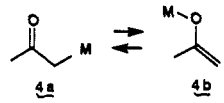
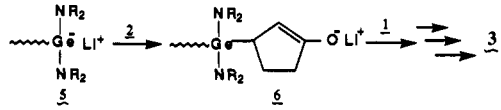
regioselectively O-germylated acyclic enolates[11] which have the potential utility for basic investigation on the stability and nucleophilic reactivity of germanium enolates.



4a 4b

At present, the copolymerization mechanism may be explained as follows. A lithium catalyst reacts with germylene **1** to produce a germyl anion species **5**,[12] to which cyclic ketone **2** adds via a Michael-type addition giving rise to an enolate anion **6**. The germyl anion is regenerated by the reaction of **6** with **1**. The steric hindrance of the bulky silyl amide group of **1** and the very poor homopolymerizability of **2** prevent the homo unit formation of **1** and of **2**, which allows the *alternating propagation* of **1** and **2** leading to copolymer **3**.



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Further investigations on the physical and chemical properties of the resulting copolymers and the mechanism of the copolymerization are now in progress.

Acknowledgment. This work was partly supported by a Grant-in-Aid for Scientific Research on a Priority Area of "New Functionality Materials, Design, Preparation, and Control" from the Ministry of Education, Science and Culture, Japan (No. 03205009).

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Novel Observation of NH···S(Cys) Hydrogen-Bond-Mediated Scalar Coupling in ^{113}Cd-Substituted Rubredoxin from *Pyrococcus furiosus*

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Understanding the nature of NH···S hydrogen bonding in iron–sulfur proteins is important since these interactions may be determinants of protein redox potential and function.[1,2] Very recently, deuteron hyperfine and quadrupole coupling were detected in ^{2}H Mims[3] pulsed ENDOR studies of ferredoxin from *Anabaena*, demonstrating that at least one (unidentified) backbone NH···S hydrogen bond contained significant covalent character.[4] To gain additional insights into the nature of NH···S hydrogen bonding in iron–sulfur proteins, we have applied ^{1}H–^{113}Cd heteronuclear spin-echo difference (HSED) NMR spectroscopy to ^{113}Cd-substituted rubredoxin [^{113}Cd(Rd)] from the hyperthermophilic archaebacterium, *Pyrococcus furiosus* (*P. furiosus*), an organism that grows optimally at 100 °C.[5,6] Of the six backbone NH protons proposed to be involved in NH···S hydrogen bonding,[7-10] four exhibit two-bond N^{1}H···S(Cys)–^{113}Cd scalar coupling, indicating that the NH···S(Cys) hydrogen bonds formed by these protons contain significant covalent character. The ^{1}H–^{113}Cd HSED signals are assigned specifically to the Ile(7), Cys(8), Ile(40), and Cys(41) backbone amide protons that are involved in types I and II NH···S tight turns. No HSED signals are detected for the Tyr(10) and Ala(43) amide protons that are believed to be involved in type II NH···S tight turns. This represents the first NMR observation of H-bond-mediated scalar coupling in a protein and provides a rationale for the high metal affinities exhibited by proteins that contain the Cys-X-X-Cys-Gly-X (X = variable amino acid) metal-binding motif.

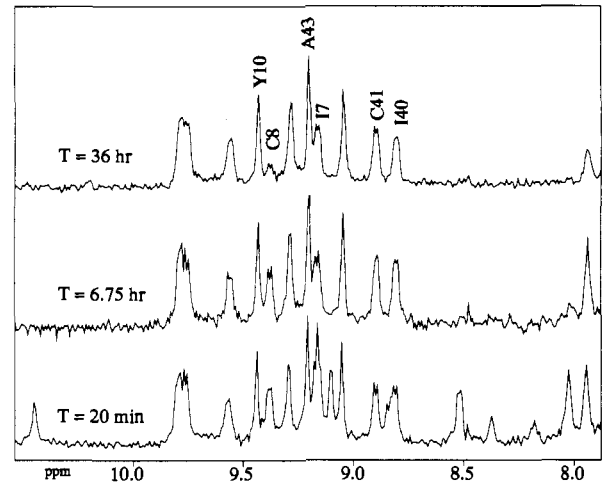

Figure 1. Downfield region of the ^{1}H NMR spectra obtained for Zn(Rd) (ca. 1.2 mM) after dissolution into D_2O at 25 °C. Data were obtained with a GE Omega-PSG 600 NMR instrument using a DANTE sequence for suppression of the residual solvent signal, followed by a SCUBA train for recovery of presaturated αH protons (referenced to internal HDO at δ = 4.773 ppm). Residues implicated in types I, II, and III NH···S hydrogen bonding are labeled.

The NMR structure of zinc-substituted *P. furiosus* Rd [Zn(Rd)] has recently been determined,[9,10] and the structure of the metal-binding site was found to be essentially identical to that observed by X-ray crystallography for native *Clostridium pasteurianum* Rd.[10,11] In both structures the NH protons of residues

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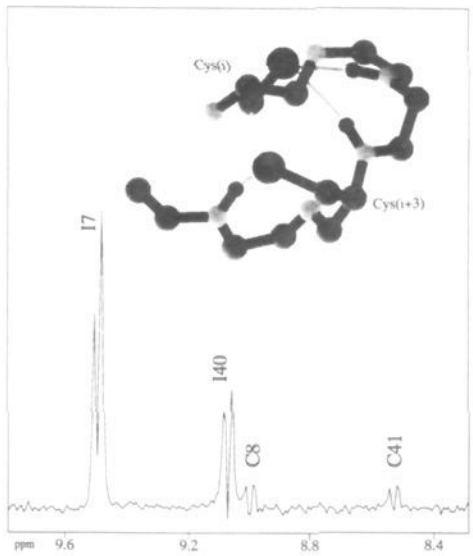

Figure 2. Downfield region of the 500-MHz ^1H–^{113}Cd heteronuclear spin-echo difference (HSED) spectrum obtained for *P. furiosus* ^{113}Cd-(Rd) (3.0 mM, 90% H_2O/10% D_2O, 25 mM acetate-d_3, pH 6.3, 250 mM NaCl, T = 45°; obtained with a GE GN-500 NMR instrument with 2 days of data acquisition; 140 000 scans) using 60-ms $J_{^1H^{113}Cd}$ evolution and refocusing periods (τ; see below). Observed HSED signals are due to two-bond NH···S mediated ^1H–^{113}Cd scalar coupling, indicating that the NH···S hydrogen bonds contain covalent character. The structure of a representative Cys(i)-X(i+1)-X(i+2)-Cys(i+3)-Gly(i+4)-X(i+5) metal-binding sequence is included in the insert, where the amide protons of residues X(i+2), Cys(i+3), and X(i+5) are from types I, III, and II NH···S tight turns, respectively. In *P. furiosus* Rd, the two metal-binding sequences are C^5-K^6-I^7-C^8-G^9-Y^{10} and C^{38}-P^{39}-I^{40}-C^{41}-G^{42}-A^{43}. The HSED sequence used is 90x(H)–τ–90x(Cd)180x(H)90±x(Cd)–τ–Acq-±x.

X(i+2), Cys(i+3), and X(i+5) [the metal-binding amino acid sequences are defined here as Cys(i)-X(i+1)-X(i+2)-Cys(i+3)-Gly(i+4)-X(i+5); in *P. furiosus* Rd, X(i+1) = Lys(6) and Pro(39), X(i+2) = Ile(7,40), X(i+5) = Tyr(10) and Ala(43)] are oriented in a manner consistent with hydrogen bonding to the Cys(i), Cys(i), and Cys(i+3) sulfurs, respectively. Deuterium exchange results obtained for *P. furiosus* Zn(Rd) are consistent with the proposal that these protons are involved in hydrogen bonding. Thus, no ^1H NMR signal loss was observed for the putative NH···S hydrogen-bonded protons after ca. 7 h of incubation in D_2O at 25 °C, and after 36 h of incubation (during which a 2D NOESY[12] spectrum was obtained in order to unambiguously assign the observed signals), only the Cys(8) NH proton exhibited substantial signal loss (Figure 1). The Tyr(10) and Ala(43) NH signals were unaffected even after further incubation for 20 min at 80 °C.

As a probe for electron-mediated metal–proton scalar coupling, ^{113}Cd substitution was performed in order to exploit the favorable nuclear (I = $^1/_2$) and electronic (S = 0) properties of this metallobioprobe.[13] Complete ^1H NMR signal assignments are made for ^{113}Cd(Rd) by standard procedures and will be published elsewhere. Although some chemical shift differences were observed for residues that comprise the metal-binding sites, cross peak patterns and intensities observed in homonuclear 2D NOESY spectra of ^{113}Cd(Rd) and Zn(Rd) matched (Figures S1 and S2 in the supplementary material), demonstrating that ^{113}Cd substitution did not cause a significant structural perturbation. ^1H–^{113}Cd HSED[14] spectra were obtained using DANTE[15] and SCUBA[16] preparation sequences, and four signals corresponding to the Ile(7), Cys(8), Ile(40), and Cys(41) backbone NH protons were observed in the downfield region of the spectrum (Figure 2). (Intense HSED signals involving the Cys-Hβ protons and very weak signals ($s/n > 2/1$) from the Cys(5) and Cys(38)-Hα protons were also observed, as expected on the basis of NMR studies of other ^{113}Cd-substituted proteins).[17-19] In ^1H–^{113}Cd HSED spectra, only signals of protons that are scalar coupled to the ^{113}Cd nucleus are detected.[14] ^1H–^{113}Cd scalar coupling via the peptide backbone is implausible since the Cys and Ile NH protons are five and eight bonds removed from the ^{113}Cd nucleus, respectively. Indeed, in previous NMR studies of $^{113}Cd_7$-metallothionein employing heteronuclear filters, no intraresidue five-bond NH(Cys)–^{113}Cd scalar coupling could be detected.[17] The observed amide HSED signals are therefore attributed to two-bond $^2J_{^1H^{113}Cd}$ scalar coupling mediated by NH···S(Cys) hydrogen bonds. These findings provide strong evidence that types I and III NH···S tight turns exist in *P. furiosus* Rd and further demonstrate that the NH···S hydrogen bonds contain significant covalent character.

The X(i+5) NH protons proposed to form type II NH···S tight turns did not give rise to detectable HSED signals, despite the fact that these protons exhibited the lowest deuterium exchange rates (Figure 1). The lack of observable HSED signals could, in principle, be attributed to rapid proton T2 relaxation, HSED signal cancellation due to homonuclear ^1H–^1H couplings, and/or weak (or absent) heteronuclear ^1H–^{113}Cd coupling. T2 measurements by the CPMG method[20] reveal that all six NH protons have similar T2 values (ca. 0.09 s; Figure S3 in the supplementary material). Although the homonuclear NH–αH coupling constants for the X(i+5) residues are small (≤3 Hz) compared to the X(i+2) and Cys(i+3) residues (>8 Hz), HSED spectra collected with $J_{^1H^{113}Cd}$ evolution delays (τ) of 20, 40, 60, 80 and 100 ms all lack signals for the X(i+5) amides; it is therefore unlikely that signal loss resulted from cancellation of homonuclear J coupling components since these components could not be antiphase for all evolution periods employed. In addition, the relative magnitudes of the observable amide HSED signals were not significantly different over the range of evolution delays employed. The combined findings suggest that the lack of an observable X(i+5) HSED signal is the result of very weak (or absent) ^1H–^{113}Cd scalar coupling and that the differences in signal intensities for the observed HSED signals reflect differences in the magnitudes of the two-bond NH–^{113}Cd coupling constants. This does *not* demonstrate that the proposed type II NH···S hydrogen bonds are weaker or that they contain less covalent character since scalar coupling is influenced by multiple factors (e.g., bond length, bond angle, electronegativity, etc.).

^1H–^{113}Cd HSED NMR spectroscopy provides a powerful direct probe of NH···S hydrogen bonding in metalloproteins. The presence of types I and III NH···S hydrogen bonds in *P. furiosus* Rd has been confirmed by direct detection. Efforts are now underway to determine the magnitudes of the $^2J_{^1H^{113}Cd}$ coupling constants, which would be useful for quantum mechanical studies of NH···S hydrogen bonding.

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instrument at UMBC was purchased with support from the NIH (Grants GM42561 and AI30917 to M.F.S.).

Note Added in Proof. Additional $^1H-^{113}Cd$ scalar coupling involving the Ala(43) methyl group, which was originally attributed to incomplete cancellation in the $^1H-^{113}Cd$ HSED spectra, has been detected and confirmed in a 2D $^1H-^{113}Cd$ heteronuclear multiple quantum correlation experiment. This scalar coupling is probably due to direct orbital overlap between the methyl protons of Ala(43) and the Cys(42) sulfur (6- and 8-bond $^1H-^{113}Cd$ scalar coupling mediated by the Ala(43) NH···S hydrogen bond or by the covalent bonds, respectively, would appear implausible) and thus may have important implications for understanding intraprotein electron transfer.

Supplementary Material Available: Figures of the full 2D NOESY spectra of ^{113}Cd- and zinc-substituted *P. furiosus* rubredoxin, the downfield region of the 500-MHz 2D NOESY spectrum of ^{113}Cd(Rd), and a stacked plot of the CPMG data for ^{113}Cd(Rd) (4 pages). Ordering information is given on any current masthead page.

Minor Groove Binding of $[Ru(phen)_3]^{2+}$ to $[d(CGCGATCGCG)]_2$ Evidenced by Two-Dimensional Nuclear Magnetic Resonance Spectroscopy

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The chiral transition metal complex $[Ru(phen)_3]^{2+}$ (phen = 1,10-phenanthroline), Figure 1, exists in two enantiomeric forms, Δ and Λ, which bind differently to DNA.[1-6] Despite extensive studies, the structural DNA-binding characteristics have remained unclear. Both minor[7-9] and major groove binding[4,8,10,11] have been suggested, and various modes of binding have been proposed, intercalation of one of the phenanthroline wings being one of them.[1,3-5,11]

Here we report the first two-dimensional NMR study on the interaction of $[Ru(phen)_3]^{2+}$ with the self-complementary decanucleotide duplex $[d(CGCGATCGCG)]_2$.[12] The results provide evidence for binding of both Δ- and Λ-$[Ru(phen)_3]^{2+}$ to the AT region in the minor groove of the oligonucleotide and strongly indicate a non-intercalative type of binding.

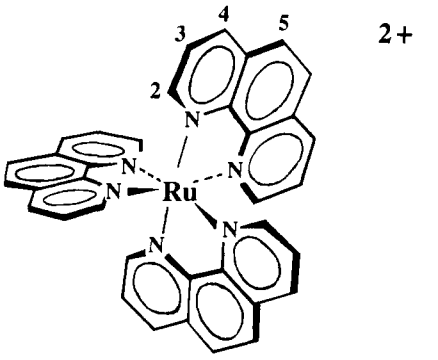

Figure 1. $[Ru(phen)_3]^{2+}$, Δ-enantiomer.

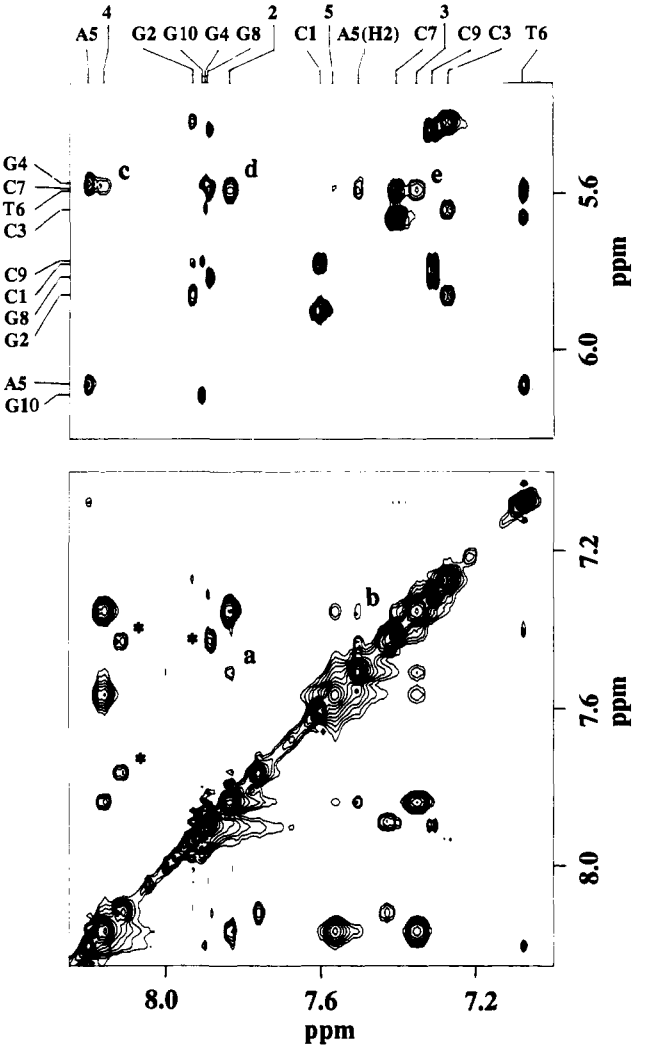

Figure 2. ^1H NOESY spectrum of Δ-$Ru(phen)_3^{2+}$-$[d(CGCGATCGCG)]_2$, molar ratio 0.3, 4 mM duplex at 41 °C, in 10 mM phosphate buffer (D_2O), uncorrected pD = 7.0. Upper panel: aromatic-H1′ region. Lower panel: aromatic-aromatic region. Cross peaks labeled as (a) 2-H2(A5), (b) H2(A5)-3, (c) 4-H1′(G4), (d) 2-H1′(G4/T6/C7), and (e) 3-H1′(T6/C7). Asterisks indicate intramolecular cross peaks from small amounts of Λ-$[Ru(phen)_3]^{2+}$ present. Assignments are indicated for the four nonequivalent protons of Δ-$[Ru(phen)_3]^{2+}$ (2, 3, 4, and 5) and for the aromatic protons H6 and H8 of the bases (horizontally) and the H1′ sugar protons (left-hand side), respectively (cf. Patel et al.[13]). The NOESY spectrum was acquired on a Bruker AM 500 spectrometer in the phase-sensitive mode with TPPI and a mixing time of 450 ms.

Selected regions of a NOESY spectrum of Δ-$[Ru(phen)_3]^{2+}$-$[d(CGCGATCGCG)]_2$ (molar ratio 0.3) are shown

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