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Calcium-binding proteins afford calibration of dihedral-angle dependence of $^3J_{NC_\gamma}$ coupling constant in aspartate and asparagine residues

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Abstract

Calibration of the $^3J_{NC_\gamma}$ couplings across the N–C_α–C_β–C_γ fragment of aspartate and asparagine residues is afforded by two interactions that produce fixed conformations of the side chains in solution. One is the binding of these side chains to calcium ions; the other is the H-bond interaction of these side chains with a backbone amide.

Keywords: Vicinal couplings; Nitrogen–carbon; Side chain; Conformation; Spin–spin couplings; Asparagine; Aspartate; Metal binding; Calmodulin

Coupling constants across three bonds (vicinal coupling) are of great interest in structural biochemistry because of their dependence on the dihedral-angle [1]. A number of the heteronuclear coupling constants relevant for amino acids in proteins have been calibrated, primarily by Bystrov [2]. Recent measurements of $^3J_{NC_\gamma}$ coupling constants across H-bonds [3–5] have also generated data for the $^3J_{NC_\gamma}$ couplings across the N–C_α–C_β–C_γ fragment of aspartate and asparagine residues. These residues often function as ligands for metal ions or are involved in protein H-bond networks in which they adopt a fixed side-chain conformation. The side-chain conformation depends on the dihedral angle (χ^1) around the C_α–C_β bond, which can be determined from the $^3J_{NC_\gamma}$ coupling constant providing that the angular dependence of the coupling is known.

Vicinal ($^3J_{NC_\gamma}$) couplings have been used for the characterization of the rotameric states of side chains around the χ^1 angle [6–9], predominantly for the aromatic and aliphatic side chains. The amino-acid specific calibration of the angular dependence over the fragment N–C_α–C_β–C_γ has been published by Perez et al. [9]. Based on the small set of data for aspartate and asparagine residues, the staggered states were characterized by 2.5–2.7 and 0.8–1.0 Hz for the *trans* ($\chi^1 = 180°$) and *gauche* ($\chi^1 = \pm 60°$) conformations, respectively. These values were outliers with respect to a Karplus-type angular dependence (consensus curve for the studied amino acids). The only theoretical calculation of the angular dependence of the $^3J_{NC}$ couplings, to our knowledge, is that of Solkan and Bystrov [10] for the peptide fragment N–C′–C_α–C_β, which reports an angular dependence approximated by the equation: $-{}^3J_{NC'} = 2.6\ \cos^2\chi^1_{N\text{–}C'\text{–}C_\alpha\text{–}C_\beta} + 0.6\ \cos\chi^1_{N\text{–}C'\text{–}C_\alpha\text{–}C_\beta}$.

To calibrate the $^3J_{NC_\gamma}$ couplings in aspartate and asparagine residues, we have identified two interactions that may produce fixed conformations of the side chain

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in solution. One is the binding of these side chains to calcium ion in a stable complex, as seen in the EF-hand motifs of calmodulin and parvalbumin (Fig. 1A). The other is the H-bond interaction of the side chain with a backbone amide (Fig. 1B) strong enough to be detected in solution by the $^{h3}J_{NC'}$ coupling across the H-bond. In Table 1, we present examples of the calcium-binding and of the H-bonding dependent conformations of the side chains. The dihedral angles seen in the corresponding X-ray crystal structures are expected to fully apply in solution because the geometry of the interaction must be preserved (coordination geometry of Ca^{2+}) or is experimentally verified as being preserved by the $^{h3}J_{NC'}$ coupling across the H-bond. At the calcium-binding sites the conformation of some side chains, for example, those of residues 51, 53, 90, and 94 in parvalbumin, is fixed by both principles.

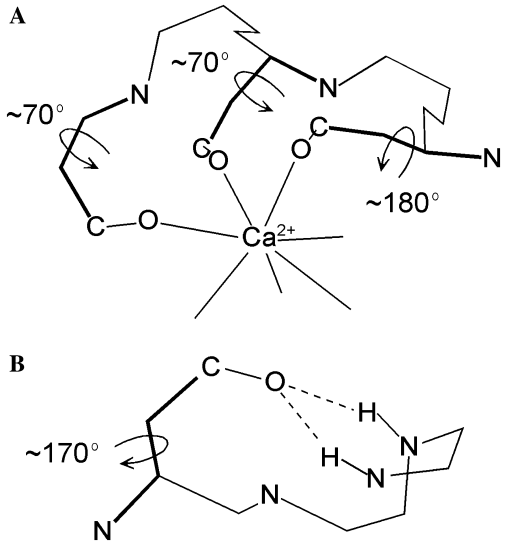


Fig. 1. Fixed side-chain conformations of aspartates and asparagines caused by coordination to Ca^{2+} (A: aspartates 20, 22, and 24, in 1cll.pdb structure) or by H-bonding to backbone amide groups (B: asparagine 25 in 4cpv structure).

The experimental data for the $^{3}J_{NC_\gamma}$ and $^{h3}J_{NC'}$ couplings in parvalbumin, and *apo*-IFABP (intestinal fatty acid binding protein) are from our earlier work [5], while the calmodulin data are new. The couplings were determined using a constant time *J*-HNCO experiment [3,11], as described previously [5]. The coupling constant values were obtained by fitting the time evolution of all observed NC′ couplings for a particular peptide–group nitrogen [12], and the reported experimental errors are standard deviations of the fit.

Based on data in Table 1, the *trans* ($\chi^1 = 180°$) conformation has the coupling constant value of about 3 Hz, which is close to the published value [9]. The overall dependence suggests minimal value of the coupling around $\chi^1 = 90°$, which is consistent with the theoretical

Table 1
$^{3}J_{NC_\gamma}$ and $^{h3}J_{NC'}$ couplings for aspartate and asparagine residues in parvalbumin [5], Ca^{2+}-calmodulin (2 mM ^{13}C,^{15}N-labeled in aqueous solution at pH 7, 20 mM Hepes, 1 mM Mg^{2+}, and 5 mM Ca^{2+}, taken at 25 °C on Bruker 600 MHz spectrometer, equipped with cryo-probe), and intestinal fatty acid binding protein (IFAPB [5])

Residue	$-^{3}J_{NC_\gamma}$ (Hz)	χ^1(°)	Ca^{2+}-bonded at position	H-bonded donor → accep.	$-^{h3}J_{NC'}$ (Hz)
Parvalbumin		4cpv			
Asp51	2.48 ± 0.08	173.1	*X*	56 → 51 s	0.15 ± 0.05
Asp53	0.56 ± 0.07	69.8	*Y*	55 → 53 s	0.30 ± 0.07
Asp90	2.9 ± 0.1	179.7	*X*	95 → 90 s	0.18 ± 0.05
Asp92	0.57 ± 0.05	69.4	*Y*		
Asp94	0.1 ± 0.1	74.7	*Z*	96 → 94 s	0.11 ± 0.05
Asn25	2.40 ± 0.06	173.9		27 → 25 s	0.30 ± 0.05
				28 → 25 s	0.10 ± 0.05
Calmodulin		1cll			
Asp20	2.8 ± 0.1	177.6	*X*		
Asp22	0 ± 0.1	78.2	*Y*		
Asp24	0 ± 0.1	83.7	*Z*		
Asp56	3.2 ± 0.1	−179.1	*X*		
Asp58	0.7 ± 0.2	65.4	*Y*		
Asn60	0 ± 0.1	78.2	*Z*		
Asp93	3.2 ± 0.1	176.4	*X*		
Asp95	0.6 ± 0.2	68.8	*Y*		
Asn97	0 ± 0.1	75.8	*Z*		
Asp129	2.8 ± 0.1	−169.4	*X*		
Asp131	0.6 ± 0.2	68.8	*Y*	139 → 131 s	0.30 ± 0.07
Asp133	0 ± 0.1	71.8	*Z*		
IFABP		1ifb			
Asn98	0.63 ± 0.05	69.0		100 → 98 s	0.22 ± 0.05
Asp74	0 ± 0.05	106.0		76 → 74	0.21 ± 0.05

The side-chain dihedral angles χ^1 are from the corresponding PDB structures (4cpv [16], 1cll [17], and 1ifb [14]).

dependence given by Solkan and Bystrov. Here, this dependence is fit by the following expression

$$^3J_{NC_\gamma} = 3.5\ \cos^2\chi_{N\text{–}C_\alpha\text{–}C_\beta\text{–}C_\gamma} + 0.4\ \cos\chi_{N\text{–}C_\alpha\text{–}C_\beta\text{–}C_\gamma} - 0.2. \quad (1)$$

This is different from the angular dependence obtained by the method of the self-consistent parameterization of the Karplus equation [9], which has a minimum around $\chi^1 = 80°$. However, data for aspartate and asparagine residues from that study fit well into Eq. (1) (Fig. 2, squares). It is conceivable that the angular dependence for aspartate and asparagine residues differs from those of the other amino-acid residues. The angular dependence represented by Eq. (1) also implies change of the coupling sign around $\chi^1 = 90°$, for which we do not have direct experimental evidence (no angles in that range). Interestingly, small negative values of $^3J_{HC'}$ couplings (protein backbone) have been observed [13] at dihedral angles around $-90°$, and the overall angular dependence appears to be similar to one observed here.

The calibration of the angular dependence of the $^3J_{NC_\gamma}$ couplings allows for critical assessment of the conformation of aspartate or asparagine residues in solution. An interesting case is *apo*-IFABP for which two forms were observed by X-ray crystal structure analysis. These forms, *apo*-I (1ifb [14]) and *apo*-II (1ifc [15]), have very similar backbone conformations, but many side-chain conformations differ. According to the experimental $^3J_{NC_\gamma}$ couplings of *apo*-IFABP (Table 2), none of the

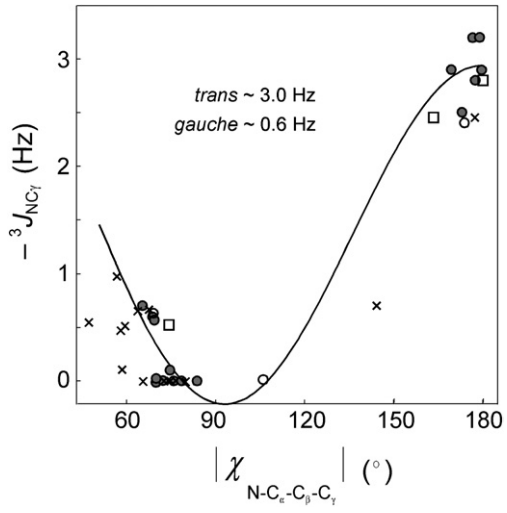


Fig. 2. Dihedral-angle (χ^1) dependence of the $^3J_{NC_\gamma}$ couplings across N–C_α–C_β–C_γ fragment in aspartate and asparagine residues. Data from three proteins (Table 1), represented by solid circles (Ca^{2+}-bonded side chains) and open circles (H-bonded side chains), were used for calibration of Eq. (1). Data from Perez et al. (Asp34, Asn114, Asp127, open squares) are given for comparison. Data from *apo*-IFABP represented by × have dihedral angles taken from the *apo*-I (1ifb) or *apo*-II (1ifc) conformations, as denoted in Table 2.

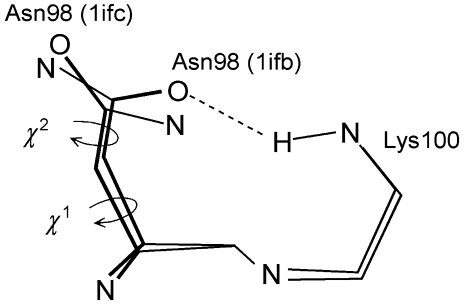
Table 2
$^3J_{NC_\gamma}$ couplings for aspartate and asparagine residues in intestinal fatty acid binding protein (*apo*-IFAPB [5])

Residue	$-{}^3J_{NC_\gamma}$ (Hz)	χ (1ifc) (°)	χ (1ifb) (°)	Preferred in solution
Asp3	0.10 ± 0.05	−41.7	−59.3	1ifb
Asp9	2.41 ± 0.07	176.0	−177.6	1ifc, 1ifb
Asn11	0.53 ± 0.08	−48.2	−49.8	1ifb, 1ifc
Asn13	0.70 ± 0.05	−145.1	−144.2	1ifb, 1ifc
Asp24	0 ± 0.05	75.6	175.0	1ifc
Asp34	0 ± 0.05	65.6	76.1	1ifb
Asn35	0.66 ± 0.05	−76.2	−65.1	1ifb
Asp45	0.51 ± 0.06	−100.3	60.4	1ifb
Asp54	0 ± 0.05	−66.4	−25.4	1ifc
Asp57	0.46 ± 0.05	−58.9	−146.3	1ifc
Asn59	0 ± 0.05	−80.5	−62.5	1ifc
Asp67	0.97 ± 0.05	74.3	−39.7	1ifc ⟺ 1ifb
Asp74	0 ± 0.05	87.5	106.0	1ifb
Asn87	0 ± 0.05	−73.2	72.4	1ifc, 1ifb
Asn98	0.63 ± 0.08	70.5	69.0	1ifb, 1ifc
Asn111	0 ± 0.05	76.6	113.0	1ifc

The side-chain dihedral angles χ^1 are from the corresponding PDB structures (1ifc [15] and 1ifb [14]).

residues are in a state of free rotation, which would correspond to a value ~1.4 Hz (equal occupancy of the staggered conformations). Therefore, they may correspond to some of the conformers seen in two forms of the crystal structures. Indeed, the $^3J_{NC_\gamma}$ coupling constant could, for the most part, be reproduced by the solid sate conformers (Fig. 2). However, deviations from the calibrating curves are overall larger than for data from Table 1, because dihedral angles taken from the solid state conformers do not necessarily apply (either due to increased mobility or change of conformation in solution).

Finally, we should emphasize that in cases when H-bonding of the side chains are detected experimentally, an estimate of the dihedral angle χ^2 is possible. An interesting example is that of Asn98 in *apo*-IFABP. On the basis of its experimentally determined $^3J_{NC_\gamma}$ coupling constant (Table 1), it has a fixed conformation in solution ($\chi^1 = 69°$); we have also detected the H-bond (Lys100 → Asn98s). This requires, $\chi^2_{C_\alpha\text{–}C_\beta\text{–}C'\text{–}O} \sim 50°$ which is compatible only with the structure *apo*-I (Scheme 1). Given the preceding, it is plausible that during X-ray



Scheme 1.

analysis an error was made that switched the identity of the O and N atoms at δ-position of Asn98 in the 1ifc structure, because an almost ideal H-bond geometry would be achieved if the identity is reversed.

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