Functional Cloning, Heterologous Expression, and Purification of Two Different N-Deoxyribosyltransferases from Lactobacillus helveticus*

Lactobacillus helveticus contains two types of N-deoxyribosyltransferases: DRTase I catalyzes the transfer of 2'-deoxyribose between purine bases exclusively whereas DRTase II is able to transfer the 2'-deoxyribose between two pyrimidine or between pyrimidine and purine bases. An Escherichia coli strain, auxotrophic for guanine and unable to use deoxyguanosine as source of guanine, was constructed to clone the corresponding genes. By screening a genomic bank for the production of guanine, the L. helveticus ptd and ndt genes coding for DRTase I and II, respectively, were isolated. Although the two genes have no sequence similarity, the two deduced polypeptides display 25.6% identity, with most of the residues involved in substrate binding and the active site nucleophile Glu-98 being conserved. Overexpression and purification of the two proteins shows that DRTase I is specific for purines with a preference for deoxyinosine (di) > deoxyadenosine > deoxyguanosine as donor substrates whereas DRTase II has a strong preference for pyrimidines as donor substrates and purines as base acceptors. Purine analogues were substrates as acceptor bases for both enzymes. Comparison of DRTase I and DRTase II activities with di as donor or hypoxanthine as acceptor and colocalization of the ptd and add genes suggest a specific role for DRTase I in the metabolism of di.

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The Journal of Biological Chemistry Vol. 277, No. 17, Issue of April 26, pp. 14400–14407, 2002

Received for publication, December 17, 2001
Published, JBC Papers in Press, February 8, 2002, DOI 10.1074/jbc.M111995200
form strain TOP10F’ (Invitrogen). Plasmid DNA from several transformants was prepared and used as DNA template in a PCR reaction with oligonucleotides gua2 (5’-NNNNCCCGGGGCAATATCTCGACGACGATG- GG-3’) and gua3 (5’-NNNNCCGGGCTTTTGGACCCTGCACTATGAATG- G3’). The parameters were 1 cycle of 5 min at 95 °C, 30 cycles with three steps (30 s at 95 °C, 30 s at 62 °C, 4 min at 72 °C), and 1 cycle of 10 min at 72 °C. The resulting PCR product was digested with restriction enzyme SmaI (underlined in the sequence of oligonucleotides gua2 and gua3), purified after migration on an agarose gel with the Qiaex extraction kit, and ligated to a 1.1-kb SmaI DNA fragment conferring resistance to gentamicin. The ligation mixture was used to transform strain β 2033. Plasmid DNA from several gentamicin-resistant transformants was prepared and used in a PCR reaction with oligonucleotides gua1 and gua4. The resulting PCR product was then purified from an agarose gel and digested for 12 h at 37 °C with the restriction enzyme DpnI. After a phenol extraction, the PCR product was precipitated with ethanol. The DNA obtained after centrifugation was resuspended in water and used to transform strain MG1655 carrying the pKOBEG plasmid (19). Transformants were selected on LB medium supplemented with ampicillin (100 μg/ml) and guanine (0.3 mM). Guanine auxotrophs that grew on LB medium supplemented with guanine (0.3 mM) and guanine auxotrophs that grew on LB medium supplemented with ampicillin (100 μg/ml), deoxyguanosine (0.3 mM), and adenine (0.3 mM).

Preparation of Crude Extracts and Rapid Measurement of the Deoxyribosyltransferase Activity—Bacteria were grown overnight at 37 °C in LB medium supplemented with ampicillin (100 μg/ml) and the appropriate nucleosides and bases depending on the strain used. Cells were centrifuged at 4000 rpm for 15 min, washed once with a 100 mM phosphate buffer (NaH2PO4/Na2HPO4, pH 7.5), and resuspended in the same buffer at one-tenth of the original volume. Bacteria were disrupted by sonication, and the extracts were cleared by two successive centrifugations at 13000 rpm for 10 min at 4 °C. The lysate activity was determined by incubation of different amounts of lysate in 20 mM of citrate buffer at pH 6.0 in the presence of a deoxyribonucleoside (3 mM) and a base (1 mM). The reaction was followed by measuring absorbance at 420 nm.

Table I

| Strain   | Genotype | Characteristics          | Source                  |
|----------|----------|--------------------------|-------------------------|
| MG1655  | F'λ-     |                          | Gift of B. Bachmann     |
| KU8     | trxA::Tn10Kan1,serB::Tn10 |                          | (20)                    |
| Se 928  | HfrH ade-11, Δlac, thi, udp, upp, ton |                          | (21)                    |
| AM2D9   | thr, leu, thi, apo-loc, ΔmelE-udp | zif9::Tn10 | Gift of A. Mironov      |
| β1308   | ΔthyA::erm |                          | This work               |
| β2033   | pro, thi, rpsL, hsdS, ΔlacZ15 lacI traD36 proA+ proB+ | This work               |
| PAK4    | ΔguaBguaA::gm |                          | This work               |
| PAK6    | ΔguaBguaA::gm Δdeo-11       |                          | This work               |
| PAK26   | ΔguaBguaA::gm Δdeo-11 ΔthyA::erm |              | This work               |
| BL21(DE3)pLysS | Δsrf::lac, F'ompT hsdR (r λdR) gal dcm Δsrl-recA306::Tn10(DE3)pLysS | Novagen                 |

Plasmids Characteristics Source
Pβam3 ColE1, bla+ Gift of C. Robert
pKOBEG OriR101 repA83 Cm' carrying the exo+, bet and gam genes from the phage λ under the control of the pBAD promoter
PET24a ColE1, lacF+ Kan' Novagen
PLH2 ColE1, lacF+ carrying the L. helveticus ntd gene This work
PLH4 ColE1, lacF+ carrying the L. helveticus ptd gene This work
pLl ColE1, lacF+ carrying the L. leichmannii ntd gene This laboratory
PETLH2 ColE1, lacF+ Km’ carrying the L. helveticus ntd gene as an NdeI-BamHI fragment from PLH2 under the control of the T7 promoter This work
PETLH4 ColE1, lacF+ Km’ carrying the L. helveticus ptd gene as an NdeI-BamHI fragment from PLH4 under the control of the T7 promoter This work

DNA fragments were then ligated into the pBAM3 plasmid digested with the restriction enzyme Smal whose 5’ extremities were dephosphorylated with alkaline phosphatase. After an incubation of 16 h at 16 °C, the ligation mixture was desalted on Millipore (0.05 μm, 13 mm) filters and used to transform strain PAK6 by electroporation according to Dower et al. (18). Bacteria were resuspended in LB medium supplemented with guanine (0.3 mM) and incubated 1 h at 37 °C. Cells were then washed twice with M9 medium before plating on M9 glucose medium (16) supplemented with ampicillin (100 μg/ml), deoxyguanosine (0.3 mM), and adenine (0.3 mM).

Protein Overexpression and Purification—Oligonucleotides PAK2, 5’-NGATAAACTATATGAAAGCAGTGATCCACACAGG and PAK3, 5’-NNGATCTTACATTGAATGCCCTGACAAGG; PAK9, 5’-NGATATACTGATGAAAGAAGACAGTATTTTGG and PAK10, 5’-NGAGGATCTTATATACAGCCTGGCCTAG were used to amplify the ptd and the ntd genes in a standard PCR reaction using plasmids pH2 or pH4, respectively, as DNA template. The parameters used were 1 cycle of 5 min at 95 °C; 25 cycles of 30 s at 95 °C; 30 s at 60 °C; and 1 min at 72 °C; and 1 cycle of 10 min at 72 °C. Each PCR product was purified by using the QiAquick PCR purification kit (Qiagen) then digested with NdeI and BamHI enzymes (underlined in the oligonucleotide sequence) over 2 h at 37 °C and purified again with the QiAquick PCR purification kit. Each PCR product was ligated with plasmid PET24a digested with the same restriction enzymes. The ligation mixtures were used to transform strain J2033. Plasmids containing an insert of the correct size were sequenced by MWG-Biotech. Those with the correct sequence, pETLH2 and pETLH4, were used to transform strain BL21(DE3)pLysS (Novagen). 500 ml of LB medium inoculated with an overnight culture of BL21(DE3)pLysS containing either pETLH2 or pETLH4 was grown at 37 °C until A600 = 0.6. Isopropyl-1-thio-β-D-galacto-
Pyranoside was added to a final concentration of 0.4 mM, and the cultures were incubated for 2.5 h. Bacteria were centrifuged, washed once with a 100 mM phosphate buffer (NaH$_2$PO$_4$/Na$_2$HPO$_4$, pH 7.5). Pellets were frozen at $-80^\circ$C. Cells were resuspended in 20 ml of phosphate buffer and broken by one passage through a French press at 14000 p.s.i. The lysate was centrifuged at 50,000 rpm for 1 h, and the supernatant was precipitated by addition of solid ammonium sulfate to 55% saturation. Proteins were pelleted by centrifugation at 18,000 rpm for 30 min and resuspended in phosphate buffer. Each protein was further purified by filtration on a Sephacryl S-200 column previously equilibrated with 0.1M NaCl. The elution was followed by UV absorption at 280 nm, and each fraction was analyzed by SDS-PAGE electrophoresis and by following the transfer activity. The most pure and active fractions were dialyzed against 100 mM citrate, pH 6.0, buffer. Protein concentration was measured by the Bradford assay using bovine serum albumin as the standard.

**Mass Spectrometry**—The mass spectrometry was performed by the PT3 proteomique of the Pasteur Institute. Ion spray mass spectra were recorded on an API 365 mass spectrometer (PerkinElmer Life Sciences-Sciex, Thornhill, Canada). Samples dissolved in water/methanol/formic acid (50/50/5) were introduced at 5 $\mu$L/min with a syringe pump (Harvard Apparatus, South Natick, MA). The mass spectrometer was scanned continuously from $m/z$ 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. Data were collected on a Power Macintosh 8600/200 and processed through the Biotoolbox 2.2 software from Sciex.

**Fig. 1. Schematic representation of the salvage pathways in E. coli.**

A, purine. The enzymes of the pathway are represented by their gene names: guaB, IMP dehydrogenase; guaA, GMP synthetase; guaC, GMP reductase; gpt, guanosine phosphoribosyltransferase; hpt, hypoxanthine phosphoribosyltransferase; gsk, guanosine kinase; deoD, purine nucleoside phosphorylase; deoB, phosphopentomutase; deoC, deoxyriboaldolase. X, xanthine, G, guanine; rG, guanosine; dG, deoxyguanosine; DR-1P, deoxyribose 1-phosphate; DR-5P, deoxyribose 5-phosphate. B, pyrimidine. thyA, thymidilate synthase; tdk, thymidine kinase; deoA, thymidine phosphorylase; udp, uridine phosphorylase; upp, uracil phosphoribosyltransferase; 1dk, uridine kinase; 1dd, cytidine deaminase; codA, cytosine deaminase; U, uracil; C, cytosine; T, thymine; dU, deoxyuridine; dT, deoxythymidine; dC, deoxycytidine; rU, uridine; rC, cytidine.
N-Deoxyribosyltransferase Assay—The standard reaction mixture contained 3 mM of the deoxyribonucleoside donor and 1 mM of the base acceptor in 20 mM of citrate buffer, pH 6.0, and the appropriate number of enzyme units to hydrolyze 1 mM of the deoxyribonucleoside donor per minute. Reactions were incubated at 40°C. After 15 and 30 min, 15-μl aliquots were added to 30-μl ethanol, and the mixture was heated at 95°C for 5 min. Samples were then diluted with water and frozen before lyophilization. The products of the reactions were analyzed by high performance liquid chromatography using a reverse-phase column (100–5C18) with a flow rate of 1 ml/min and a linear gradient of 5–25% CH3CN (or 5–15% CH3CN in the case of purine exchange) in 10 mM triethylammonium acetate, pH 7.5, buffer for 20 min.

RESULTS

A Unique Genetic Selection for Cloning the Genes Coding for the Two Classes of N-Deoxyribosyltransferases: Construction of a Guanine Auxotrophic E. coli Strain Unable to Grow with Deoxyguanosine as a Source of Guanine—A functional screen allowing selection for the production of guanine was established in E. coli. The two genes of the guaBA operon code for IMP dehydrogenase and for GMP synthetase, respectively, which govern the conversion of IMP to xanthosine 5'-phosphate and to GMP. These genes were deleted along with the genes of the deoCABD operon involved in the catabolism of nucleosides. In the resulting strain, designated PAK6, GMP can only be synthesized from guanine by the product of the gpt gene coding for the guanine phosphoribosyltransferase, because the purine nucleoside phosphorylase coded by the deoD gene of the deoC-ABD operon was deleted (Fig. 1A). The PAK6 strain is auxotrophic for guanine (G) and this requirement cannot be satisfied by deoxyguanosine (dR-G); only a residual growth was observed (Fig. 2). This could be due to the xanthosine phosphorylase (coded by xapA) activity, which, like other purine nucleoside phosphorylases, is able to carry out both phosphorolysis and synthesis of purine deoxy- and ribonucleosides (22). Deoxyguanosine (dR-G) will be a source of guanine only if a N-deoxyribosyltransferase activity is expressed in strain PAK6. Because both class I and class II N-deoxyribosyltransferase catalyze the transfer of deoxyribose between two purine bases, deoxyguanosine and adenine (A) were chosen as substrates expecting the reaction, dR-G + A → dR-A + G.

**FIG. 2. Requirement of a deoxyribosyl hydrolase activity for PAK6 (guaBA::gm, deo-11) growth.** Colonies were first isolated on glucose mineral medium supplemented with chloramphenicol (25 μg/ml) and guanine (0.3 mM) and then streaked on either the same medium (A) or on glucose mineral medium supplemented with ampicillin (100 μg/ml), thymidine (0.3 mM), and guanine (0.3 mM) and then streaked on either the same medium (A) or on glucose mineral medium supplemented with ampicillin (100 μg/ml), deoxyguanosine (0.3 mM), and thymine (0.3 mM). Plates were incubated 48 h at 37°C. a, PAK6 carrying pBam3 plasmid; b, PAK6 carrying pH2 plasmid (L. helveticus ntd); c, PAK6 carrying pH4 plasmid (L. helveticus ptd); d, PAK6 carrying pLL plasmid (L. leichmannii ntd).

**FIG. 3. Requirement of a N-deoxyribosyltransferase activity for PAK6 (guaBA::gm deo-11 ΔthyAerm Δ(udp-metE) zif9::Tn10) growth.** Colonies were first isolated on glucose mineral medium supplemented with ampicillin (100 μg/ml), thymidine (0.3 mM), and guanine (0.3 mM) and then streaked on either the same medium (A) or on glucose mineral medium supplemented with ampicillin (100 μg/ml), deoxyguanosine (0.3 mM), thymine (0.3 mM). Plates were incubated 48 h at 37°C. a, PAK6 carrying pBam3 plasmid; b, PAK6 carrying pH2 plasmid (L. helveticus ntd); c, PAK6 carrying pH4 plasmid (L. helveticus ptd); d, PAK6 carrying pLL plasmid (L. leichmannii ntd).
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**Table II**

Growth of strain PAK6 carrying different plasmids on glucose mineral medium (in vivo) and enzymatic activity of the corresponding crude extracts (in vitro)

|        | A     | G     | dG    | dG + A | dC + T | dG + A | dC + A |
|--------|-------|-------|-------|--------|--------|--------|--------|
| PAK6 pBam3<sup>+</sup> | -     | +     | ±     | -      | -      | -      | -      |
| PAK6 pLL (ndt L)  | -     | +     | +     | -      | +      | +      | -      |
| PAK6 pLH2 (ndt Lh) | -     | +     | +     | -      | +      | +      | +      |
| PAK6 pLH4 (ptd Lh) | -     | +     | +     | -      | +      | +      | +      |

<sup>*</sup> PAK6, MG1655 ΔguaB:A::gm, ΔdeoCABD; ntd L, gene coding for L. leichmannii N-deoxyribosyltransferase; ntd Lh, gene coding for L. helveticus N-deoxyribosyltransferase; ptd L.h, gene coding for L. helveticus purine trans-deoxyribosylase; A, adenine; G, guanine; T, thymine; dG, deoxyguanosine; dC, deoxycytidine.

PAK6. Colonies expressing a deoxyribosyltransferase activity were selected by their ability to grow on glucose mineral medium supplemented with deoxyguanosine (dR-G) and adenine. Fig. 2 illustrates the growth differences for PAK6 transformants expressing a DRTase activity (Fig. 2, A and B, parts b, c, and d) compared with the parent (part a). To distinguish the two deoxyribosyltransferase activities, plasmid DNA from different selected colonies was extracted and used to transform the thymidine and guanine auxotrophic strain PAK26. In strain PAK26, dTMP cannot be synthesized from dUMP, because the thymidylate synthase encoded by thyA gene has been inactivated. In addition, thymine cannot be a source of thymidine, because the thymidine phosphorylase encoded by deoA and the uridine phosphorylase encoded by udp genes have been deleted (Fig. 1B) (22). Deoxyguanosine (dR-G) and thymine (T) will be sources of guanine and thymidine only if a DRTase II activity is expressed in strain PAK26 to catalyze the exchange reaction dG + T ↔ dT + G. Only colonies expressing a DRTase II activity can grow on glucose mineral medium supplemented with deoxyguanosine and thymine as sources of guanine and thymidine (Fig. 3, A and B, parts b and d). This second screening allowed the correlation of DRTase II activity with plasmid pLH2 and DRTase I with plasmid pLH4. To confirm the in vivo activities, in vitro, cell-free extracts from strains PAK6, PAK6 pLH2, and PAK6 pLH4 were prepared. As expected from the in vivo selection, extracts from strain PAK6 pLH4 could transfer the 2'-deoxyribose between guanine and adenine but not between cytosine and thymine or cytosine and adenine (Table II). This was in contrast to extracts of PAK6 pLH2 and PAK6 pLH (pLH contains the L. leichmannii ntd gene under the control of the lac promoter) clearly indicating that pLH4 codes for DRTase I. The inserts of plasmids pLH2 and pLH4 were sequenced. Plasmid pLH2 contained a 1286-bp insert. A 474-bp open reading frame (GenBank<sup>TM</sup> accession number AY064167) beginning at position 209 relative to the insertion site was identified. This open reading frame coded for a 158-amino acid polypeptide of 18,145 Da, designated NTD Lh with 83 and 81% identity with the corresponding N-deoxyribosyltransferase from L. helveticus ATCC 8018 (23) and L. leichmannii (14). NTD is probably expressed from its own promoter as predicted by Neural Network Promoter Prediction. No other significant homology was found in the data bank for the 1-kb DNA fragment following ntd.

Plasmid pLH4 contained a 1465-bp insert with two open reading frames (GenBank<sup>TM</sup> accession number AY064166). The first one was incomplete but displayed 38% identity with E. coli adenosine deaminase. In particular, the His-197, Cys-245, Asp-278, and Asp-279 residues that may participate to the catalytic site of the E. coli deaminase (24) were also conserved in the L. helveticus ADD-like sequence. The second open reading frame separated by 52 bp from the first and located in the same orientation was 501 bp long and coded for a 167-amino acids polypeptide, designated PTD (for purine trans-deoxyribosylase), with an apparent molecular mass of 18,713 Da. PTD displayed 25% identity with NTD Lh.

Alignments of the four amino acid sequences with ClustalW 1.7 (Fig. 4) shows that the active site nucleophile Glu-98 is conserved in the four sequences as well as residues Asp-72 and Asp-123, however, Asp-92 and Gln-46, involved in substrate binding, are only found in three of the four sequences. This conservation suggests a similar active site for the four proteins, although structural differences must exist because pyrimidine is not a substrate for PTD.

**Overexpression and Purification of PTD and NTD**—To further characterize the PTD and NTD enzymes, the two genes were amplified as Ndel-BamHI fragments and cloned into the pET24a, which has a T7 promoter inducible by isopropyl-1-thio-β-D-galactopyranoside. The ptd gene was expressed at a high level and purified by ammonium sulfate fractionation (Fig. 5A, lane 2) and gel filtration (Fig. 5A, lane 3) to homogeneity as judged by SDS-PAGE. By comparison, the ntd gene seemed to be expressed at a lower level. The purification was not as homogenous as for PTD, because a polypeptide of about 32 kDa was still present (Fig. 5B). Homogeneity could be obtained after a short incubation at 65 °C, which denatured the 32-kDa polypeptide without affecting the NTD activity (data not shown). Both PTD and NTD have a similar apparent molecular mass of 24 kDa, a higher value than the deduced 18-kDa molecular mass. However their molecular mass estimated by electrospray ionization mass spectrometry (18,712.91 ± 0.84 and 18,149.10 ± 1.58) was in agreement with that calculated from the sequence (18,713.18 and 18,148.57). As estimated by their elution volume during the gel filtration both proteins should be hexameric, which is in agreement with previous reports (15, 25).

**Substrate Specificity and Activity**—PTD is a strict purine-purine deoxyribosyltransferase, because no deoxyribose exchange was detected with a purine base as donor and a pyrimidine base as acceptor or with a pyrimidine base as donor and a purine or a pyrimidine base as acceptor. Table III summarizes the specific activities of PTD with the different purine couples. Specific activities were approximately equal regardless of the purine pairs, although a preference order of dT > dA > dG as deoxyribonucleoside donor was observed. The purine → purine activity of PTD was at least 20 times higher than that of NTD, if one considers the dI + A ↔ dA + I exchange reaction (82.8 units/mg versus 3 units/mg). However, deoxyinosine (dI) does not seem to be a very good substrate for NTD, because the difference of purine ↔ purine activity between NTD and PTD was significantly reduced when deoxyguanosine (dG) was used as deoxyribonucleoside donor. Furthermore, although hypoxanthine (Hx) was as good as guanine or adenine as acceptor base for PTD, it was a bad substrate for NTD whatever the deoxyribonucleoside donor (Tables III and IV). As
previously shown for purified NTD from *L. leichmannii*, pyrimidine deoxyribonucleosides and purine bases seemed to be the preferred substrates for *L. helveticus* NTD (Table IV).

**Synthesis of Purine Nucleoside Analogues**—Most purine nucleoside analogues synthesized enzymatically were prepared from *L. leichmannii* and *L. helveticus* crude extracts or partially purified enzymes (26–29).

Having DRTase I and II purified, it was possible to evaluate

![Amino acids sequences alignment of lactobacilli deoxyribosyltransferases. Sequences were aligned using the ClustalW 1.7 program.](image)

**L. helveticus Deoxyribosyltransferases**

![Purification of *L. helveticus* PTD and NTD. A, different steps of the purification of PTD. Lane 1, 10 μg of total proteins after lysis of BL21(DE3)pLysS pETLH4 cells and centrifugation. Lane 2, 10 μg of proteins after precipitation with 55% ammonium sulfate. Lane 3, 10 μg of proteins after gel filtration on Sephacryl S-200 column. B, 2.5 μg of proteins from BL21(DE3)pLysS pETLH2 cells after lysis, centrifugation ammonium precipitation, and gel filtration. Samples were separated on a 12% SDS-PAGE stained with Coomassie Blue. Molecular weight markers on the left side of each gel (Prestained SDS-PAGE standards low range from Bio-Rad) contained phosphorylase B (116 kDa), bovine serum albumin (80 kDa), ovalbumin (52.5 kDa), carbonic anhydrase (34.9 kDa), soybean trypsin inhibitor (29.9 kDa), and lysozyme (21.8 kDa).](image)

**Specific activities of the *L. helveticus* purine transdeoxyribosylase PTD**

Activities are expressed in nanomoles of deoxyribonucleoside (row 1, dl; row 2, dA) formed per min per mg of protein.

|   | dI | dG | dA |
|---|----|----|----|
| Hx | 82.8 | 54 | 61 |
| A  | 82.8 | 50 |    |
the activities of the two proteins for such analogues. 2-Aminopurine and 2,6-diaminopurine (Fig. 6) were chosen as unnatural purines; 4-amino-5-carboxamide-imidazole (AICA) and 5-amicarboxamide imidazole (ICA) (Fig. 6), because they represent simplified purines resulting from the opening of the six-membered ring and elimination of C2 and N3 (AICA) and C2 (ICA). AICA and ICA were converted to their deoxyribo derivative by using crude extracts from L. leichmannii (30).

2-Aminopurine and 2,6-diaminopurine were found to be converted to the corresponding deoxyribonucleoside at a similar rate as canonical purine bases for both PTD and NTD. AICA and ICA were poorer substrates. Their conversion to dR-AICA and dR-ICA required more enzyme units and extended incubation times. In the four transglycosylation reactions, the specific activity of PTD was higher than that of NTD (data not shown).

**DISCUSSION**

The existence of two deoxyribosyltransferases in L. helveticus and L. leichmannii was suggested for a long time and was only confirmed by the comparison of the first N-terminal amino acids of the two L. leichmannii DRTases (15). Here, we cloned the genes coding for these two types of activities by their capacity to restore the guanine auxotrophy of an E. coli strain unable to grow with deoxyguanosine as the source of guanine. This genetic selection may be applied to clone genes coding for an N-deoxyribosyltransferase or deoxyribosyl hydrolase from any organism. Furthermore, the use of a double-guanine thymidine auxotroph strain as a second screen allowed for discrimination between DRTase I and DRTase II activities and rapid determination of their substrate specificities. N-Deoxyribosyltransferase is not restricted to Lactobacilli, because they were also found in some Pediococcus, Aerococcus, Leuconostoc, and Streptococcus strains (31). Whether they contain the two types of activities remains unknown for the moment. A purine trans-N-deoxyribosyltransferase similar to DRTase I was purified from the protozoan parasite Crithidia luciliae (32, 33). Because this parasite does not synthesize purines de novo and is deficient in adenosine deaminase, it was postulated that the deoxyribosyltransferase may regulate the pool of nucleotides. A similar role could be proposed for the L. helveticus or L. Leichmannii deoxyribosyltransferases, because adenine was shown to inhibit cell growth. This might be related to adenine inhibition of the deoxyribose transfer reaction (34). If so, this would explain the existence of two DRTases with different expression levels and activities. Several lines of evidence suggest a specific role for DRTase I in the eradication of deoxynosine from the cellular nucleoside pool by converting deoxynosine to hypoxanthine, which can then be recycled in IMP by hypoxanthine phosphoribosyltransferase and to a DRTase I-deoxyribose complex that can be combined with another purine. Indeed, the specific activity of PTD was more than 20 times higher than that of NTD when deoxynosine was the donor substrate. Such a difference of activity was not observed with deoxyinosine as donor. Furthermore, hypoxanthine, compared with the other bases, is not a good substrate for NTD but is a substrate for PTD and hypoxanthine phosphoribosyltransferase. Another argument in favor of a specific role for PTD in the eradication of dI is brought by the colocalization of its gene (ptd) with the add gene (coding for adenosine deaminase) on the same DNA fragment, suggesting a link in their function. Adenosine deaminase catalyzes the deamination of adenosine and deoxyadenosine to inosine and deoxyinosine, respectively. Thus, in the lactobacilli having a requirement for bases and deoxyribonucleosides for growth, in the absence of a purine trans-deoxyribosylase, deoxynosine would not be catabolized efficiently by NTD alone (in these strains, nucleoside phosphorylase, nucleoside hydrolase, phosphopentomutase, and deoxyriboaldolase are absent).

**TABLE IV**

| Activities of the L. helveticus deoxyribosyltransferase NTD | Specific activities of the L. helveticus the N-deoxyribosyltransferase NTD |
|-------------------------------------------------------------|--------------------------------------------------------------------------|
| Activities are expressed in nanomoles of deoxyribonucleoside (row 1, dI; row 2, dA; row 3, dU; row 4, dT; row 5, dC) formed per min per mg of prot. | Activities are expressed in nanomoles of deoxyribonucleoside (row 1, dI; row 2, dA; row 3, dU; row 4, dT; row 5, dC) formed per min per mg of prot. |
|-------------------------------------------------------------|--------------------------------------------------------------------------|
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dI would then be subject to phosphorylation by a purine deoxyribonucleoside kinase. Deoxyribonucleoside kinases have not yet been characterized in L. helveticus, but they have been identified in the closely related species L. leichmannii and L. acidophilus (35, 36). These lactobacilli possess kinases for the four deoxyribonucleotides and in L. acidophilus R-26, three of the four activities are organized into two heterodimers deoxyadenosine/deoxycytidine kinase and deoxyadenosine/deoxyguanosine kinase (36). This latter enzyme could phosphorylate deoxyinosine to give dIMP, similar to the Bacillus subtilis deoxyguanosine kinase (37), which in turn would be converted to dITP through the successive action of guanylate kinase and nucleoside diphosphate kinase. Incorporation of dITP into DNA would be mutagenic and consequently detrimental for the cell. Thus, the necessity of two deoxyribosyltransferases, one with deoxynosine specificity and closely linked to the level of adenine deaminase, would favor the eradication of dI and prevent its incorporation into DNA.

The comparison of the amino acids sequences of DRTases I and II revealed a low degree of conservation. The crystal structure of L. leichmannii NDT and two ligand-bound forms of the enzyme have been determined (13). Structural and biochemical data indicate that Glu-98 is the nucleophile (12, 14) and that Gln-46, Asp-72, and Asp-92 may be involved in substrate binding. These residues, with the exception of Gln-46, are also found in the Gln-like residue of Gln-46 (replaced by a glycine residue in PTD) was proposed to make two hydrogen bonds with a pyrimidine substrate and one with a purine (13). Thus, the substitution of Gln-46 by Gly could explain why DRTase I is unable to transfer deoxyribose between a purine donor and a pyrimidine base as acceptor. Neither mutagenesis of the Gly residue to Gln nor the random mutation of the whole gene converted the DRTase I activity to that of DRTase II (data not shown). Thus, this conversion likely requires extensive structural changes, because a single amino acid change was not sufficient. However, PTD represents an alternative to NTD and to purine nucleoside phosphorylase for the enzymatic synthesis of purine nucleoside analogues, considering its substrate specificity and its activity. Crystallization of PTD with different ligands and comparison with the NDT structure should provide a more complete picture of the enzymes reaction mechanism, the residues involved in purine and pyrimidine binding, and the basis of its substrate specificity. The combination of structural studies and genetic selection should help to improve the NDT enzyme as a biocatalyst for nucleoside synthesis.

Acknowledgments—I thank P. Tailliez, R. Cotaya for L. helveticus cultures, S. Perrier for technical assistance in protein purification, O. Helyncn for high performance liquid chromatography, S. Pochet for helpful discussions, and D. M. Rowe for critical reading and correction of the manuscript.

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