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ARTICLE

Pressure response of amide one-bond *J*-couplings in model peptides and proteins

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Abstract The pressure dependence of the one-bond indirect spin–spin coupling constants $^1J_{N-H}$ was studied in the protected tetrapeptides Ac-Gly-Gly-Xxx-Ala-NH_2 (with Xxx being one of the 20 proteinogenic amino acids). The response of the $^1J_{N-H}$ coupling constants is amino acid type specific, with an average increase of its magnitude by 0.6 Hz at 200 MPa. The variance of the pressure response is rather large, the largest pressure effect is observed for asparagine where the coupling constant becomes more negative by −2.9 Hz at 200 MPa. The size of the *J*-coupling constant at high pressure is positively correlated with its low pressure value and the β-propensity, and negatively correlated with the amide proton shift and the first order nitrogen pressure coefficient and the electrostatic solvation free energy.

Keywords Tetrapeptide · High pressure · NMR spectroscopy · Random coil · One-bond *J*-coupling · Amide group

Introduction

High pressure nuclear magnetic resonance (HP-NMR) spectroscopy allows to manipulate thermodynamic equilibria of biologically active molecules in a reversible way (for recent reviews see Akasaka 2006 and Kitahara et al. 2013). In biology it can be used to study protein folding/unfolding (Inoue et al. 2000), polymerization (Kachel et al. 2006; Munte et al. 2013) or ligand interactions and drug design (Urbauer et al. 1996; Kalbitzer et al. 2013). Although pressure may influence indirectly all NMR parameters, mainly chemical shift changes and cross peak volume changes in HSQC-spectra are usually considered. For getting meaningful results it is important to separate chemical shift changes of relevant pressure induced protein conformational transitions from more trivial direct compression effects. The subtraction of pressure induced random-coil shift changes from the observed experimental shifts before analyzing the data in detail was a crucial step before analyzing the pressure induced chemical changes in the human prion protein (Kachel et al. 2006) and now represents a well-established procedure in high pressure NMR spectroscopy. The first high pressure NMR data set from model peptides Gly-Gly-Xxx-Ala (with Xxx being one of the 20 proteinogenic amino acids) was introduced by Arnold et al. (2002). More recently, a more detailed chemical shift data base from the protected tetrapeptides Ac-Gly-Gly-Xxx-Ala-NH_2 has been published by Koehler et al. (2012) that also includes hetero atoms. The chemical shifts observed for random-coil peptides usually do not show a linear dependence on pressure but can be fitted with a second order polynomial. The first and second order coefficients B_1 and B_2 (corresponding to a second order Taylor expansion) can be interpreted in thermodynamical terms in a two-state model provided the Gibbs free energy difference $|\Delta G^0| \ll 2\ RT$: the ratio of B_2/B_1 corresponds to $-\Delta\beta'^0/\Delta V^0$, with $\Delta\beta'_0$ the difference of the molar compressibility factors and ΔV^0 the partial molar volume difference (Beck Erlach et al. 2014).

A parameter seldom studied in proteins is the one-bond indirect spin–spin coupling constant $^1J_{N-H}$ of backbone

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amide groups. For now, only a single study of the backbone $^1J_{N-H}$ pressure dependence has been reported for the histidine containing protein (HPr) from *S. carnosus* (Kalbitzer et al. 2000). It shows that the value of $^1J_{N-H}$ varies from residue to residue and exhibits a clear pressure dependence. From theoretical studies it is known that $^1J_{N-H}$ is negative and becomes more negative by hydrogen bonding (Pecul et al. 2000; Sakhayan et al. 2008). Sakhayan et al. (2008) predicted that the magnitude of the one-bond amide coupling constant is linearly dependent on the electric field component parallel to the NH-bond caused by the nearby electric dipoles. A small contribution to the amide *J*-splitting is due to the dynamic frequency shift that depends on the magnetic field and the motional correlation time. With increasing magnetic field and larger rotational correlation times the magnitude of the observed *J*-couplings decreases somewhat (Tjandra et al. 1996). The magnitude of the $^1J_{N-H}$ coupling constants in proteins is somewhat larger and hence the $^1J_{N-H}$ coupling constants are more negative in hydrogen-bonded amide groups (Xiang et al. 2013).

As a reference for residue specific effects in unstructured polypeptides the random-coil model peptides Ac-Gly-Gly-Xxx-Ala-NH_2 appear to be a good choice since already pressure dependent chemical shift data were published (Koehler et al. 2012). It is unknown if the one-bond indirect spin–spin coupling constants $^1J_{N-H}$ of random-coil peptides are pressure dependent, a question we will answer in the following.

Methods

Synthesis of peptides and sample preparation

The tetrapeptides Ac-Gly-Gly-Xxx-Ala-NH_2 were synthesized as described earlier by Koehler et al. (2012). The amino acid Xxx was uniformly ^{15}N and ^{13}C enriched. The peptide concentration was 5 mM in aqueous solution of 90 % H_2O and 10 % D_2O. 20 mM perdeuterated Tris–HCl (tris(hydroxymethyl)-aminomethane hydrochloride) and 0.5 mM DSS (4,4-di-methyl-4-silapentane-sulfonic acid) were added. The pH value was adjusted to 6.7 by adding suitable quantities of HCl or NaOH to the solution. Only the histidine containing peptide was measured at pH 4.0. The pH-values were measured with a glass electrode (Spintrode, Hamilton) and have not been corrected for the deuterium isotope effect.

High pressure system

All high pressure data were recorded with a homebuilt online-pressure system using the Yamada-method (Yamada 1974). Pressure was either applied by a home-made manually operated piston compressor or by an air-to-liquid-pressure intensifier (Barocycler® HUB440, Pressure BioSciences Inc., South Easton, MA, USA) controlled by the spectrometer. The pressure was transmitted via a high pressure line (High Pressure Equipment Company, Linden, PA, USA) by methylcyclohexane or de-ionized water to the high pressure ceramic cell (with an outer diameter of 5 mm and an inner diameter of 3 mm) from Daedalus Innovations LLC (Aston, PA, USA). The high-pressure cell was joined to the high-pressure lines by a safety titan autoclave developed in our laboratory (for details see Koehler et al. 2012). The Barocycler® was coupled via a microprocessor unit with the NMR spectrometer that controlled and monitored the output pressures. In a Bruker Topspin auxiliary (AU) program a user-defined series of high pressure NMR experiments was started including an automated shimming of the sample after changing the pressure before the actual experiment(s) were started. Pressure data were recorded from 0.1 to 200 MPa in steps of 20 MPa.

NMR spectroscopy

Most NMR experiments were carried out on a Bruker 800 MHz Avance spectrometer equipped with a QXI probe at 283 K. Temperature calibration was done before each sample-change via the difference of the resonance lines of the hydroxyl- and methyl-group protons in pure methanol according to Raiford et al. (1979). For the measurements of $^1J_{N-H}$ coupling constants in peptides and proteins a number of different experimental methods have been proposed. The simplest method used here corresponds to a modification of the standard ^1H–^{15}N-HSQC pulse sequence (Davis et al. 1992) with omitting the nitrogen decoupling in the direct dimension. Measuring the coupling constant in the direct dimension has the advantage that the digital resolution is not limiting the accuracy but the amide proton resonances are broadened by exchange with the water signal. Measuring the splittings in the indirect dimension without proton decoupling has the advantage that the lines are considerably narrower but a optimal digital resolution requires long measurement times. The digital resolution in the ^1H and ^{15}N dimensions were 0.12 and 0.16 Hz, respectively. Proton resonances were additionally measured with the PURGE (Presaturation Utilizing Relaxation Gradients and Echoes) sequence (Simpson and Brown 2005) with a digital resolution of 0.02 Hz. ^1H frequencies were referenced to DSS used as internal standard, ^{15}N chemical shifts were referenced indirectly to DSS using a $^{15}N/^1H$–ratio (Wishart et al. 1995) of 0.101329118. Data were filtered with a Lorentzian-to-Gaussian transformation in the direct dimension before quantification. The *J*-couplings were determined by manual peak picking in the δ_2-

Table 1 Experimental amide $^1J_{N-H}$ coupling constants in the tetrapeptides Ac-Gly-Gly-Xxx-Ala-NH_2 at 0.1 MPa and 200 MPa[a]

Xxx	$^1J_{N-H}$ 0.1 MPa (Hz)	$^1J_{N-H}$ 200 MPa (Hz)	Δ^1J_{N-H} (Hz)
Ala	−95.4 ± 0.2	−97.7 ± 0.2	−2.3
Arg	−95.2 ± 0.2	−95.5 ± 0.1	−0.3
Asn	−96.3 ± 0.2	−99.2 ± 0.2	−2.9
Asp	−95.1 ± 0.3	−96.0 ± 0.2	−0.9
Cys	−95.6 ± 0.4	−97.8 ± 0.2	−2.3
Gln	−94.4 ± 0.5	−97.4 ± 0.3	−3.0
Glu	−94.9 ± 0.4	−94.6 ± 0.3	0.3
Gly	−96.4 ± 0.3	−96.8 ± 0.6	−0.4
His[b]	−95.5 ± 0.7	−94.1 ± 0.2	1.3
Ile	−93.7 ± 0.2	−96.0 ± 0.5	−2.3
Leu	−94.6 ± 0.3	−94.5 ± 0.2	0.1
Lys	−94.4 ± 0.2	−94.7 ± 0.7	−0.3
Met	−93.3 ± 0.1	−94.0 ± 0.5	−0.7
Phe	−94.9 ± 0.3	−93.5 ± 0.4	1.4
Ser	−95.3 ± 0.1	−95.1 ± 0.7	0.2
Thr	−94.5 ± 0.4	−94.2 ± 0.6	0.3
Trp	−94.7 ± 0.3	−95.1 ± 0.2	−0.4
Tyr	−93.6 ± 0.2	−94.2 ± 0.4	−0.6
Val	−93.9 ± 0.4	−92.5 ± 0.1	1.4
Mean[c]	−94.8 (0.8)	−95.4 (1.7)	−0.6 (1.4)

[a] 5 mM Ac-Gly-Gly-Xxx-Ala-NH_2 in 20 mM perdeuterated Tris–HCl, pH 6.7, 0.5 mM DSS, 90 % H_2O and 10 % D_2O. The amino acid Xxx was uniformly ^{15}N and ^{13}C enriched. Temperature 283 K. The errors correspond to the standard errors calculated from different measurements (see "Methods")

[b] Data measured at pH 4.0

[c] Values in brackets represent the standard deviation σ

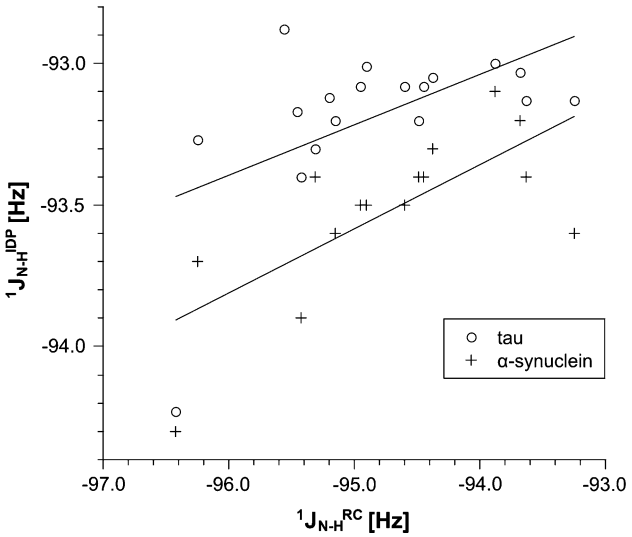

Fig. 1 Correlation between amino acid type specific $^1J_{N-H}$ coupling constants in the model peptide and intrinsically disordered proteins (IDPs). The amino acid specific couplings $^1J_{N-H}^{IDP}$ of the IDPs tau and α-synuclein are plotted as function of the amino acid specific couplings $^1J_{N-H}^{RC}$ of the random-coil model peptides Ac-Gly-Gly-Xxx-Ala-NH_2. The corresponding correlation coefficients are 0.54 and 0.71, respectively

projection using TOPSPIN (Bruker Biopspin, Karlsruhe), by automated peak picking in the 2D-HSQC spectra (AUREMOL, Gronwald and Kalbitzer 2004) and by a new routine implemented in the program AUREMOL that also allows an estimation of the precision of the obtained values. The coupling constants given are the means of the values obtained with the different methods, the errors given represent the corresponding standard errors calculated from the data.

Results and discussion

High pressure NMR-spectroscopy on the model peptides Ac-Gly-Gly-Xxx-Ala-NH_2

Here, we investigate the pressure dependence of $^1J_{N-H}$ coupling constants of backbone amide groups in the ^{15}N, ^{13}C-enriched model peptides Ac-Gly-Gly-Xxx-Ala-NH_2 by ^1H–^{15}N-HSQC spectroscopy. We had reported $^1J_{N-H}$ couplings at ambient pressure earlier but unfortunately the table given contained a number of transcription errors (Koehler et al. 2012) that are corrected here. The individual peptides show significantly different direct amide *J*-couplings at ambient pressure meaning that the kind of side chain of the amino acid Xxx influences the coupling constant. The largest negative values of the coupling constants are observed for Gly (−96.4 Hz) and Asn (−96.3 Hz), the smallest for Ile (−93.7 Hz), Met (−93.3 Hz), Tyr (−93.6 Hz) and Val (−93.9 Hz) (Table 1). This leads to amino acid dependent variation of the *J*-couplings up to 3.1 Hz. As a rule amino acids with small side chains seem to have larger negative values than amino acids with large side chains but there are also exceptions from this simple pattern e.g. the hydrophobic amino acids Leu, and Phe or the hydrophilic amino acid Arg. Charge per se does not appear to be a significant factor since Gln and Glu have almost the same coupling constants (Table 1).

The mean value of the *J*-couplings at ambient pressure is −94.8 Hz, significantly different from the mean values −93.2 and −93.6 Hz reported by Xiang et al. (2013) for the different amino acid types of the intrinsically disordered proteins (IDPs) tau and α-synuclein, respectively. A factor that could partly explain the higher average value of the *J*-couplings in the tetrapeptides is the dynamic frequency shift contribution that should lead to a small decrease (<0.5 Hz) of the negative value of the *J*-coupling constant in proteins (Tjandra et al. 1996). The $^1J_{H-N}$ couplings of the individual amino acid types in our model peptides show an

intermediate to good correlation to the amino acid type specific mean values for the two intrinsically disordered proteins tau and α-synuclein (Fig. 1). The correlation coefficients are 0.54 for the tau protein and 0.71 for α-synuclein. Nevertheless, this indicates that with respect to the $^1J_{H-N}$ coupling the random-coil model peptide behaves differently to the intrinsically disordered proteins. This may be due to sequence specific effects of neighboring amino acids or to residual secondary structures in the IDPs. The last explanation is supported by the fact that the correlations between the amino acid specific *J*-couplings of the two IDPs is with 0.89 rather high indicating that they are determined by similar physical processes. In this interpretation, α-synuclein would be more random-coil like than tau. Another factor that also leads to smaller correlations as expected is the accuracy of the data that is limited in both studies and varies from amino acid to amino acid type.

When pressure is increased, the *J*-couplings change their magnitude, for most of the residues the absolute values of *J*-couplings increase continuously with pressure. Exceptions are Glu, His, Leu, Phe, Ser, Thr and Val but with respect to the experimental errors involved only for Phe and Val a decrease of the magnitude of the *J*-coupling constant can be considered as clearly significant (Table 1). At 200 MPa the spread of *J*-coupling values is significantly increased compared to ambient pressure. The smallest negative value at 200 MPa is found for Val with −92.5 Hz, the largest for Asn with −99.2 Hz (Table 1).

Correlation of the pressure dependence of the amide ^1J-coupling constant with other properties

A general method for finding the possible origin for an experimental observation (in our case the size of the amide *J*-couplings and their pressure dependence) represents correlation analysis (Table 2). There is a good correlation (−0.59) between the size of the *J*-coupling constant at ambient pressure and the proton chemical shift. Such a correlation is predicted by Sahakyan et al. (2008). According to a hybrid density functional theory method applied by the authors both parameters should show a linear dependence on the electric field component parallel to the NH-bond. Essentially, part of the functional form of the electric field dependence of $^1J_{N-H}$-coupling constants is similar to that derived earlier for chemical shifts (Buckingham 1960). In contrast, the nitrogen chemical shifts should not be influenced much by the electric field component parallel to the N–H bond vector. In line with these calculations, the amide nitrogen chemical shifts are only weakly correlated to the $^1J_{N-H}$ coupling constants. If the electric field component is mainly caused by the electric dipole moment of the hydrogen-bonded water, the observed pattern would also agree with the observation that hydrogen bonding leads to a more negative amide *J*-coupling constant and a downfield shift of the amide proton but not to a significant shift change of the nitrogen resonances (Buckingham 1960).

The electric field close to the backbone atoms is related to the solvation dependent electrostatic contribution to the conformational energy (ESF) (Avbelj et al. 2004) and the statistical and thermodynamic β-strand propensities Pβ (Fasman 1989) and ΔΔG (Kim and Berg 1993). Both parameters show significant correlations to the low pressure value of the *J*-couplings (Table 2) but also to the proton chemical shifts at ambient pressure. In addition, high β-strand propensities are associated with strong pressure responses of the amide proton shifts in the random coil peptides indicated by large first and second order pressure coefficients.

The thermodynamic β-propensities ΔΔG give the difference of the free energies for formation of a β-structure from an unfolded structure for a given amino acid X minus the corresponding free energy differences for a glycine residue. In contrast to the statistical β-strand propensities the ΔΔG-values are also correlated to the nitrogen shifts and their pressure response (Table 2). The ratio B_2/B_1 is related to the difference of the compressibility factors $\Delta\beta'$ and the partial molar volumes ΔV (Beck Erlach et al. 2014). It is positively correlated to ΔΔG as well as the size of the pressure induced *J*-coupling changes.

Pressure dependence of amide one-bond couplings in proteins

Comparing the amino acid specific $^1J_{H-N}$ couplings in the tetrapeptides with the amino acid specific average values obtained in folded proteins leads to vanishing correlations: in ubiquitin (Xiang et al. 2013) the correlation coefficient is −0.04, indicating that other factors than the type of amino acid determine the size of the $^1J_{H-N}$ couplings in folded proteins. One of the factors is the existence of internal hydrogen bonds that leads to more negative coupling constants in ubiquitin. For a hydrogen bonded amide group typically a decrease by more than −0.35 Hz relative to the amino acid specific mean value can be observed (Xiang et al. 2013). However, it is not characteristic for a specific type of secondary structure and thus it does not depend significantly on the backbone dihedral angles. As already reported earlier, in well-defined secondary structures of HPr from *S. carnosus* the magnitude of the pressure dependent changes of the one-bond amide coupling constants is positively correlated to the magnitude of the H-bond energies (Kalbitzer et al. 2000).

With increasing pressure the water density increases [at 283 K and 200 MPa by 7.3 % (Chen et al. 1977)]. The

Table 2 Correlation analysis of the pressure dependent one-bond *J*-couplings in Ac-Gly-Gly-Xxx-Ala-NH_2

	$^1J_{N-H}$ 200 MPa	Δ^1J_{N-H}	δ_0^{15N}	B_1^{15N}	B_2^{15N}	δ_0^{1H}	B_1^{1H}	B_2^{1H}	B_2/B_1 ^{15}N	B_2/B_1 ^{1}H	Pβ[a]	ΔΔG[b]
$^1J_{N-H}$ 0.1 MPa	**0.59**	0.12	0.36	**−0.40**	−0.20	**−0.59**	**0.45**	−0.26	−0.38	−0.12	**0.59**	**−0.63**
$^1J_{N-H}$ 200 MPa		**0.87**	0.02	−0.01	**−0.41**	**−0.41**	0.29	−0.23	**−0.49**	−0.16	**0.44**	**−0.44**
Δ^1J_{N-H}			−0.20	0.23	−0.38	−0.15	0.07	−0.13	−0.36	−0.12	0.18	−0.15
δ_0^{15N}				**−0.60**	0.12	−0.23	0.12	−0.04	−0.27	−0.03	−0.05	**−0.61**
B_1^{15N}					**−0.54**	0.14	−0.05	−0.20	−0.13	−0.23	−0.25	**0.51**
B_2^{15N}						**0.42**	−0.37	**0.67**	**0.91**	**0.68**	−0.26	0.26
δ_0^{1H}							**−0.62**	**0.47**	**0.55**	0.36	**−0.82**	**0.51**
B_1^{1H}								**−0.80**	**−0.43**	**−0.65**	**0.60**	**−0.67**
B_2^{1H}									**0.68**	**0.96**	**−0.40**	**0.51**
B_2/B_1 ^{15}N										**0.69**	**−0.42**	**0.51**
B_2/B_1 ^{1}H											−0.28	**0.41**
Pβ[a]												**−0.51**

The Pearson correlation coefficients were calculated for the *J*-coupling data presented in Table 1 and the pressure dependence of amide proton and nitrogen chemical shifts published earlier (Koehler et al. 2012), with δ_0, B_1 and B_2 chemical shift at 0.1 MPa, first and second order pressure coefficients, respectively

Bold letters refer to correlation coefficients ≥0.4, underlined entries refer to correlation coefficients ≥0.8

[a] β-propensities Pβ are taken from Fasman (1989)

[b] Experimentally derived β-propensities ΔΔG are taken Kim and Berg (1993)

compression of the solvent leads to a shortening of hydrogen bonds, a decrease of the O–N distance and in turn to an elongation of the covalent NH-bond (Orekhov et al. 2000). That partly explains the downfield shift of the amide nitrogen resonances with pressure. From the data given by Koehler et al. an average downfield shift of 0.49 ppm at 200 MPa can be observed, that would correspond to a pressure induced lengthening of the NH-bond by 1.2 pm assuming a value of 2.5 pm/ppm (Kuroki et al. 1990).

In summary, in random coil model peptides Ac-Gly-Gly-Xxx-Ala-NH_2 the one-bond *J*-couplings are different for different amino acids and thus depend on the type of their side chains. In general, more negative values are found for small side chains, less negative values for large side chains. However there are some exceptions from this simple rule. The size of the *J*-couplings in the random-coil peptides is positively correlated to their statistical β-strand propensity Pβ and negatively correlated to their thermodynamical β-propensities ΔΔG, that is amino acids that preferentially form β-pleated sheets in folded proteins have less negative values in the random-coil model peptides.

The thermodynamical β-propensities ΔΔG are known to correlate with the electrostatic solvation free energies of the peptide group (ESF) that mainly describes the shielding of a peptide group by its own side chain from water (Avbelj et al. 2004). Again aromatic and β-branched side chains representing class L of amino acids with the highest electrostatic solvation free energies (Penkett et al. 1997; Avbelj and Baldwin 2004) show the largest shielding of the electric field of the water dipols. The ESF and the thus the water dipols close to the amide group determine the electric field parallel to the N–H-bond. This local electric field effect is an important determinant of the $^1J_{N-H}$ coupling constant (Sahakyan et al. 2008) as well as the amide proton shifts but has a small influence in the amide nitrogen shifts. In accordance with that we find a negative correlation between the $^1J_{N-H}$ coupling constant, the amide proton chemical shift, and the thermodynamical β-propensities ΔΔG.

In average, the coupling constants become more negative with pressure by approximately 1 % at 200 MPa, but also a significant increase of the coupling constant can be observed for some amino acids, namely Phe and Val. Usually, pressure would lead to a stronger hydrogen bonding and should thus lead to decreased local electric field component parallel to the NH-bond vector and to a more negative *J*-coupling. However, if the *J*-coupling gets less negative in this model, this could mean that pressure induced solvation free energy may also increase with pressure for these residues. However, only a more detailed analysis of the data and additional experimental evidence would be required.

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References

Akasaka K (2006) Probing conformational fluctuation of proteins by pressure perturbation. Chem Rev 106:1814–1835

Arnold MR, Kremer W, Luedemann H-D, Kalbitzer HR (2002) ^{1}H NMR parameters of common amino acid residues measured in aqueous solutions of the linear tetrapeptides Gly-Gly-X-Ala at pressures between 0.1 and 200 MPa. Biophys Chem 96:129–140

Avbelj F, Baldwin RL (2004) Origin of the neighboring residue effect on peptide backbone conformation. Proc Natl Acad Sci USA 101:10967–10973

Avbelj F, Kocjan D, Baldwin RL (2004) Protein chemical shifts arising from α-helices and β-sheets depend on solvent exposure. Proc Natl Acad Sci USA 101:17394–17397

Beck Erlach M, Koehler J, Moeser B, Horinek D, Kremer W, Kalbitzer HR (2014) Relationship between non-linear pressure induced chemical shift changes and thermodynamic parameters. J Chem Phys B 18:5681–5690

Buckingham AD (1960) Chemical shifts in the nuclear magnetic resonance spectra of molecules containing polar groups. Can J Chem 38:300–307

Chen CT, Fine RA, Miller FJ (1977) The equation of state of pure water determined from sound speeds. J Chem Phys 66: 2142–2144

Davis AL, Keeler J, Laue ED, Moskau D (1992) Experiments for recording pure-absorption heteronuclear correlation spectra using pulsed field gradients. J Magn Reson 98:207–216

Fasman GD (1989) Prediction of protein structure and the principles of protein conformation. Plenum Press, New York

Gronwald W, Kalbitzer HR (2004) Automated structure determination of proteins by NMR spectroscopy. Prog NMR Spectrosc 44:33–96

Inoue K, Yamada H, Akasaka K, Herrmann C, Kremer W, Maurer T, Döker R, Kalbitzer HR (2000) Pressure-induced local unfolding of the ras binding domain of RalGDS. Nat Struct Biol 7:547–550

Kachel N, Kremer W, Zahn R, Kalbitzer HR (2006) Observation of intermediate states of the human prion protein by high pressure NMR spectroscopy. BMC Struct Biol 16:6

Kalbitzer HR, Görler A, Li H, Dubovskii P, Hengstenberg W, Kowolik C, Yamada H, Akasaka K (2000) ^{15}N and ^{1}H NMR study of histidine containing protein (HPr) from *Staphylococcus carnosus* at high pressure. Protein Sci 9:693–703

Kalbitzer HR, Rosnizeck IC, Munte CE, Puthenpurackal Narayanan S, Kropf V, Spoerner M (2013) Intrinsic allosteric inhibition of signaling proteins by targeting rare interaction states detected by high-pressure NMR spectroscopy. Angew Chem Int Ed 52: 14242–14246

Kim CA, Berg JM (1993) Thermodynamic β-sheet propensities measured using a zinc-finger host peptide. Nature 362:267–270

Kitahara R, Hata K, Li H, Williamson MP, Akasaka K (2013) Pressure-induced chemical shifts as probes for conformational fluctuations in proteins. Prog NMR Spec 71:35–58

Koehler J, Beck Erlach M, Crusca E Jr, Kremer W, Munte CE, Kalbitzer HR (2012) On the pressure dependence of ^{15}N chemical shifts in the model peptides Ac-Gly-Gly-X-Ala-NH_2. Materials 5:1774–1786

Kuroki S, Ando S, Ando I (1990) Hydrogen-bonding effect on ^{15}N NMR chemical shifts of the glycine residue of oligopeptides in the solid state as studied by high-resolution solid-state NMR spectroscopy. J Mol Struct 240:19–29

Munte CE, Beck Erlach M, Kremer W, Koehler J, Kalbitzer HR (2013) Distinct conformational states of the alzheimer β-amyloid peptide can be detected by high pressure NMR spectroscopy. Angew Chem Int Ed 52:8943–8947

Orekhov VY, Dubovskii PV, Yamada H, Akasaka K, Arseniev A (2000) Pressure effect on the dynamics of an isolated α-helix studied by ^{15}N–^{1}H NMR relaxation. J Biomol NMR 17:257–263

Pecul M, Leszczynski J, Sadlej J (2000) The shielding constants and scalar couplings in N–H···O=C and N–H···N=C hydrogen bonded systems: an ab initio MO study. J Phys Chem A 104:8105–8113

Penkett CJ, Redfield C, Dodd I, Hubbard J, McBay DL, Mossakowska DE, Smith RAG, Dobson CM, Smith LJ (1997) NMR analysis of main-chain conformational preferences in an unfolded fibronectin-binding protein. J Mol Biol 274:152–159

Raiford DS, Fisk CL, Becker ED (1979) Calibration of methanol and ethylene glycol nuclear magnetic resonance thermometers. Anal Chem 51:2050–2051

Sahakyan AB, Shahkhatuni AG, Shahkhatuni AA, Panosyan HA (2008) Electric field effects on one-bond indirect spin–spin coupling constants and possible biomolecular perspectives. J Phys Chem A 112:3576–3586

Simpson AJ, Brown SA (2005) Purge NMR: effective and easy solvent suppression. J Magn Reson 175:340–346

Tjandra N, Gresziek S, Bax A (1996) Magnetic field dependence of nitrogen-proton *J* splittings in ^{15}N-enriched human ubiquitin resulting from relaxation interference and residual dipolar coupling. J Am Chem Soc 118:6264–6272

Urbauer JL, Ehrhardt MR, Bieber RJ, Flynn PF, Wand AJ (1996) High resolution triple-resonance NMR spectroscopy of a novel calmodulin peptide complex at kilobar pressures. J Am Chem Soc 118:11329–11330

Wishart DS, Bigam CG, Yao J, Abildgaard F, Dyson HJ, Oldfield E, Markley JL, Sykes BD (1995) ^{1}H, ^{13}C and ^{15}N chemical shift referencing in biomolecular NMR. J Biomol NMR 6:135–140

Xiang S-Q, Narayanan RL, Becker S, Zweckstetter M (2013) N–H spin–spin couplings: probing hydrogen bonds in proteins. Angew Chem Int Ed 52:3525–3528

Yamada H (1974) Pressure-resisting glass cell for high-pressure, high-resolution NMR measurement. Rev Sci Instrum 45: 640–642