Vicinal $^{113}Cd,^{1}H^{\beta}$-Cysteine Coupling in Cd-Substituted Metalloproteins Follows a Karplus-Type Dependence

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Heteronuclear ($^{113}Cd,^{1}H$) multiple quantum coherence (HMQC) has frequently been used to obtain metal–ligand connectivities in cadmium-substituted metalloproteins.[1,2] However, no attempt has been made to obtain information concerning the position of the metal ion. Our NMR studies on ^{113}Cd-substituted metallothionein and ^{113}Cd-substituted rubredoxin indicate a Karplus-type correlation[3] between the $^{3}J(^{113}Cd,^{1}H)$ coupling constants for the cysteine C^{β} protons and the H^{β}–C^{β}–S^{γ}–Cd dihedral angle. Similar relationships have been established for vicinal couplings between lighter nuclei, such as $^{1}H,^{1}H$,[3] $^{15}N,^{1}H$,[4] and $^{13}C,^{1}H$.[4] In this work, we report the first demonstration of such a dependence in a system involving a heavy nucleus, i.e., $^{113}Cd,^{1}H$.

Heteronuclear $^{3}J(^{113}Cd,^{1}H)$ coupling constants were determined for cysteine C^{β} protons in ($^{113}Cd_7$)metallothionein (Cd_7-MT) from rat liver and ^{113}Cd-substituted *Desulfovibrio gigas* rubredoxin (Cd-Rd)[5,6] (see Table I). In both cases, the structures of the metal binding sites have been defined at high resolution by X-ray crystallography. In the case of metallothionein, a structural model of rat liver Zn_2Cd_5-MT at 2.0-Å resolution is available,[7] and comparison with earlier NMR studies on rat liver Cd_7-MT show that both metalloforms exhibit identical molecular architectures.[8] This protein contains three- and four-metal clusters with a total of 12 terminal and 8 bridging cysteine ligands. In this study, $^{3}J(^{113}Cd,^{1}H)$ coupling contants were extracted for terminal cysteines which bind cadmium in the crystallographically defined metalloform (see Table I). The structure of *D. gigas* rubredoxin, which contains a mononuclear $Fe(CysS)_4$ center, is known to 1.4-Å resolution,[9] and we have been able to demonstrate previously that the Cd-derivative is isostructural with the native protein.[10]

Table I. Cysteine $^{3}J(^{113}Cd,^{1}H^{\beta a})$ and $^{3}J(^{113}Cd,^{1}H^{\beta b})$ Coupling Constants for Rat Liver ($^{113}Cd_7$)Metallothionein and *D. gigas* (^{113}Cd)Rubredoxin (10% D_2O/H_2O v/v, pH 7.6, 300 K)

	C^{α}–C^{β}–S^{γ}–Cd (deg)	$^{3}J(^{113}Cd,^{1}H)$ (Hz)[a,b]	
		$H^{\beta a}$	$H^{\beta b}$
		$^{113}Cd_7$-MT	
Cys5	−46.6	51.5 (−166.6)	<1 (73.4)
Cys21	−170.6	<1 (69.4)	5.0 (−50.6)
Cys33	−81.8	47.5 (158.2)	12.5 (38.2)
Cys36	−79.9	74.3 (160.1)	10.3 (40.1)
Cys41	159.8	9.6 (39.8)	<1 (−80.2)
Cys48	−62.9	49.0 (177.1)	15.5 (57.1)
Cys57	−149.8	<1 (90.2)	19.2 (−29.8)
Cys59	−57.3	45.0 (−177.3)	4.0 (62.7)
		^{113}Cd-Rd	
Cys6	−172.0	0.5 (68.0)	3.0 (−52.0)
Cys9	−91.0	38.0 (149.0)	17.0 (29.0)
Cys39	−178.0	0.5 (62.0)	2.5 (−58.0)
Cys42	−94.0	37.0 (146.0)	18.0 (26.0)

[a] Errors are estimated to be approximately 1.5 Hz. [b] The calculated H^{β}–C^{β}–S^{γ}–Cd dihedral angles (ϕ_a and ϕ_b, as illustrated in Figure 1) are given in parentheses.

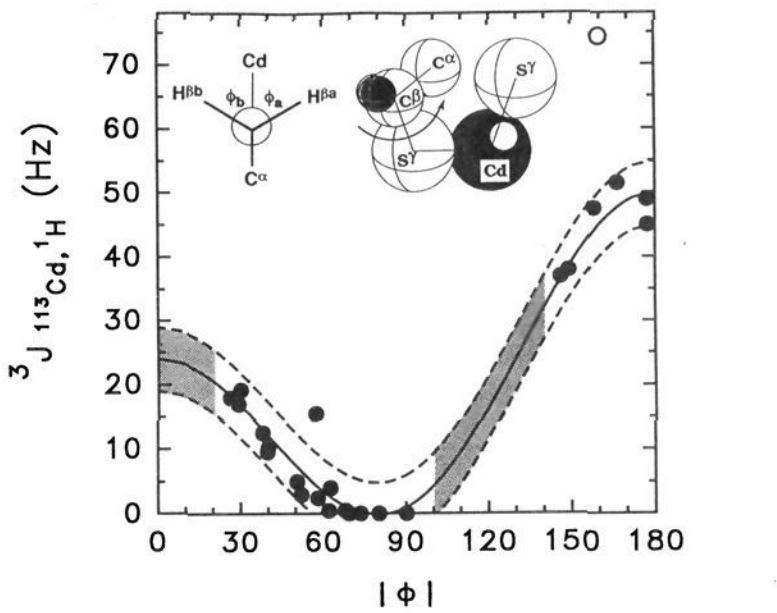

Figure 1. Correlation between the cysteine H^{β} $^{3}J(^{113}Cd,^{1}H)$ coupling constant and the H^{β}–C^{β}–S^{γ}–Cd dihedral angle ϕ. The solid line represents a nonlinear least-squares fit of the form $c(\cos^2\phi) - b(\cos\phi) - a$. The values calculated for the a, b, and c constants are 36, 13, and 1 Hz, respectively, with $r^2 = 98.7\%$. The dashed lines illustrate 95% confidence limits. The point shown as a hollow circle was omitted from the calculation (see text). The shaded areas represent sterically hindered conformations (see text).

Figure 1 illustrates the Karplus-type relationship obtained when the cysteine H^{β} $^{3}J(^{113}Cd,^{1}H)$ coupling constants are plotted against the H^{β}–C^{β}–S^{γ}–Cd dihedral angles (ϕ_a, ϕ_b) (see Figure 1, inset), derived from the respective crystal structures by assuming tetrahedral geometry around C^{α} (see Table I). Although in general heteronuclear couplings involving heavy nuclei depend on orbital angular momentum, electron–nucleus dipole–dipole interaction, and Fermi contact terms,[11] it seems that, in this case, the H^{β}–C^{β}–S^{γ}–Cd dihedral angle is the principal determinant in the latter term and the dominant variable influencing the system. The absence of values in the range of ϕ between approximately 100° and 140° and between 0° and 20° (shaded areas in Figure 1), corresponding to C^{α}–C^{β}–S^{γ}–Cd or H^{β}–C^{β}–S^{γ}–Cd angles of less than about 20°, may be due to steric hindrance caused by the interaction of either C^{α} or H^{β} with the bulky Cd(II) ion or atoms of the adjacent cysteine ligand, resulting in these angles being strongly disfavored (see Figure 1, inset). The data point at 74-Hz coupling (see Figure 1, hollow circle), corresponding to Cys36 $H^{\beta a}$ of Cd_7-MT, clearly lies outside the expected range.

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(5) Rat liver Zn_2Cd_5-metallothionein-2 was isolated as described in ref 13, and the $^{113}Cd_7$-derivative was prepared as in ref 14. Heteronuclear $^{3}J(^{113}Cd,^{1}H)$ coupling contants were determined as in ref 10, using a Bruker AMX 600-MHz NMR spectrometer. Conditions used for the NMR experiments were identical to those given in ref 1b, from which the sequence-specific proton assignments were taken. The stereospecific assignments of cysteine $H^{\beta a}$ and $H^{\beta b}$ in Table I were confirmed on the basis of the $^{3}J(^{1}H^{\alpha},^{1}H^{\beta})$ coupling constants and comparison with the crystal structure data.
(6) ^{113}Cd-substituted *D. gigas* rubredoxin was prepared as in ref 10, in which details of the sequence-specific proton assignments and the determination of the $^{3}J(^{113}Cd,^{1}H)$ coupling contants are given.
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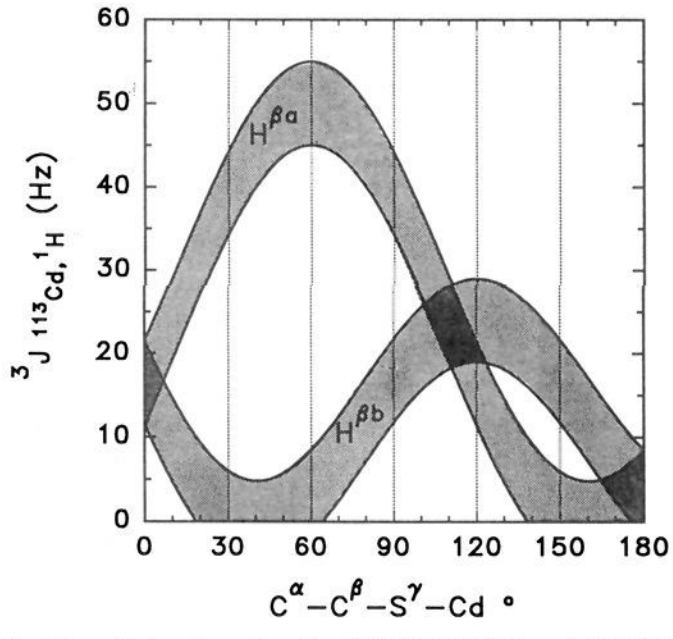

Figure 2. Expected values for the $^3J(^{113}Cd,^1H^{\beta a})$ and $^3J(^{113}Cd,^1H^{\beta b})$ coupling constants over the range of C^α–C^β–S^γ–Cd dihedral angles from 0° to 180°. As the relationship is identical for C^α–C^β–S^γ–Cd angles between 0° and −180° with $H^{\beta a}$ and $H^{\beta b}$ being exchanged, this region of the plot has been omitted.

As the $^3J(^{113}Cd,^1H)$ coupling constant obtained for Cys36 $H^{\beta b}$ is consistent with the relationship (see Figure 1), this unusual behavior is presumably caused by a distorted geometry around C^β.

It should be noted that each possible C^α–C^β–S^γ–Cd angle[12] gives rise to a unique pair of $H^{\beta a}$–C^β–S^γ–Cd and $H^{\beta b}$–C^β–S^γ–Cd angles and hence a unique pair of $^3J(^{113}Cd,^1H)$ coupling contants. In Figure 2, the ranges of the $^3J(^{113}Cd,^1H^{\beta a})$ and $^3J(^{113}Cd,^1H^{\beta b})$ coupling constants expected are defined over the range of possible C^α–C^β–S^γ–Cd dihedral angles. Thus, by determining the magnitudes of these coupling constants, an estimate of the C^α–C^β–S^γ–Cd angle can be obtained. This relationship will clearly be of great use in defining accurately the geometries of metal binding sites in ^{113}Cd-substituted metalloproteins, such as zinc-finger proteins, which contain cysteine ligands. Such information may prove useful as an additional input parameter in three-dimensional structure analysis by NMR methods, permitting a more precise determination of the position of the metal ion.

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(12) The dihedral angle C^α–C^β–S^γ–Cd corresponds to $-\chi^2$ in the protein structure nomenclature.

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