The influence of various parameters on triple-helix formation was examined. Addition of polyamines, such as spermine, at constant NaCl concentration, stabilized the triple helix as previously reported.[3,4] An increase in sodium chloride concentration, at constant spermine concentration, disfavored complex formation as shown in previous reports.[3,5] A pH decrease favored triplex formation as expected due to the requirement for cytosine protonation to form two Hoogsteen hydrogen bonds with a G·C Watson–Crick base pair (results not shown).

The present study shows that it is possible to form a stable complex on a single-stranded nucleic acid by an oligonucleotide capable of forming both Watson-Crick and Hoogsteen hydrogen bonds with a homopurine sequence. Most of the hydrogen-bonding sites on the purine bases of the target sequence are involved in complex formation: four hydrogen bonds are formed with A and five with G (Figure 1B). In addition we have shown that an intercalating agent attached to the 5'-end of the dimeric oligonucleotide strongly stabilizes the complex when the oligonucleotide is chosen such that the Watson–Crick sequence extends over a few base pairs (two in our study) outside the triple-helix-forming region. Formation of a triple-stranded structure on a single-stranded nucleic acid such as messenger RNA or viral RNA or DNA might prove more efficient to arrest translation, reverse transcription, or replication than double-helix formation.

(4) Moser, H.; Dervan, P. B. *Science* **1987**, *238*, 645–650.
(5) Maher, L. J.; Dervan, P. B.; Wold, B. J. *Biochemistry* **1990**, *29*, 8820–8826.
(6) Durand, M.; Chevrier, M.; Chassignol, M.; Thuong, N. T.; Maurizot, J. C. *Nucleic Acids Res.* **1990**, *18*, 6353–6359.

Stereochemical and Mechanistic Studies of CDP-D-glucose Oxidoreductase Isolated from *Yersinia pseudotuberculosis*

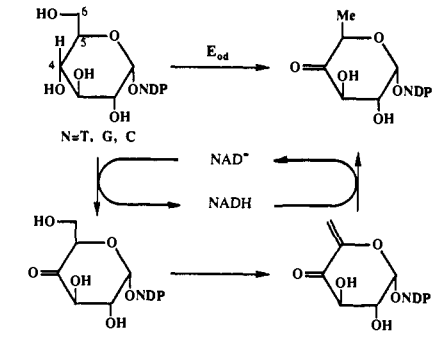
Raymond N. Russell and Hung-wen Liu*

Department of Chemistry, University of Minnesota
Minneapolis, Minnesota 55455

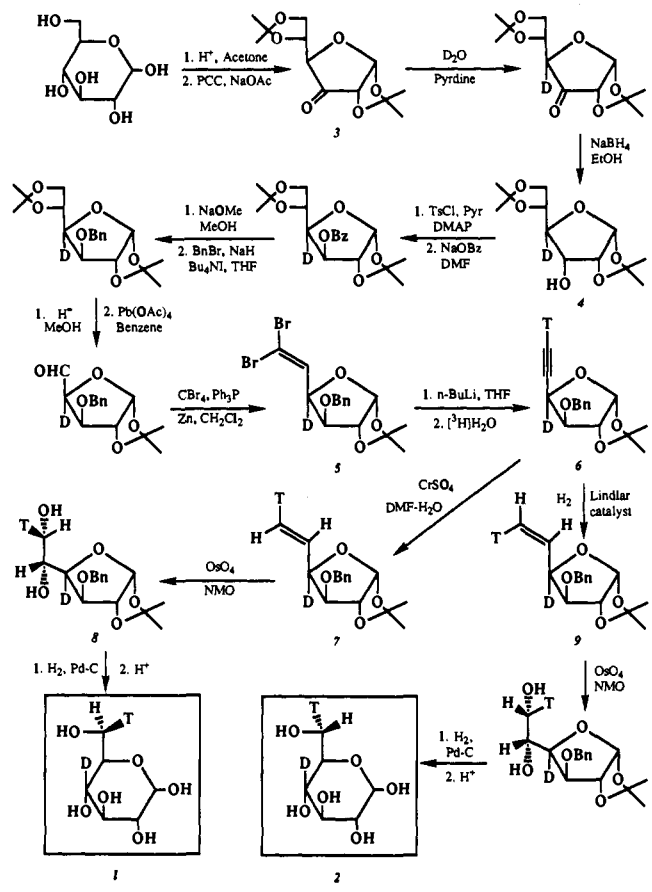
Received May 20, 1991

The great majority of deoxy sugars are 6-deoxyhexoses, which are commonly encountered as components of bacterial antigens and are also found widely in plant and animal tissues.[1] Studies of several naturally occurring deoxy sugars have shown that the first step unique to their formation involves the conversion of a nucleoside diphosphohexose to the corresponding 4-keto-6-deoxyhexose mediated by an oxidoreductase.[2] As depicted in Scheme I, studies of TDP-D-glucose[2] and GDP-D-mannose oxidoreductase[3] established that the reaction proceeds with an oxidation at C-4, followed by a dehydration between C-5 and C-6 and then a reduction at C-6.[4] An active-site-bound NAD^+ in these enzymes serves as a hydride carrier which mediates the internal hydrogen transfer from C-4 to C-6.[2] The stereochemistry of the displacement at C-6 of this intramolecular oxidation–reduction has been shown to occur with inversion.[5] However, study of the biosynthesis of 3,6-dideoxyhexoses in *Pasturella pseudotuberculosis*[6] led to the isolation of a CDP-D-glucose oxidoreductase which is readily resolved from its cofactor by simple purification steps.[6] The absolute requirement of NAD^+ for activity makes this enzyme fundamentally distinct from most of its counterparts[7] and thus throws into doubt whether its catalysis still follows the mechanism shown in Scheme I. As part of our efforts to study the biosynthesis of 3,6-dideoxyhexoses,[8] we have recently

Scheme I



Scheme II



(1) (a) Lüderitz, O.; Staub, A. M.; Westphal, O. *Bacteriol. Rev.* **1966**, *30*, 192. (b) Hanessian, S. *Adv. Carbohydr. Chem. Biochem.* **1966**, *21*, 143. (c) Williams, N. R.; Wander, J. D. In *The Carbohydrates: Chemistry and Biochemistry*; Pigman, W., Horton, D., Eds.; Academic Press: New York, 1980; Vol. 1B, p 761.
(2) (a) Glaser, L.; Zarkowsky, H. In *The Enzymes*; Boyer, P. D., Ed.; Academic Press: New York, 1972; Vol. 5, p 465. (b) Gabriel, O. In *Carbohydrates in Solution*; Gould, R., Ed.; Advances in Chemistry 117; American Chemical Society: Washington, DC, 1973; p 387. (c) Gabriel, O.; VanLenten, L. In *Biochemistry of Carbohydrates II*; University Park Press: Baltimore, 1978; Vol. 16, p 1–36. (d) Frey, P. A. In *Pyridine Nucleotide Coenzyme, Chemical, Biochemical, and Medical Aspects*; Dolphin, D., Poulson, R., Avramovic, O., Eds.; Wiley-Interscience: New York, 1987; Part B, p 461.
(3) (a) Liao, T-h.; Barber, G. A. *Biochim. Biophys. Acta* **1972**, *276*, 85. (b) Broschat, K. O.; Chang, S.; Serif, G. *Eur. J. Biochem.* **1985**, *153*, 397.
(4) Exemplified in Scheme I is the reaction mechanism of a generic nucleoside diphosphate-D-glucose oxidoreductase. NDP represents the nucleoside diphosphate group.
(5) (a) Snipes, C.; Brillinger, G.-U.; Sellers, L.; Mascaro, L.; Floss, H. G. *J. Biol. Chem.* **1977**, *252*, 8113. (b) Oths, P. J.; Mayer, R. M.; Floss, H. G. *Carbohydr. Res.* **1990**, *198*, 91.
(6) (a) Gonzalez-Porque, P.; Strominger, J. L. *J. Biol. Chem.* **1972**, *247*, 6748. (b) Gonzalez-Porque, P. In *Vitamin B_6 Pyridoxal Phosphate, Chemical, Biochemical, and Medical Aspects*; Dolphin, D., Poulson, R., Avramovic, O., Eds.; Wiley-Interscience: New York, 1986; Part B, p 392 and references cited therein.
(7) The other exception of this class enzyme is a TDP-D-glucose oxidoreductase isolated from *Saccharopolyspora erythraea*, which also requires added NAD^+ for activity (Vara, J. A.; Hutchinson, C. R. *J. Biol. Chem.* **1988**, *263*, 14992).

0002-7863/91/1513-7777$02.50/0

purified a CDP-D-glucose oxidoreductase from *Yersinia pseudotuberculosis*, which is also NAD^+ dependent.[9] In view of the central position of this enzyme as a branch point in 3,6-dideoxyhexose biosynthesis and its unusually weak binding of the nicotinamide cofactor, we have carried out a stereochemical analysis of this enzymatic process aimed at elucidating its reaction course in detail. Reported in this paper are the results of this analysis and their implication on this enzyme's mechanism.

Crucial to this study is the availability of the labeled substrates (6*S*)- and (6*R*)-CDP-[4-^{2}H,6-^{3}H]-D-glucose. It is anticipated that the replacement of the hydroxyl group at C-6 by the deuterium migrating from C-4 is intramolecular and stereospecific; therefore, the resulting product will have hydrogen, deuterium, and tritium in a chiral arrangement at C-6 that can be analyzed by the method of Cornforth, Arigoni, and co-workers.[10] The requisite substrates were obtained from the corresponding chirally labeled precursors **1** and **2**, which were synthesized as outlined in Scheme II.[11] The deuterium at C-4 was introduced by repetitive incubation of **3** in a pyridine/D_2O (5:1) solution followed by evaporation.[12] The deuterium content estimated by NMR of the reduced product **4** was greater than 95%. Stereospecific incorporation of tritium labeling into the C-5 hydroxymethyl group was effected according to a method developed by Kakinuma with minor modification.[13] The common precursor, a 5,6-yne derivative **6**, was obtained from the dibromo olefin **5** upon treatment with *n*-butyllithium in THF at −78 °C followed by quenching with $[^3H]H_2O$ (30 mCi, 0.3 mL). Transformation of **6** with a specific radioactivity of 0.46 Ci/mol to the *E* olefin **7** was achieved by reduction using chromous sulfate in aqueous DMF.[14] Dihydroxylation with a catalytic amount of OsO_4 in the presence of *N*-methylmorpholine *N*-oxide (NMO) afforded the desired *cis*-diol **8** in 72% yield.[15] The protected glucose was converted to free (6*S*)-[4-^{2}H,6-^{3}H]-D-glucose (**1**) by hydrogenolysis (10% Pd/C) and subsequent acid hydrolysis. The 6*R*-labeled glucose was prepared analogously from **6** via the *Z* olefin **9**, which was produced by hydrogenation over Lindlar catalyst poisoned with quinoline. Conversion of the 6*S*- and 6*R*-labeled glucose to the corresponding CDP derivatives was accomplished by a sequence commonly used to make nucleoside diphosphohexoses.[16] The specific radioactivities of the final products were 89 and 74 mCi/mol, respectively.[17]

The (6*S*)- and (6*R*)-CDP-[4-^{2}H,6-^{3}H]-D-glucose were each mixed with CDP-[*U*-^{14}C]-D-glucose, diluted with excess unlabeled CDP-glucose (1:9), and then incubated with homogeneous CDP-D-glucose oxidoreductase in the presence of NAD^+. The CDP-4-keto-6-deoxy-D-glucose products isolated by paper chromatography (EtOH/BuOH/H_2O, 5:5:2) were subjected to Kuhn-Roth oxidation. The nascent acetic acid samples were formed in radiochemical yields of 52-54%, and their chiralities were determined by the method of chiral methyl analysis.[10,18] An *F* value of 71 corresponding to a 72% ee *R* configuration and an *F* value of 30 corresponding to a 69% ee *S* configuration were obtained for the two acetates derived from the 6*S*- and 6*R*-labeled glucose, respectively.[19] These results unequivocally show that the hydrogen migration from C-4 to C-6 does proceed intramolecularly and the displacement of the hydroxyl group at C-6 is stereospecific and occurs with inversion.[20] Notwithstanding the high enantiomeric purity of the substrates, the moderate ee found for the acetic acid samples may directly reflect the weak binding of the cofactor to this enzyme. Nevertheless, our general conclusions completely parallel those found for all of the other sugar oxidoreductases characterized so far[5,21] and, thus, suggest that this class of enzymes, regardless of their source, evolved from a common progenitor whose stereochemical course has persevered throughout the enzyme's subsequent diversification.

Acknowledgment. This work is supported by National Institutes of Health Grant GM 35906. H.-w.L. also thanks the National Institutes of Health for a Research Career Development Award (GM 00559).

(8) (a) Han, O.; Liu, H.-w. *J. Am. Chem. Soc.* **1988**, *110*, 7893. (b) Han, O.; Miller, V. P.; Liu, H.-w. *J. Biol. Chem.* **1990**, *265*, 8033. (c) Shih, Y.; Yang, D.-y.; Weigel, T. M.; Liu, H.-w. *J. Am. Chem. Soc.* **1990**, *112*, 9652.

(9) Although *Pasturella pseudotuberculosis* has now been reclassified as *Yersinia pseudotuberculosis*, comparison of the enzymes isolated from these two strains suggested that the bacterial stain used in Strominger's studies was different from that used in our current research.

(10) (a) Cornforth, J. W.; Redmond, J. W.; Eggerer, H.; Buckel, W.; Gutschow, C. *Eur. J. Biochem.* **1970**, *14*, 1-13. (b) Luthy, J.; Rétey, J.; Arigoni, D. *Nature (London)* **1969**, *221*, 1213. (c) Floss, H. G.; Tsai, M.-D. *Adv. Enzymol.* **1979**, *50*, 243.

(11) Compounds **1** and **2** had been prepared heretofore by methods based on complex enzymatic manipulations (see ref 5a and references cited therein).

(12) Ritchie, R. G. S.; Cyr, N.; Korsch, B.; Koch, H. J.; Perlin, A. S. *Can. J. Chem.* **1975**, *53*, 1424.

(13) (a) Kakinuma, K. *Tetrahedron* **1984**, *40*, 2089. (b) Kakinuma, K. *Tetrahedron Lett.* **1971**, 4413.

(14) Castro, C. E.; Stephens, R. D. *J. Am. Chem. Soc.* **1964**, *86*, 4358.

(15) The minor 5*S*,6*R* isomer was readily removed by flash chromatography on silica gel (30% acetone/hexane). The stereochemical assignments of this compound and the following products were made by comparing the NMR spectra of these samples with those of the respective unlabeled and/or deuterated standards that were prepared in parallel.

(16) This sequence involved the treatment of each of the labeled free glucose with hexokinase, phosphoglucomutase, glucose 1,6-diphosphate, and inorganic pyrophosphatase to furnish glucose 1-phosphate, which was then incubated with CTP and CDP-D-glucose pyrophosphorylase to form the requisite substrates, (6*S*)- and (6*R*)-CDP-[4-^{2}H,6-^{3}H]-D-glucose in an 18-20% overall yield. The pyrophosphorylase used in these incubations was partially purified from the same strain of *Y. pseudotuberculosis*.

(17) Since cold carrier was added to increase the sample mass at the later stage of the synthesis, the specific radioactivities of the final products were lower than that of **6**. However, every tritiated molecule still carries a deuterium at C-4.

(18) The standard (*S*)-[2-^{2}H,^{3}H]acetate was synthesized chemically from *pro-S*-tritium-labeled glycine (Kajiwara, M.; Lee, S.-F.; Scott, A. I.; Akhtar, M.; Jones, C. R.; Jordan, P. M. *J. Chem. Soc., Chem. Commun.* **1978**, 967), which was generated by the incubation of [2-^{3}H]glycine with alanine aminotransferase (Besmer, P. Ph.D. Dissertation No. 4435, ETH, Zürich, 1970). An identical sequence using deuterated buffer afforded the (*R*)-[2-^{2}H,^{3}H]-acetate. Chiral methyl analysis performed on these standards gave *F* values of 30 and 74 for the (*S*)- and (*R*)-acetates, respectively.

(19) Malate synthetase was purified (Durchschlag, H.; Biedermann, G.; Eggerer, H. *Eur. J. Biochem.* **1981**, *114*, 255) using freshly pressed bakers' yeast kindly donated by Pillsbury Company (Minneapolis, MN).

(20) In view of the large dilution of the labeled precursor by the unlabeled species, the methyl group of the resulting acetic acid will be chiral only if the migration of deuterium from C-4 to C-6 is strictly intramolecular and stereospecific.

(21) (a) Snipes, C.; Chang, C.-J.; Floss, H. G. *J. Am. Chem. Soc.* **1979**, *101*, 701. (b) Otsuka, H.; Mascaretti, O. A.; Hurley, L. H.; Floss, H. G. *J. Am. Chem. Soc.* **1980**, *102*, 6817. (c) Lee, J.-J.; Lee, J. P.; Keller, P. J.; Cottrell, C. E.; Chang, C.-J.; Zahner, H.; Floss, H. G. *J. Antibiot.* **1986**, *39*, 1123.

A New Catalytic Reaction Involving Oxidative Addition of Iodotrimethylsilane (Me_3SiI) to Pd(0). Synthesis of Stereodefined Enynes by the Coupling of Me_3SiI, Acetylenes, and Acetylenic Tin Reagents

Naoto Chatani, Nobuyoshi Amishiro, and Shinji Murai*

Department of Applied Chemistry, Faculty of Engineering Osaka University, Suita, Osaka 565, Japan

Received June 17, 1991

Oxidative addition reactions of a carbon-halogen (C-X) bond to a low valent transition metal complex are essential in catalytic and stoichiometric application of transition-metal reagents for organic synthesis.[1] Oxidative addition of a hydrogen-silicon bond (H-Si) is known for a number of transition metals and is an obligatory step in catalytic reactions such as hydrosilylation of olefins.[2] Recently, important advances have been made in the

(1) For general reviews, see: (a) Collman, J. P.; Hegedus, L. S. *Principles and Applications of Organotransition Metal Chemistry*; University Science Books: Mill Valley, CA, 1980. (b) Colquhoun, H. M.; Holton, J.; Thompson, D. J.; Twigg, M. V. *New Pathways for Organic Synthesis. Practical Applications of Transition Metals*; Plenum Press: New York, NY, 1984. (c) Tsuji, J. *Organic Synthesis with Palladium Compounds*; Springer-Verlag: Berlin, 1980.

0002-7863/91/1513-7778$02.50/0