and in **2b** vs **1**. The β-C electrophilicity of the F-enolates is rationalized by their relatively low lying LUMOs combined with the small positive or slightly negative charge on their β-carbon atoms. The lower LUMO level of **2b** relative to that of **1** nicely accounts for its enhanced C-electrophilicity, and its comparatively *lower* HOMO level is consistent with its aldol unreactivity.[7]

In conclusion, the procedure described herein provides a facile synthesis of F-enolates whose reactivities markedly differ from those of their hydrocarbon analogues but can be anticipated by ab initio molecular orbital calculations. Further studies on the mechanism of enolate formation and synthetic utility are in progress.

Acknowledgment. This research was partially supported by a Grant-in-Aid for Scientific Research from the Ministry of Education, Japan, and the Chemical Materials Research & Development Foundation, which are gratefully acknowledged. We also thank Asahi Glass Company for the gift of C_2F_5I.

Supplementary Material Available: Experimental details for generating the F-enolates and their reactions, spectral characterization of the products, and the computed atom coordinates for the enolates (4 pages). Ordering information is given on any current masthead page.

(12) The HOMOs of these enolates have the largest electron densities in the out-of-plane p orbitals on O and C_β. There is some delocalization into the out-of-plane p orbitals bonded to C_β.

Nickel-Mediated Sequence-Specific Oxidative Cleavage of DNA by a Designed Metalloprotein

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We recently designed a sequence-specific DNA-cleaving metalloprotein consisting wholly of naturally occurring α-amino acids.[1] *The tripeptide copper-binding domain, Gly-Gly-His* (GGH), was attached to the NH_2-terminus of the DNA-binding domain of Hin recombinase (residues 139–190) to afford a new 55-residue protein, GGH(Hin139–190).[1] This protein is capable of binding DNA at four 13 base pair sites (termed *hixL* and secondary).[1] Cu·GGH(Hin139–190) in the presence of excess hydrogen peroxide and sodium ascorbate cleaves DNA predominantly at one of the four Hin binding sites.[1] We report here that, in the presence of $Ni(OAc)_2$ and monoperoxyphthalic acid, the sequence specificity and efficiency of the DNA cleavage by GGH(Hin139–190) are remarkably altered. The nickel-mediated DNA cleavage process occurs at all four binding sites, is more rapid and efficient, and is chemically activated by 1 equiv of an oxygen atom donor. At the *hixL* site, cleavage occurs predominantly at a *single* deoxyribose position on *one strand* of each binding site, indicating a nondiffusible oxidizing species.

From X-ray diffraction analysis, the tripeptide GGH is known to bind Cu(II) in a square-planar complex with coordination from an imidazole nitrogen, two deprotonated peptide nitrogens, and the terminal amino group (Figure 1).[2] Although a crystal structure of GGH·Ni(II) is not available, the Ni(II) complex of GGH has been studied by other techniques.[3-5] Crystal structures

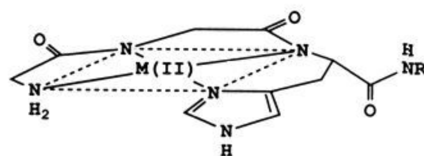

Figure 1. Model for GGH ligand bound to Cu(II) or Ni(II) in a square-planar complex[2-5] (M = Cu(II) or Ni(II)).

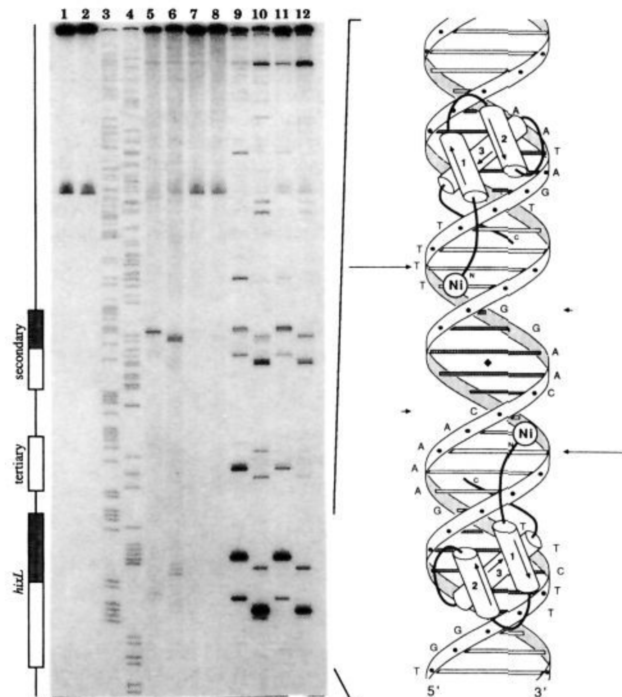

Figure 2. Left: Autoradiogram of high-resolution denaturing gel of Cu·GGH(Hin139–190) and Ni·GGH(Hin139–190) cleavage of a ^{32}P end-labeled fragment (*XbaI*/*EcoRI*) from pMFB36.[21] Reaction conditions were 20 mM NaCl, 20 mM phosphate, pH 7.5, calf thymus DNA (100 μM in base pair), and ~15 000 cpm end-labeled DNA in a total volume of 20 μL. Reactions for Cu·GGH(Hin139–190) and Ni·GGH(Hin139–190) were run for 90 and 15 min, respectively (25 °C). Nickel-mediated reactions were treated with 0.1 M $BuNH_2$ for 30 min at 90 °C.[13] Cleavage products were analyzed on an 8%, 1:20 cross-linked, 50% urea polyacrylamide gel, 0.4 mm thick. Odd-numbered and even-numbered lanes are DNA labeled at the 5' and 3' ends with ^{32}P, respectively. Lanes 1 and 2 are DNA control lanes. Lanes 3 and 4 are A specific sequencing reactions.[22] Lanes 5 and 6 contain $CuCl_2$ (2.5 μM) and GGH(Hin139–190) (5 μM) followed by sodium ascorbate (1 mM) and hydrogen peroxide (1 mM). Lanes 7 and 8 are identical with lanes 5 and 6 except that they contain $CuCl_2$ (0.5 μM) and GGH(Hin139–190) (1 μM). Lanes 9 and 10 contain $Ni(OAc)_2$ (5 μM) and GGH(Hin139–190) (5 μM) followed by monoperoxyphthalic acid, magnesium salt (5 μM). Lanes 11 and 12 are identical with lanes 9 and 10 except that they contain $Ni(OAc)_2$ (1 μM), GGH(Hin139–190) (1 μM), and monoperoxyphthalic acid (1 μM). Right: Model of Ni·GGH(Hin139–190) binding to the *hixL* site based on the helix-turn-helix motif in ref 9. The sequence of Hin(139–190) is GRPRAINKHEQEQISRLLEKGHPRQQLAIIFGIGVSTLYRYFPASSIKKRMN. Arrows represent location and extent of cleavage produced by Ni·GGH(Hin139–190) in the presence of monoperoxyphthalic acid.

of tetraglycine with Cu(II) or Ni(II) indicate that the metal ions are bound by peptide ligands in a similar fashion.[6-8]

GGH(Hin139–190) at 1.0 μM concentrations (pH 7.5, 25 °C, 20 mM NaCl) in the presence of $Ni(OAc)_2$ (1.0 μM) and mon-

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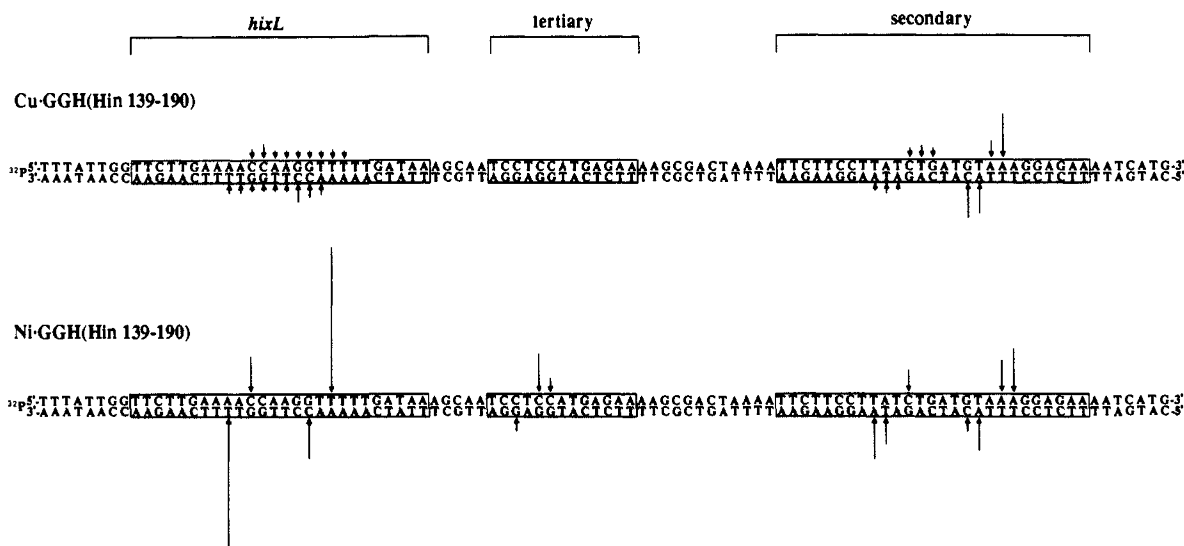

Figure 3. The sequence left to right represents the cleavage data from the bottom to the middle of the gel in Figure 2. Boxes indicate the dimeric *hixL* and secondary Hin binding sites. Arrows represent location and the extent of cleavage. Top: Cu·GGH(Hin139–190) (5 μM) in the presence of sodium ascorbate (mM) and hydrogen peroxide (mM) (Figure 2, lanes 5 and 6). Bottom: Ni·GGH(Hin139–190) (1 μM) in the presence of monoperoxyphthalic acid (1 μM) (Figure 2, lanes 11 and 12). Extent of cleavage was determined by densitometric analysis of the gel autoradiogram.

operoxyphthalic acid (1.0 μM) cleaves DNA at all four sites upon base workup (0.1 M n-BuNH_2, 90 °C, 30 min) (Figures 2 and 3). The cleavage patterns observed at the *hixL* sites are strong and occur predominantly on one strand of each DNA site with single-base specificity, while the cleavage at the secondary sites is modest and covers one to two base positions on both DNA strands (Figures 2 and 3). Maximal cleavage on opposite strands of the DNA is asymmetric to the 3′ side, consistent with the known location of the NH_2-terminus of Hin(139–190) in the minor groove.[9] Upon base workup, the DNA termini at the cleavage site are 3′- and 5′-phosphate, consistent with oxidative degradation of the deoxyribose backbone.[10]

The small number of deoxyribose cleavage positions within each binding site implies that a nondiffusible oxidant is generated by both the Ni(II)·GGH(Hin139–190)–peracid and Cu(II)·GGH-(Hin139–190)–H_2O_2–ascorbate reactions. However, differences in cleavage specificity are evident (Figure 3). Cu·GGH-(Hin139–190) affords modest cleavage at one-half of the secondary site and weak cleavage at the other three sites (Figure 3). Ni·GGH(Hin139–190) affords strong cleavage at the *hixL* sites and modest cleavage at the secondary sites. Footprinting data suggest that all four sites are occupied at these concentrations.[1] One explanation for the difference observed for the two metalloproteins is that the structure of the DNA is different at each site, and the reactive moieties on the metalloprotein are sensitive to these sequence-dependent conformational differences.

There exist significant differences in the reactivities of Cu-(II)·GGH–H_2O_2–ascorbate and the Ni(II)·GGH–peracid systems toward DNA. The nickel-mediated reaction in the presence of 1 equiv of a peracid is complete in 1 min (25 °C).[12] For copper-mediated cleavage in the presence of a 500-fold excess of ascorbate and hydrogen peroxide, 1.5-h reaction times are necessary (25 °C).[11] Moreover, the extent of cleavage obtained from the Ni(II)·GGH–peracid reaction is significantly greater than that for the Cu(II)·GGH–H_2O_2–ascorbate reaction (Figure 2).

The highly reactive nature of the oxidizing species, the precision of the cleavage reaction, and the identity of the DNA termini created upon treatment with butylamine[13] imply that the species may be a high valent nickel bound oxygen[14-16] that abstracts a specific hydrogen atom on the deoxyribose backbone.[17] Similarities of end-product analysis with bleomycin·Fe cleavage of DNA suggest that hydrogen atom abstraction may occur (at least in part) at the 4′-position of the deoxyribose ring. To test this, an oligonucleotide duplex containing the *hixL* site was synthesized with a single deuterium incorporated at the deoxyribose 4′-position of one thymidine nucleotide corresponding to the strong cleavage site in *hixL*.[18] A deuterium isotope effect (k_H/k_D) of 1.6 was observed for the cleavage reaction.[20]

Although considerable work remains to clarify the mechanistic details of this reaction, this result has implications for design of protein catalysts. The tripeptide GGH is a metal-specific structural domain consisting of naturally occurring amino acids that could be incorporated at the NH_2-terminus of a variety of recombinant proteins (such as other DNA binding proteins, receptors, or antibodies) with the function of precise, efficient

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(10) End-product analysis was carried out by high-resolution 20% polyacrylamide gel electrophoresis.

(11) The reaction of Cu·GGH(Hin139–190) with monoperoxyphthalic acid affords sequence-specific cleavage similar to that afforded by Ni·GGH-(Hin139–190), but 100-fold-higher concentrations (0.1 mM) of peracid are required, and reaction times are much longer, 1.5 h.

(12) In the nickel-mediated cleavage reaction, monoperoxyphthalic acid can be replaced with H_2O_2 or PhIO. Cleavage specificity is the same as with peracid, indicating a common intermediate. However, Ni(II)·GGH–H_2O_2 requires significantly longer reaction times (h) and is less efficient, indicating that a putative oxygen atom transfer is likely rate determining. Ni(II)·GGH(Hin139–190) in the presence of H_2O_2 (mM) and ascorbate (mM) does not cleave DNA to any appreciable extent.

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(16) Consistent with the possibility that a high-valent nickel–oxo species is formed, we find that Ni(II)·GGH in the presence of PhIO epoxidizes olefins such as styrene and norbornene in H_2O/MeOH.

(17) The preferential cleavage on *one* strand of each *hixL* site suggests some sort of diastereofacial selectivity (Figures 1 and 2). Ni(II)·GG(L-His→D-His)(Hin139–190) was synthesized and found to afford a different cleavage pattern. Moreover, Ni(II)·AibAibH(Hin139–190), which might create a steric blockade to oxygen transfer, does not cleave the DNA.

(18) We thank M. Greenberg for a sample of [4-^{2}H]thymidine[19] phosphoramidite.

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(20) This primary kinetic isotope effect can be compared with that obtained for Fe·bleomycin (k_H/k_D = 2.1–4.0), which cleaves DNA by abstracting the 4′-hydrogen by a putative high-valent iron–oxo species.[19]

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substrate-directed oxidation, activated in the presence of Ni(II) and peracid (25 °C, pH 7.5).

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Batch Oscillation in the Reaction of Chlorine Dioxide with Iodine and Malonic Acid[1]

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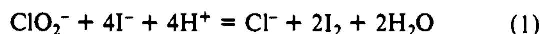
The reaction of chlorite, iodide, and malonic acid (MA) is one of a handful of chemical reactions to oscillate in a closed (batch) system.[3] This reaction has recently been used in the first experimental demonstration[4] of the symmetry-breaking, reaction-diffusion structures predicted by Turing[5] nearly 40 years ago. We have discovered that the source of oscillation in the ClO_2^-–I^-–MA reaction is the chlorine dioxide–iodine–malonic acid reaction. From this knowledge, we can construct a simple model that explains the temporal behavior of the system and should serve as a basis for theoretical studies of the Turing structures.

Batch oscillations in the ClO_2^-–I^-–MA reaction were studied at 25 °C and 400 rpm in an HP8452A diode array spectrophotometer. The diode array instrument enabled us to monitor many wavelengths simultaneously, making it possible to follow several intermediates and products during the reaction.

In Figure 1, we show a representative experiment at two different wavelengths: 468 nm, the isosbestic point of I_2 and I_3^- (ϵ = 740 cm^{-1} M^{-1}), and 280 nm, where iodomalonic acid (IMA) and triiodide ion (ϵ = 32 000 cm^{-1} M^{-1}) both have significant absorptivity. The envelope of minima in the 280-nm curve corresponds to a first-order increase in [IMA] at a rate equal to that of the disappearance of I_2 in the 468-nm curve. In view of the much larger molar absorptivity of I_3^- at 280 nm, we conclude that $[I_2] \gg [I_3^-]$ and that the oscillations result from changes in $[I_3^-]$.

In the preoscillatory period, the autocatalytic increase in $[I_2]$ followed by a sharp decrease and a slow recovery resembles the behavior of the chlorite–iodide reaction in the absence of MA.[6] This similarity suggests that in the presence of MA the ClO_2^-–I^- reaction is complete before oscillations start, and one or more products of this reaction plays a crucial role in the subsequent periodic behavior. From $[I_2]$ determined from the absorbance at 468 nm, we can estimate $[IO_3^-]$ using the stoichiometric equations



$$ClO_2^- + 4I^- + 4H^+ = Cl^- + 2I_2 + 2H_2O \quad (1)$$

$$5ClO_2^- + 2I_2 + 2H_2O = 5Cl^- + 4IO_3^- + 4H^+ \quad (2)$$

Rábai and Beck[7] showed that reaction 2 is accompanied by formation of chlorine dioxide. The $[ClO_2^\bullet]$ formed during the chlorite–iodide reaction (at the same initial concentrations used in the chlorite–iodide–malonic acid oscillator) can be determined from the spectrum of the reaction mixture. If we mix the amounts of I_2, IO_3^-, and $ClO_2^\bullet$ formed in the chlorite–iodide reaction with MA and H_2SO_4 at the concentrations used in the oscillatory reaction, oscillation appears with no induction period. Further study reveals that iodate ion is not required for oscillation. These experiments demonstrate that *malonic acid, chlorine dioxide, and iodine* are responsible for the oscillation. In Figure 2, we show three oscillatory curves at different $[ClO_2^\bullet]_0$.

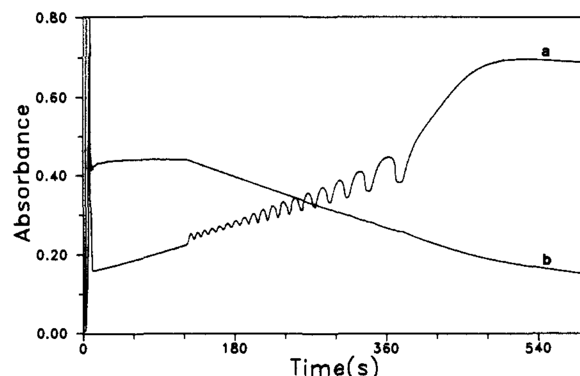


Figure 1. Absorbance at (a) 280 nm and (b) 468 nm in the chlorite–iodide–malonic acid reaction. $[ClO_2^-]_0 = 5.71 \times 10^{-3}$ M, $[I^-]_0 = 4.57 \times 10^{-3}$ M, $[MA]_0 = 1.0 \times 10^{-3}$ M, $[H_2SO_4]_0 = 5.0 \times 10^{-3}$ M.

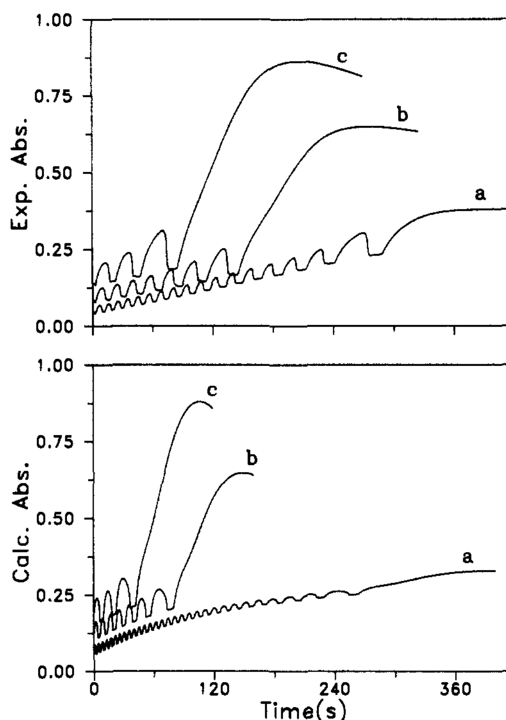


Figure 2. Experimental and calculated oscillations in absorbance (280 nm) in the chlorine dioxide–iodine–malonic acid reaction. Molar absorbances for I_3^-, I_2, $ClO_2^\bullet$, and iodomalonic acid are 32000, 100, 88, and 300 M^{-1} cm^{-1}, respectively. $[I_2]_0 = 5.0 \times 10^{-4}$ M, $[MA]_0 = 1.0 \times 10^{-3}$ M, $[H_2SO_4]_0 = 5.0 \times 10^{-3}$ M, $[ClO_2^\bullet]_0 = 1.7 \times 10^{-4}$ (a), 1.0×10^{-4} (b), and 7.1×10^{-5} M (c). For clarity, curves b and c have been shifted upward by 0.05 and 0.10 absorbance units, respectively, since in the absence of a shift the curves overlap significantly.

Malonic acid can be replaced by ethyl acetoacetate at the same concentration. This suggests that MA serves primarily to generate I^- from I_2 and that in an open system the reaction of chlorine dioxide with iodide should be oscillatory. Our experiments confirm this prediction over a broad range of initial concentrations and residence times.[8]

On the basis of these observations, we propose a model consisting of three component processes. The first is the reaction between (MA) and I_2.

(1) Part 65 in the series Systematic Design of Chemical Oscillators. Part 64: Rábai, Gy.; Orbán, M.; Epstein, I. R. *Acc. Chem. Res.* Submitted for publication.

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(8) A typical reaction mixture consists of $[ClO_2]_0 = 1 \times 10^{-4}$ M, $[I^-]_0 = 1 \times 10^{-4}$ M, $[H_2SO_4]_0 = 5 \times 10^{-3}$ M at a reciprocal residence time $k_0 = 5 \times 10^{-3}$ s^{-1}. The oscillations were monitored with an iodide-selective electrode.

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