for mutants of the E. coli DNA polymerase I (43). Substitution of E. coli DNA polymerase I Phe-762 with Tyr results in a dramatic decrease of dideoxynucleotide discrimination. This
absence of specificity is due to the compensation of the missing 3′-OH group of the nucleotide by the phenolic OH of Tyr (43). This resembles the principle of substrate-assisted catalysis (44, 45). Although the initial amino acid was not essential for catalysis, the activity with deoxynucleoside was lower than the wild-type enzyme. The OH group of serine or threonine does not replace the OH group of 2′-deoxynucleosides in terms of H-bonds. However, it compensates this loss by stabilizing the intermediate in a different way, allowing a gain of activity.

The LF-NDT A15T enzyme was used to prepare at the mmol scale and in good yield (up to 70%) 2′,3′-didehydro-2′,3′-dideoxyadenosine and 2′,3′-didehydro-2′,3′-dideoxynosine from d4U (data not shown). This enzymatic synthesis represents an efficient alternative method to organic syntheses (46–48) that often require several steps from ribonucleosides. Furthermore, radiolabeled derivatives could be synthesized for metabolic studies in both animal models and human subjects.

Our selection strategy should be applicable to other nucleosides analogs as those substituted at 3′ or 5′ positions of the sugar moieties. The newly selected variants will allow the diversification and expansion of the number of analogs of biological interest.

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