Addition of Deoxyribose to Guanine and Modified DNA Bases by *Lactobacillus helveticus* *trans-N*-Deoxyribosylase

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The use of bacterial *trans-N*-deoxyribosylase was evaluated as an alternative method for deoxyribosylation in the synthesis of deoxyribonucleosides containing potentially mutagenic adducts. A crude enzyme preparation was isolated from *Lactobacillus helveticus* and compared to *Escherichia coli* purine nucleoside phosphorylase. *trans-N*-deoxyribosylase was more regioselective than purine nucleoside phosphorylase in the deoxyribosylation of Gua at the N9 atom, as compared to N7, as demonstrated by NMR analysis of the product. 5,6,7,9-Tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine was efficiently deoxyribosylated by *trans-N*-deoxyribosylase but not at all by purine nucleoside phosphorylase. Other substrates for *trans-N*-deoxyribosylase were N^2-(2-oxoethyl)Gua, pyrimido[1,2-*a*]purin-10(3*H*)-one, 1,N^2-ϵ-Gua, N^2,3-ϵ-Gua, 3,N^4-ϵ-Cyt, 1,N^6-ϵ-Ade, C^8-methylGua, and C^8-aminoGua, most of which gave the desired isomer (bond at the nitrogen corresponding to N9 in Gua) in good yield. Neither N^7-alkylpurines nor C^8-(arylamino)-substituted guanines were substrates. The approach offers a relatively convenient method of enzymatic preparation of many carcinogen−DNA adducts at the nucleoside level, for either use as standards or incorporation into oligonucleotides. *trans-N*-deoxyribosylase can also be used to remove deoxyribose from modified deoxyribonucleosides in the presence of excess Cyt.

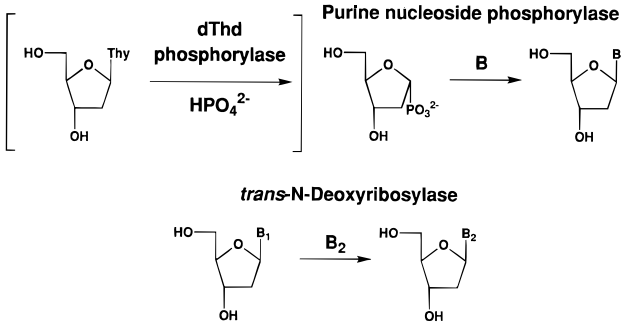
Introduction

Many carcinogens either are electrophiles or are biologically transformed to electrophiles that react with DNA bases to form covalent adducts. This process is generally considered to be important in chemical carcinogenesis (*1*−*3*). Understanding the chemistry and biology related to the roles of these modified DNA bases in mutagenesis and cancer has been challenging. DNA bases, nucleosides, and nucleotides appropriately modified with carcinogens are needed for analysis of biological samples and for biochemical and physical studies involving oligonucleotides.

Strategies for synthesis of such modified deoxyribonucleosides are often most logical when the desired chemical modifications are done on the base and then the deoxyribose group is attached. This approach usually allows for better control of the modification chemistry because the sugar hydroxyls may react with an electrophile, the nucleoside may not be soluble in certain solvents, or the glycosidic bond may be unstable to modification conditions. Chemical approaches exist for attachment of a deoxyribose moiety to a modified base (*4*). However, there are inherent problems in controlling both the regiochemistry (e.g., N7 vs N9 atom of a purine) and stereoselectivity (e.g., an S_N2 attack is needed on a sugar isomer to generate the desired linkage).

One approach to attaching deoxyribose to modified purines has been the use of the enzyme *Escherichia coli* purine nucleoside phosphorylase (EC 2.4.2.1) (Scheme 1). This enzyme has been used in a number of applications (*5*−*10*), including some in our own group (*11, 12*). However, we were totally unsuccessful in achieving deoxyribosylation of 5,6,7,9-tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine, a key intermediate in our strategy to introduce the vinyl chloride-derived DNA adduct N^2-(2-oxoethyl)Gua[1] into oligonucleotides (*12*). As an alternative, we considered the enzyme *trans-N*-deoxyribosylase (EC 2.4.2.6, nucleoside:purine (pyrimidine) deoxyribosyltransferase) (Scheme 1).

Scheme 1. Reactions Catalyzed by Purine Nucleoside Phosphorylase and *trans-N*-Deoxyribosylase (B = Base)



The activity of *trans-N*-deoxyribosylase in Lactobacillus was first reported in 1952 (*13*). The enzyme has been purified (*14, 15*) and preparations have been used in syntheses of 2-aminopurine (*16, 17*). In contrast to

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[1] Abbreviations: NOE, nuclear Overhauser effect; M_1G, pyrimido-[1,2-*a*]purin-10(3*H*)-one. We refer to N^2-(2-oxoethyl)Gua and its nucleoside using that convention for simplicity, although the predominant form appears to be the cyclized hemiaminal, 5,6,7,9-tetrahydro-7-hydroxy-9-oxoimidazo[1,2-*a*]purine (*12*).

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purine nucleoside phosphorylase, this enzyme also transfers pyrimidines, in a reversible reaction (*13*, *15*, *18*). Other deoxyribonucleosides are cosubstrates, so an auxiliary enzyme system is not required for synthetic applications (Scheme 1). Xanthine, hypoxanthine, N^1-methylhypoxanthine, uric acid, kinetin, 5-methylCyt, 8-azaAde, 6-mercaptopurine, N^6-methylaminopurine, 2-ethyl-6-hydroxypurine, 5-fluoroUrd, 2-aminopurine, and (possibly) 2,6-diaminopurine are reported substrates for the enzyme (*14*−*18*). We have examined the usefulness of this system in the formation of several DNA−carcinogen adducts, as an alternative to purine nucleoside phosphorylase. The regioselectivity of coupling has also been analyzed in many of these reactions.

Experimental Procedures

Enzymes. *E. coli* purine nucleoside phosphorylase and thymidine phosphorylase (EC 2.4.2.4) were purchased from Sigma Chemical Co. (St. Louis, MO).

trans-N-Deoxyribosylase was partially purified from *Lactobacillus helveticus* (from American Type Culture Collection, Rockville, MD) using the procedure described by Uerkvitz (*15*). Cells were grown in stationary culture on Difco Lactobacillus medium (Difco, Detroit, MI) containing 20 μM dThd, and the enzyme was fractionated through the heat and ammonium sulfate precipitation steps exactly as described (*15*). The sample was dialyzed and protein concentration was estimated using a bicinchoninic acid assay according to the supplier's instructions (Pierce Chemical Co., Rockford, IL). The preparation derived from 1 L of bacteria contained 8 mg of protein.

Chemicals. Gua, dGuo, dCyd, dThd, N^7-methylGua, N^7-methylAde, Cyt, 3,N^4-ϵ-Cyt, and 1,N^6-ϵ-Ade were purchased from Sigma and used without further purification. [4,5,8-$^{13}C_3$]-Gua was synthesized as described by Scheller *et al.* (*19*). N^2-(2-Oxoethyl)Gua was prepared from 2-fluorohypoxanthine as described elsewhere (*12*) and treated with a mixture of pyridine and $(CH_3CO)_2O$ at room temperature to form 5,6,7,9-tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine in quantitative yield: fast atom bombardment MS, *m*/*z* (assignment and relative abundance in parentheses) 236 (MH^+, 58), 176 [$MH^+ - CH_3CO_2 - H_2O$ (ϵ-Gua), 20]; ^{1}H-NMR ([$^2H_6$]Me_2SO) δ 2.03 (s, 3 H, CH_3-CO), 3.51 (d, 1 H, H-6), 3.91 (dd, 1 H, H-6), 6.91 (d, 1 H, H-7), 7.68 (s, 1 H, NH), 8.14 (s, 1 H, H-2), 12.53 (s, 1 H, NH). C^8-(*N*-Anilino)Gua, C^8-[*N*-(2-aminofluorenyl)]Gua, and C^8-[*N*-(4-aminobiphenyl)]Gua (all labeled with ^{3}H in the aromatic rings) were gifts of Dr. F. F. Kadlubar, National Center for Toxicology Research, Jefferson, AR. 1,N^2-ϵ-Gua and N^2,3-ϵ-Gua and their deoxyribonucleosides were prepared as described previously (*12*, *20*). C^8-MethylGua was prepared by acid hydrolysis (70 °C, 1 N aq HCl, 1 h) of C^8-methylGuo (*21*). The product precipitated on cooling to ambient temperature and was recovered by centrifugation and washed with H_2O; analytical HPLC indicated that the recovered base was homogeneous: fast atom bombardment MS, *m*/*z* 166 (MH^+, 100). The same procedure was used to prepare C^8-aminoGua from C^8-aminoGuo (Sigma): UV (H_2O, pH 7) λ_{max} 291 nm (*22*); fast atom bombardment MS, *m*/*z* 167 (MH^+, 100).

Instrumental Analysis. HPLC analyses and preparative work were done with reversed-phase octadecylsilane columns (Phenomenex Partisil 5 ODS (3), 4.6 × 250 mm, 5 μm, Phenomenex, Torrance, CA, or—for larger samples—10 × 250 mm Beckman Ultrasphere, 5 μm, Beckman, San Ramon, CA) connected to a Spectra-Physics 8700 pumping system (Thermo-Separation Products, Piscataway, NJ), with the effluent passing through a Hewlett Packard 1040A diode array detector (Hewlett Packard, Palo Alto, CA). The separations used here all involved increasing gradients of CH_3OH in 50 mM NH_4HCO_2 (pH 5.0), usually 0−50% CH_3OH over 25 min, with a flow rate of 1.0 mL min^{-1} (2.5 mL min^{-1} for preparative work) and monitoring absorbance at 254 nm or the λ_{max} of the product. ^{1}H NMR spectra were recorded in [$^2H_6$]Me_2SO or H_2O/^2H_2O using a Bruker AM-400 spectrometer (Bruker, Billerica, MA) in the Vanderbilt facility. UV spectra (other than HPLC diode array) were recorded with a modified OLIS/Cary 14 instrument (On-Line Instrument Systems, Bogart, GA). Mass spectra were collected using a Kratos Concept II HH instrument (Kratos, Manchester, U.K.) with fast atom bombardment ionization and a mixture of glycerol, Me_2SO, and 3-nitrobenzyl alcohol as the matrix or with an electrospray instrument (Finnigan TSQ 7000, Finnigan, Sunnyvale, CA).

Assays. Incubations with purine nucleoside phosphorylase (95 μg of protein mL^{-1}) were done as described elsewhere, in the presence of thymidine phosphorylase (*10*).

The general procedure used for deoxyribosylation of bases involved a mixture of 5 or 25 μg of partially purified *trans-N*-deoxyribosylase protein (*vide supra*) and 5.3 mM dCyd (as the deoxyribose donor) in a total volume of 1.0 mL of 0.1−0.5 M potassium 2-(*N*-morpholino)ethanesulfonate buffer (pH 6.0) and containing 0.3−0.5 mM Gua or a modified base, dissolved either in 50−100 μL of 0.1−1 N HCl or Me_2SO. Incubations proceeded for 3 or 15 h (under Ar), and products were analyzed by HPLC. In some cases the pH was adjusted or the scale of the reaction was increased (for preparative work).

The synthesis of N^2-(2-oxoethyl)dGuo on a semipreparative scale is presented as an example. N^2-(2-Oxoethyl)Gua (10 mg, 52 μmol) (*12*) was dissolved in 0.5 mL of 0.1 N HCl and diluted in 10 mL of 0.5 M potassium 2-(*N*-morpholino)ethanesulfonate buffer (pH 6.0) containing 180 mg (790 μmol) of dCyd. The mixture was incubated with 250 μg of the *trans-N*-deoxyribosylase enzyme preparation for 15 h at 37 °C under an Ar atmosphere (to prevent bacterial growth). The mixture was filtered through a 0.22 μm filter, and aliquots were injected onto a semipreparative HPLC column as described above. The product, eluting at t_R 14.1 min (both N9 and N7 addition products), was collected and the salts were removed by repeated lyophilization to yield 15.3 mg of N^2-(2-oxoethyl)dGuo [cyclized form (*12*)]: 95% yield, ratio of N9:N7 products 95:5 as determined by ^{1}H NMR; UV λ_{max} 280 nm (*12*); ^{1}H NMR (N9 coupled product) (^2H_2O) δ 2.46 (m, 1 H, H-2′$_a$), 2.70 (m, H-2′$_b$), 3.74 (m, 3 H, H-5′$_a$, H-5′$_b$, H-6$_a$), 4.08 (m, 2 H, H-4′, H-6$_b$), 4.56 (m, 1 H, H-3′), 6.19 (t, 2 H, H-1′, H-7), 7.91 (s, 1 H, H-2); electrospray mass spectrometry, *m*/*z* 310 (MH^+, 100), 194 (MH^+ − deoxyribose, 26).

Results and Discussion

Preparation of *trans-N*-Deoxyribosylase. *L. helveticus* cells were grown in the presence of dThd, and *trans-N*-deoxyribosylase was partially purified as described (*15*). In our experience, the enzyme activity is stable for at least 6 months at 4 °C. The specific activity of the preparation we made, using the heat and ammonium sulfate precipitation steps, was 6.7 μmol of dGuo formed h^{-1} (mg of protein)$^{-1}$, which compared favorably with an activity of 1.0 (same units) prepared by an alternate streptomycin sulfate precipitation method (*17*) and kindly provided by Dr. L. C. Sowers, City of Hope Medical Center, Duarte, CA. *trans-N*-Deoxyribosylase can be further purified by DEAE chromatography and crystallization (*15*); however, we have not observed any reactions with the bases or nucleosides that would interfere with the use of the crude enzyme preparation for most routine preparative work (except possibly in the case of 1,N^6-ϵ-Ade, *vide infra*).

In studies on deoxyribosylation of the Gua, the extent of nucleoside formation in a 3 h period was linear up to 7 μg of protein mL^{-1} (results not shown). We saw little difference in rates over the pH range of 6−8, and the addition of 5% Me_2SO (v/v) to the reaction did not change the rate. Our findings on the pH dependence are more in line with those of Kanda and Takag (*18*) than Beck and Levin (*14*), who reported a much sharper profile.

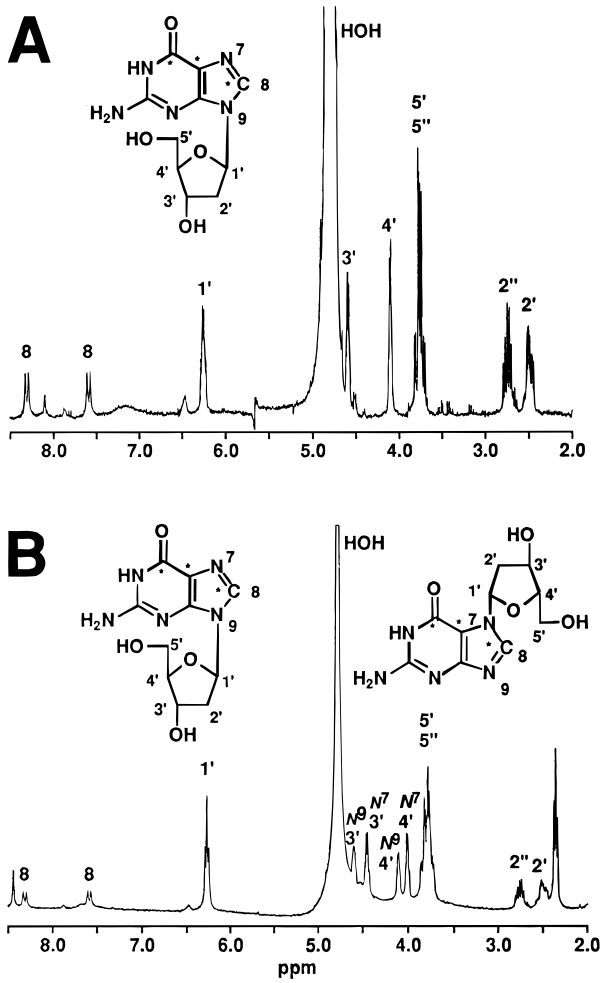

Figure 1. ^{1}H NMR spectra of dGuo prepared from [4,5,8-$^{13}C_3$]-Gua by (A) *trans-N*-deoxyribosylase and (B) purine nucleoside phosphorylase. Spectra were recorded in $^2H_2O/H_2O$. In part B, the assignments of the 3′ and 4′ protons are indicated for the two forms with deoxyribose attached at the N7 and N9 atoms. Asterisks (*) denote ^{13}C atoms. The "prime" and "double prime" denote the anomeric hydrogens at the 2′ and 5′ positions of the deoxyribose.

Deoxyribosylation of Gua. In the course of preparing [4,5,8-$^{13}C_3$]dGuo by deoxyribosylation of the base with purine nucleoside phosphorylase, we found that a significant fraction of the product contained the deoxyribose moiety attached to the Gua N7 atom instead of the N9 atom, a problem noted previously in the synthesis of deoxyribosylpyrimido[1,2-*a*]purin-10(3*H*)-one (M_1G) with this enzyme (*10*). The two products can be separated by HPLC using a shallow gradient, after initial HPLC fractionation with a sharper gradient, but the need for the second step limits the usefulness of the procedure.

The crude dGuo peak was collected from the initial HPLC from reactions done with either *trans-N*-deoxyribosylase or purine nucleoside phosphorylase and analyzed by ^{1}H NMR (Figure 1). In the case of the product formed by *trans-N*-deoxyribosylase (Figure 1A), the spectrum is consistent with the presence of only dGuo. The spectrum of the product obtained with purine nucleoside phosphorylase (Figure 1B) contains ~40% of the isomer with the deoxyribose attached at the N7 atom, as best demonstrated by the shifts of the 3′ and 4′ sugar protons (*23*). (In both cases the H-2 proton is split by the geminal ^{13}C.) The yield of total "dGuo" (N9 and N7 linkage) was essentially quantitative with both enzymes under these conditions.

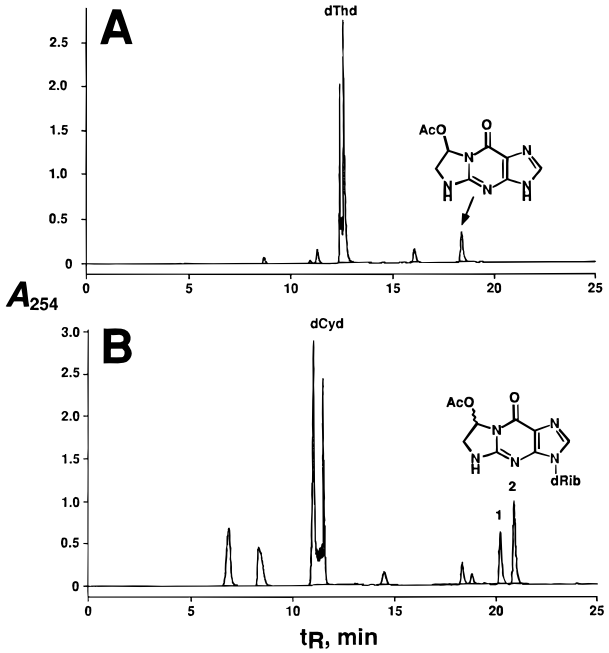

Figure 2. Deoxyribosylation of 5,6,7,9-tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine (structure shown) using (A) purine nucleoside phosphorylase and (B) *trans-N*-deoxyribosylase. The reactions were done for 18 h using either 93 μg (A) or 50 μg (B) (protein, mL^{-1}) of the designated enzyme fraction, and the products were analyzed by HPLC. The diastereomeric products **1** and **2** are denoted in part B. (The split peaks for dThd and dCyd are due to an overload of absorbance for the detector.)

Deoxyribosylation of 5,6,7,9-Tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine. In the course of work with N^2-(2-oxoethyl)Gua, a DNA adduct formed from reaction of dGuo or DNA with 2-chlorooxirane (*12*), we found that purine nucleoside phosphorylase could catalyze the deoxyribosylation of N^2-(2-oxoethyl)Gua but not the *O*-acetyl derivative, 5,6,7,9-tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine. Other lots of the enzyme, a preparation of recombinant enzyme (provided by Burroughs-Wellcome through Prof. L. J. Marnett of this department), and calf spleen purine nucleoside phosphorylase (Sigma) were all unable to catalyze this reaction. However, *trans-N*-deoxyribosylase efficiently converted the base to a nucleoside (Figure 2).

Two peaks of equal size were detected as products. Both had nearly identical UV, mass, and ^{1}H NMR spectra, and they do not appear to be positional isomers. The carbon (C7) attached to the acetoxy group is chiral, and the addition of chiral deoxyribose generates diastereomers. In support of this view, the two products had opposite CD spectra (data not presented). The CD spectra of both disappeared upon treatment with 0.1 N NaOH at ambient temperature, consistent with the view that after hydrolysis of the acetoxy group epimerization occurred at the C7 atom of the 5,6,7,9-tetrahydro-7-hydroxy-9-oxoimidazo[1,2-*a*]purine through opening to N^2-(2-oxoethyl)Gua and reclosing of the ring.

Deoxyribosylation of 1,N^2-ϵ-Gua by *trans-N*-Deoxyribosylase. The above experiments with Gua and 5,6,7,9-tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine indicated considerable regioselectivity in deoxyribosylation of bases, at least Gua derivatives, by *trans-N*-deoxyribosylase. 1,N^2-ϵ-Gua was examined as a substrate. The reaction proceeded to 86% total deoxyribosylation under the conditions used (25 μg of protein mL^{-1}, overnight at 37 °C). HPLC analysis indicated the presence of a minor

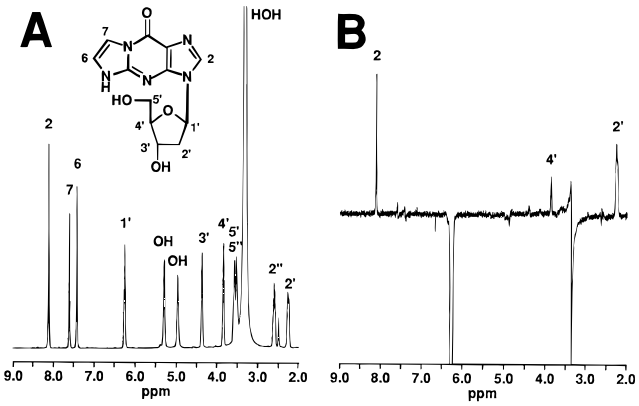

Figure 3. ^{1}H NMR spectra of 1,N^2-ϵ-dGuo formed by *trans*-*N*-deoxyribosylase. (A) 1-Dimensional spectrum ($^2H_2O/H_2O$ mixture). (B) NOE difference spectrum. Irradiation was at H-1′. The "prime" and "double prime" denote the anomeric hydrogens at the 2′ and 5′ positions of the deoxyribose.

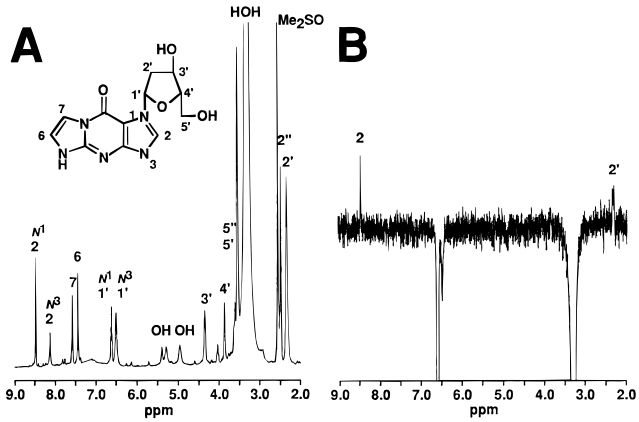

Figure 4. ^{1}H NMR of the minor product of the deoxyribosylation of 1,N^2-ϵ-Gua with *trans*-*N*-deoxyribosylase. The product was separated by HPLC and eluted immediately following 1,N^2-ϵ-dGuo. The structure is consistent with coupling of the deoxyribose to the N7 atom of 1,N^2-ϵ-Gua. (A) 1-Dimensional spectrum ($^2H_2O/H_2O/Me_2SO$ mixture). (B) NOE difference spectrum, with irradiation at H-1′. The "prime" and "double prime" denote the anomeric hydrogens at the 2′ and 5′ positions of the deoxyribose.

component eluting just after 1,N^2-ϵ-dGuo. The major peak showed the expected UV spectra diagnostic of 1,N^2-ϵ-(d)Guo, including the strong shift in alkali (*24*). NMR and nuclear Overhauser effect (NOE) analysis indicated proximity of the H-1′ proton to H-2′ and H-2, as expected (Figure 3).

We considered the possibility that coupling of the deoxyribose could have occurred at either the N1 or N5 atom (corresponding to N7 and N2 of Gua, respectively). The minor product was separated and analyzed by NMR spectroscopy (Figure 4). The spectrum indicated some 1,N^2-ϵ-dGuo as a contaminant, but the major component had additional signals at δ 6.6 and 8.5. When the 1′ proton was irradiated, NOE changes were seen in the signals assigned as H-2 and H-2′. Thus, the structure of this compound involves linkage of the deoxyribose to the N1 atom of 1,N^2-ϵ-Gua. Linkage at the N5 atom is not an option, because the NOE would have been expected at H-6 instead of H-2.

Deoxyribosylation of Other Bases by *trans*-*N*-Deoxyribosylase. Several bases were tested as substrates for *trans*-*N*-deoxyribosylase (Table 1). The products were either available as standards, have been characterized above, or else were analyzed by UV, ^{1}H NMR, and mass spectrometry. The apparent fraction linked at the N9 atom instead of N7 (using the numbering system for Gua) was estimated from ^{1}H NMR measurements, using the chemical shifts of the sugar protons as a guide (e.g., Figures 1 and 4). Different enzyme concentrations and reaction times were used.

Table 1. Deoxyribosylation of Bases with *trans*-*N*-Deoxyribosylase

base	combined yield (total N9 and N7 coupling)[a] 5 μg of protein 3 h	5 μg of protein 15 h	25 μg of protein, 15 h	ratio coupling at N9 and N7 atoms[a]
Gua			>95	100:0
N^2-(2-oxoethyl)Gua	36	88	>95	95:5
5,6,7,9-tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine			90	100:0
M_1G	11	58	97	85:15
1,N^2-ϵ-Gua	15	64	86	86:14
N^2,3-ϵ-Gua (pH 6.0)	1	5	14	50:50
N^2,3-ϵ-Gua (pH 8.0)	2	16	49	92:8
3,N^4-ϵ-Cyt[b]	12	66	79[b]	*c*
1,N^6-ϵ-Ade	23	68	94[d]	*c*
C^8-methylGua	40	85	91	*c*
C^8-aminoGua			58[e]	*c*
C^8-(*N*-anilino)Gua	<1	<1	<1[f]	
C^8-[*N*-(4-aminobiphenyl)]-Gua	<1	<1	<1[f]	
C^8-[*N*-(2-aminofluorenyl)]-Gua	<1	<1	<1[f]	
N^7-methylGua	<1	<1	<1[f]	
N^7-methylAde	<1	<1	<1[f]	

[a] The N7/N9 nomenclature indicates the atoms in the basic purine nucleus. In some derivatives the numbering system changes (e.g., ϵ-Gua, 3,N^4-ϵ-Cyt). [b] 3,N^4-ϵ-dCyd: MS (fast atom bombardment), *m*/*z* 274 (M + Na, 28), 252 (MH^+, 54), 223 (M − 28, 100). [c] A single HPLC peak was seen, but the regioselectivity was not examined by NMR. [d] The product 1,N^6-ϵ-dAdo had the correct retention time as an authentic sample (Sigma), and its identity was further verified by its characteristic UV (λ_{max} 275, 266 nm at pH 7) and fluorescence spectra (emission maximum at 418 nm with excitation at 225 nm) (*25*). [e] UV λ_{max} 288, 255 nm (ratio 0.6, pH 5) (*22*). [f] No new peaks were found (with the approximate expected t_R values); further, none of the characteristic Cyt was formed from dCyd in these reactions.

The reactions with N^2-(2-oxoethyl)Gua and M_1G proceeded in high yield and high, but not complete, regioselectivity.

The reaction with N^2,3-ϵ-Gua did not work very well at pH 6.0 but was considerably better at pH 8.0, in terms of total yield and apparent regiochemistry. This result can be rationalized by the instability of the glycosidic bond of N^2,3-ϵ-dGuo at acid pH (*26*).[2]

trans-*N*-Deoxyribosylase also transfers pyrimidines (*15*), and 3,N^4-ϵ-Cyt was a substrate.

1,N^6-ϵ-Ade was a good substrate, although an unidentified peak, of unknown origin, eluted after the product 1,N^6-ϵ-dAdo and it is not clear if this is a degradation product. Neither the UV nor the fluorescence spectra suggested any relationship to 1,N^6-ϵ-dAdo.

C^8-MethylGua was deoxyribosylated in high yield, with no extra HPLC peaks. However, the C^8-Gua arylamine adducts were not utilized by the enzyme. This could be due to steric bulk, even with the anilino derivative, or

[2] The difference between the N9:N7 coupling ratios of 86:14 for 1,N^2-ϵ-dGuo and 92:8 for N^2,3-ϵ-dGuo (pH 8.0) is not considered to be very great. Steric hindrance is not the only determinant of the glycosydic instability of N^2,3-ϵ-dGuo. The H-5 proton does show an NOE with the H-1′ proton (*12*), but space-filling models indicate that the sugar is easily accommodated, and H-1′ proximity to the H-5 atom is consistent with an *anti* conformation. Also, the glycosydic stability of N^2,3-ϵ-dGuo is influenced by pH, 5′-phosphorylation, and 2′-hydroxylation (ribose substitution) (*26*).

due to electrostatic effects. In order to consider the possibility of an electronic effect, we examined C^8-aminoGua, which has been reported to be deoxyribosylated by mammalian purine nucleoside phosphorylase (*22*). This base was also deoxyribosylated by *trans-N*-deoxyribosylase (58% yield, Table 1). Apparently the bulk of even C^8-(*N*-anilino)Gua is a problem for purine nucleoside phosphorylases (*10*) and *trans-N*-deoxyribosylase.

Neither N^7-methylGua nor N^7-methylAde was a substrate. The addition of a sugar at the N9 atom requires an electronically different transition state, since the product bears a positive charge.

Use of *trans-N*-Deoxyribosylase in Removal of Deoxyribose. In all of the studies thus far, the object has been to add deoxyribose to a modified base. The reaction of *trans-N*-deoxyribosylase is one in which an equilibrium is reached between two bases (Scheme 1), and we considered the possibility that there might be situations in which it would be desirable to use an enzymatic reaction in removing the deoxyribose to generate a base, without the use of acid or heat. The deoxyribosylation of C^8-methyl dGuo was considered as an example. The general reaction conditions were used (i.e., pH 6.0, 0.5 mM C^8-methyl dGuo, and a 10-fold molar excess of Cyt). In the presence of 5 μg of *trans-N*-deoxyribosylase mL^{-1}, yields of C^8-methyl Gua were 26% and 86% after 3 and 20 h, respectively, at 37 °C. With 25 μg of protein mL^{-1}, the yield was 92% after 20 h.

Conclusions. *L. helveticus trans-N*-deoxyribosylase is a useful enzyme for attaching deoxyribose to normal and modified purines and pyrimidines. The bacterial preparation is relatively easy to obtain and is stable. Comparisons of purines examined previously with purine nucleoside phosphorylase indicate comparable yields and regioselectivity (*10*). In some cases (e.g., Gua) regioselectivity appears better (Figure 1). The lack of regioselectivity may be a thermodynamic rather than a kinetic factor, which could be exacerbated in efforts to increase yields. A dramatic example of the broader specificity of *trans-N*-deoxyribosylase was seen in the case of the deoxyribosylation of 5,6,7,9-tetrahydro-7-acetoxy-9-oxoimidazo[1,2-*a*]purine, a requisite intermediate in the synthesis of oligonucleotides modified with N^2-(2-oxoethyl)Gua.

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