Enzymatic Incorporation of a New Base Pair into DNA and RNA

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The discovery of catalytic ribonucleic acid supports the hypothesis that an early form of life relied exclusively on RNA catalysis.[1] We have suggested[2] that one way of investigating the scope of RNA catalysis is to prepare nucleic acids with an enlarged pool of mononucleotide building blocks possessing catalytic groups. Toward this end, we report here that both a DNA and an RNA polymerase direct the incorporation of isoguanosine (*iso*-G) into an oligonucleotide opposite isocytidine (*iso*-C) in a DNA template. These two molecules form a base pair with a hydrogen-bonding pattern distinct from those occurring in the natural A-T(U) and G-C pairs (Figure 1).

Protected deoxyribo-*iso*-C is suitable as a building block for the chemical synthesis of DNA, and both ribo- and deoxyribo-*iso*-GTPs were synthesized by direct extensions of published methods.[3,4] Two templates containing d-*iso*-C were synthesized (templates 1 and 4, Figure 2).[5,6] Templates 2, 3, and 5 were synthesized for use in control experiments (vide infra). An 8-mer primer was annealed to templates 1–3 to provide a double-stranded binding site for the Klenow fragment of DNA polymerase I (*Escherichia coli*).[7] Templates 4 and 5 were annealed to an 18-mer to give the double-stranded promoter region required by T7 RNA polymerase.[8]

Incubation of primed template 1 with the Klenow enzyme and dNTPs led to full-length product formation only in the presence of d-*iso*-GTP (compare lanes 1 and 4, Figure 3). d-*iso*-G was found at the correct position in the product oligonucleotide by a "nearest-neighbor" analysis[9] and by the "minus" sequencing method of Sanger.[10]

Experiments were carried out to determine the specificity with which the new bases pair. Pairing of d-*iso*-C with the natural purine nucleotides dATP and dGTP was investigated first. In an incubation of primed template 1 with dATP, dCTP, and TTP, full-length product was observed only to the extent anticipated by the low level of dU present in the template (lane 2, Figure 3).[6] Comparison with lane 4 shows that incorporation of dA accounts for full-length product formation. In a similar incubation of primed template 1 with dGTP, dCTP, and TTP, no full-length product was observed within the limits of detection (ca. 1%) (lane 3, Figure 3). It is thus concluded that essentially no dA or dG is incorporated opposite d-*iso*-C. Pairing of d-*iso*-GTP with the natural pyrimidine bases T and C was investigated next. Undesired pairing between d-*iso*-GTP and T was anticipated due to the presence of the minor "phenolic" tautomer of *iso*-G in addition to the major N^1-H tautomer;[11] this minor tautomer is, in principle,

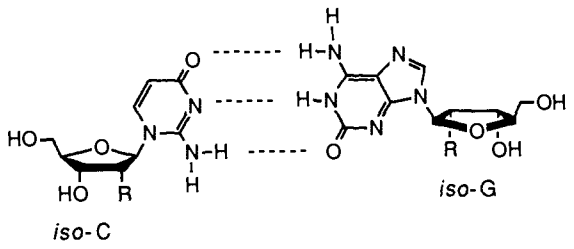

Figure 1. Putative Watson–Crick pairing interaction between *iso*-C and *iso*-G.

Template 1	d-5'-GATTTTGA d-3'-CTAAAACTGG*iso*-CGA
Template 2	d-5'-GATTTTGA d-3'-CTAAAACTGGTGA
Template 3	d-5'-GATTTTGA d-3'-CTAAAACTGGCGA
Template 4	d-5'-TAATACGACTCACTATAG d-3'-ATTATGCTGAGTGATATCGCGGC*iso*-CCGA
Template 5	d-5'-TAATACGACTCACTATAG d-3'-ATTATGCTGAGTGATATCGCGGCCCGA

Figure 2. Template/primers and template/promoters used in the nucleotide incorporation experiments.

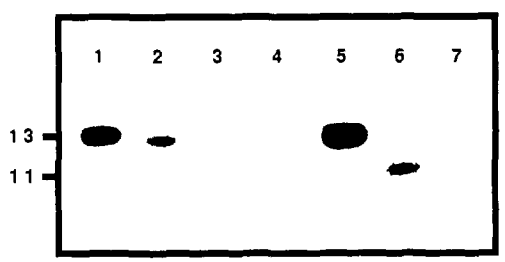

Figure 3. Polyacrylamide gel electrophoretic (PAGE) assay of the incorporation of d-*iso*-G into DNA by the Klenow enzyme, and the specificity with which the new bases pair. Incubations were conducted by using the protocol of Cobianchi and Wilson.[7] Template and nucleotide components: lane 1, template 1, d-*iso*-GTP, dCTP, [α-^{32}P]TTP; lane 2, template 1, dATP, dCTP, [α-^{32}P]TTP; lane 3, template 1, dGTP, dCTP, [α-^{32}P]TTP; lane 4, template 1, dCTP, [α-^{32}P]TTP; lane 5, template 2, d-*iso*-GTP, dCTP, [α-^{32}P]TTP; lane 6, template 2, dCTP, [α-^{32}P]TTP; lane 7, template 3, d-*iso*-GTP, dCTP, [α-^{32}P]TTP.

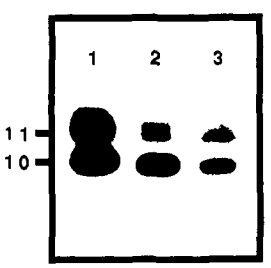

Figure 4. PAGE assay of the incorporation of *iso*-G into RNA by T7 RNA polymerase. Incubations were conducted by using the protocol of Milligan et al.[8] Template and nucleotide components: lane 1, template 5, GTP, CTP, [α-^{32}P]UTP; lane 2, template 4, *iso*-GTP, GTP, CTP, [α-^{32}P]UTP; lane 3, template 4, GTP, CTP, [α-^{32}P]UTP.

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(3) Synthesis of (unprotected) d-*iso*-C: (a) Watanabe, K. A.; Reichman, C. K.; Fox, J. J. *Nucleic Acid Chemistry*; Tipson, R. S., Townsend, L. B., Eds.; John Wiley and Sons: New York, 1978; Part 1, p 273. (b) Kimura, J.; Yagi, K.; Suzuki, H.; Mitsunobu, O. *Bull. Soc. Chem. Jpn.* **1980**, *53*, 3670. N^2-Benzoyl-5'-dimethoxytrityl-d-*iso*-C diisopropyl phosphoramidite was prepared from d-*iso*-C by the general procedure of Atkinson and Smith: Atkinson, T.; Smith, M. *Oligonucleotide Synthesis: A Practical Approach*; Gait, M. J., Ed.; IRL Press: Oxford, 1985; pp 35.

(4) Synthesis of ribo- and deoxyribo-*iso*-GTPs: Mantsch, H. H., et al. *Biochemistry* **1975**, *14*, 5593.

(5) The oligonucleotides were synthesized on an Applied Biosystems 380B DNA synthesizer.

(6) Incorporation of d-*iso*-C into the synthetic DNA molecules was verified by digestion of template samples (Eritja, R., et al. *Nucl. Acids Res.* **1986**, *14*, 8135). When this method was used, a small amount (ca. 15%) of dU was found to be present in the templates, presumably arising from the hydrolysis of d-*iso*-C under the alkaline conditions used for deprotecting the synthetic oligonucleotides.

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(8) Milligan, J. F.; Groebe, D. R.; Witherell, G. W.; Uhlenbeck, O. C. *Nucl. Acids Res.* **1987**, *15*, 8783.

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capable of Watson-Crick pairing with T. In fact, incubation of primed template 2 (containing T in place of d-*iso*-C) with d-*iso*-GTP, dCTP, and TTP did yield full-length product (lane 5, Figure 3). Comparison with lane 6 shows that incorporation of d-*iso*-G accounts for full-length product formation. In an incubation of primed template 3 (containing dC in place of d-*iso*-C) carried out in the same way as with primed template 2, no full-length product was detected (lane 7, Figure 3). Therefore, while d-*iso*-GTP showed undesired pairing with T, no incorporation of d-*iso*-G was observed opposite dC.

T7 RNA polymerase was also shown to accept the new base pair. Thus, template 4 possessing the T7 consensus promoter yielded 75% more full-length product in the presence of *iso*-GTP than in its absence (compare lanes 2 and 3, Figure 4).[12] The "read-through" in the absence of *iso*-GTP observed in this case is consistent with the lower fidelity of RNA polymerases relative to DNA polymerases.[13] Sequencing of the product RNA transcript using a standard protocol[14] positively established incorporation of *iso*-G at the expected position.

These experiments demonstrate for the first time that both a DNA polymerase (Klenow enzyme) and an RNA polymerase (T7) will incorporate into a growing oligonucleotide a nucleotide with a novel pattern of hydrogen-bonding groups, under the direction of its intended partner in a template. We are currently extending this work to include other base pairs with novel pairing schemes.

Acknowledgment. We thank Dr. J. A. Piccirilli for many helpful discussions. Financial support for this work was provided by the Swiss National Science Foundation, Sandoz AG, and the Swiss Federal Institute of Technology.

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(12) The two product bands observed in these experiments are a common feature of transcriptions with T7 RNA polymerase.[8]
(13) Battula, N.; Loeb, L. *J. Biol. Chem.* **1975**, *250*, 4405.
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Ligand Oxidation in a Nickel Thiolate Complex: A Model for the Deactivation of Hydrogenase by O_2

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Many hydrogenases (H_2ases) possess Ni centers with S-donor ligands that have unusual redox properties.[1] Upon exposure to O_2, these enzymes are deactivated but may be reductively activated in a multistep process to yield active enzyme.[1] We have recently described oxidations of the dimeric complex **1** involving one-half and two electrons per Ni that lead to an EPR-active dimeric radical and a monomeric disulfide complex, respectively.[2] These oxidation products support a redox role for thiolate ligands in the H_2ase active site. Herein we report a four-electron oxidation of a related Ni complex upon exposure to molecular oxygen. The resulting Ni(II) sulfinato complex is a rare example of a structurally characterized product of thiolate oxidation employing molecular oxygen and provides a plausible chemical model for the deactivation of H_2ase by O_2.

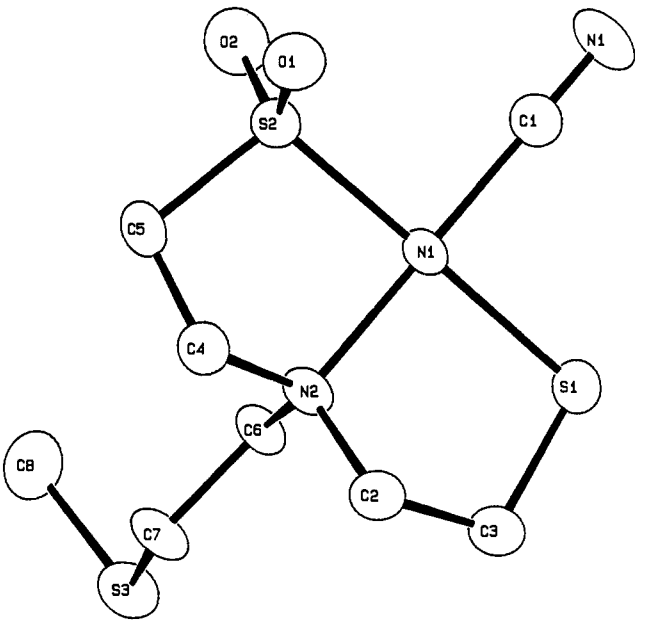
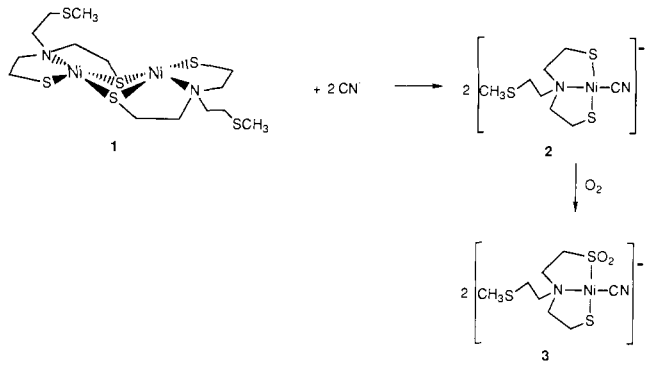


Figure 1. ORTEP plot of **2** with thermal ellipsoids at the 30% probability level. Selected bond distances in Å are as follows: Ni-S1, 2.175 (3); Ni-S2, 2.146 (3); Ni-N2, 1.973 (7); Ni-C1, 1.875 (10); S2-O1, 1.488 (8); S2-O2, 1.419 (7). Selected bond angles in degrees are as follows: S1-Ni-N2, 89.6 (2); S1-Ni-C1, 90.5 (3); S2-Ni-N2, 89.0 (2); S2-Ni-C1, 91.0 (3); S1-Ni-S2, 176.2 (1); N2-Ni-C1, 180.0 (8); Ni-S2-O1, 111.2 (3); Ni-S2-O2, 118.3 (4); O1-S2-O2, 114.4 (4); O1-S2-C5, 103.6 (5); O2-S2-C5, 105.6 (5).

Scheme I



Reaction of **1** with 2 equiv of $Et_4N(CN)$ in DMF[3] (Scheme I) leads to the formation of a structurally characterized square-planar complex (**2**),[4] in analogy with a similar system employing thiophenolate as the fourth ligand.[5] Upon exposure to air or an oxygen atmosphere, **2** undergoes oxidation to the sulfinato complex **3** (Figure 1).[6] This novel diamagnetic Ni(II) complex, a four-electron-oxidation product of **2** that features one thiolate ligand and one sulfinate ligand, can be isolated in 84% yield upon addition of toluene to a DMF solution of **2** stirred under O_2 overnight.[7]

(1) (a) Fauque, G.; Peck, H. D., Jr.; Moura, J. J. G.; Huynh, B. H.; Berlier, Y.; DerVartanian, D. V.; Teixeira, M.; Przybyla, A. E.; Lespinat, P. A.; Moura, I.; LeGall, J. *FEMS Microbiol. Rev.* **1988**, *54*, 299-344. (b) Gogotov, I. N. *Biochimie* **1986**, *68*, 181-187. (c) Hausinger, R. P. Microbiol. Rev. **1987**, *51*, 22-42. (d) Moura, J. J. G.; Moura, I.; Teixeira, M.; Xavier, A. V.; Fauque, G.; LeGall, J. *Met. Ions Biol. Syst.* **1988**, *23*, 285-314.
(2) Kumar, M.; Day, R. O.; Colpas, G. J.; Maroney, M. J. *J. Am. Chem. Soc.* **1989**, *111*, 5974-5976.
(3) **CAUTION**: Solutions of $Et_4N(CN)$ are extremely toxic by skin absorption.
(4) Under oxygen-deficient conditions, cocrystals of **2** and **3** that are isomorphous with pure **3** and contain ca. 63% **2** are obtained from DMF/toluene. Lattice constants: a = 12.762 (9) Å, b = 12.740 (7) Å, c = 14.797 (8) Å, β = 117.56 (4)°. Details of this structure will be reported elsewhere.
(5) Krüger, H.-J.; Holm, R. H. *Inorg. Chem.* **1989**, *28*, 1148-1155.
(6) X-ray analysis on orange crystals obtained from slow aerobic evaporation of a DMF/MeOH solution of **2**: monoclinic space group $P2_1/c$, a = 12.784 (4) Å, b = 12.757 (2) Å, c = 14.847 (4) Å, β = 115.00 (3)°, V = 2194.5 Å^3, Z = 4. The present values of R = 0.056 and R_w = 0.068 are based on anisotropic unit-weighted refinement of non-hydrogen atoms. Full details will be published elsewhere.

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