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Partial alignment and measurement of residual dipolar couplings of proteins under high hydrostatic pressure

Yinan Fu and **A. Joshua Wand***
Johnson Research Foundation and Department of Biochemistry & Biophysics, University of Pennsylvania, Philadelphia, Pennsylvania 19104-6059

Abstract

High-pressure NMR spectroscopy has emerged as a complementary approach for investigating various structural and thermodynamic properties of macromolecules. Noticeably absent from the array of experimental restraints that have been employed to characterize protein structures at high hydrostatic pressure is the residual dipolar coupling, which requires the partial alignment of the macromolecule of interest. Here we examine five alignment media that are commonly used at ambient pressure for this purpose. We find that the spontaneous alignment of Pf1 phage, d(GpG) and a C12E5/*n*-hexnanol mixture in a magnetic field is preserved under high hydrostatic pressure. However, DMPC/ DHPC bicelles and collagen gel are found to be unsuitable. Evidence is presented to demonstrate that pressure-induced structural changes can be identified using the residual dipolar coupling.

Keywords

Residual dipolar coupling; High hydrostatic pressure; Alignment media; NMR spectroscopy; Structure calculation

As a fundamental thermodynamic variable, pressure can be used to modify the free energy landscape of biological macromolecules such as proteins (Weber and Drickamer 1983; Akasaka 2006). Recent developments in high-pressure NMR spectroscopy have allowed for the investigation of fundamental properties involving local protein structure, dynamics, cooperativity and thermodynamics (Fu et al. 2012; Kamatari et al. 2004; Kitahara et al. 2005; Fuentes and Wand 1998; Roche et al. 2012). Indeed, high-resolution protein structure determination under high hydrostatic pressure has been undertaken (Kitahara et al. 2005). However, residual dipolar couplings (RDCs), which arguably form the basis for obtaining structural models of the highest precision and accuracy, have not been employed. Introduction of weak partial alignment in solution NMR recovers dipolar couplings that are normally averaged to zero by macromolecular tumbling (Tjandra and Bax 1997; Tolman et al. 1995). The resulting residual dipolar coupling provides important global orientational information that significantly complements classical short-range restraints obtained from the chemical shift, the scalar coupling constant and the nuclear Overhauser effect in the determination of macromolecular structure (Prestegard et al. 2000; Prestegard et al. 2004;

Corresponding Author Information, wand@mail.med.upenn.edu, Tel: 215-573-7288, Fax: 215-573-7290.

Ethical Standards
A.J.W. declares that the experiments comply with the current laws of U.S.A.

Conflict of Interest
A.J.W. declares a competing financial interest as Member of Daedalus Innovations, LLC, a manufacturer of high-pressure and reverse micelle NMR apparatus.

Bax and Grishaev 2005). RDCs can also provide valuable information regarding internal dynamics over a wide range of timescales (Tolman and Ruan 2006; Meirovitch et al. 2012; Lange et al. 2008). The requisite partial alignment can be achieved by several means such as by taking advantage of intrinsic or attached paramagnetic centers (Prestegard et al. 2004; Barbieri et al. 2002), or by introduction of an external alignment medium that most often acts through anisotropic excluded volume effects (Tjandra et al. 1997). Different types of partial alignment media have been developed to serve this purpose. In some cases, more than one alignment media are preferred in order to generate multiple alignment tensors. Selection of a suitable weak alignment medium for RDC measurement is molecule specific. Many factors influence the efficiency and properties of this weak alignment, including temperature, pH, charge, concentration, detergent compatibility, ionic-strength tolerance and potentially, pressure (Prestegard et al. 2004; Prestegard and Kishore 2001).

In an attempt to extend the application of high-pressure NMR to the measurement of RDCs, we examined the viability of five commonly used alignment media under various pressures up to 2.5 kbar, including Pf1 filamentous bacteriophage (Hansen et al. 1998), the dinucleotide 2′-deoxyguanylyl-(3′,5′)-2′-deoxyguanosine (d(GpG)) (Lorieau et al. 2008), a C12E5/*n*-hexanol mixture (Ruckert and Otting 2000), DMPC/DHPC bicelles (Ottiger and Bax 1999) and collagen gel (Ma et al. 2008). The pressure response of the alignment was monitored by deuterium quadrupolar splittings induced by partially aligning D_2O. Human ubiquitin and β-cyclodextrin bound *E.coli* maltose binding protein (MBP) were used as model proteins to demonstrate the feasibility of obtaining RDCs under pressure. Our results show that Pf1 phage, d(GpG) and C12E5/*n*-hexanol are largely pressure tolerant, making them good candidates for RDC measurement under high hydrostatic pressure. They provide a starting point for developing suitable weak alignment media covering a broad range of experimental conditions under pressure for macromolecules with diverse properties.

Pf1 phage is one of the most commonly used liquid crystalline alignment media. It is a rod-shaped, negatively charged particle that can be used to weakly align negatively charged proteins and nucleic acids with its long axis aligned parallel to the magnetic field (Hansen et al. 1998). It is effective over ranges of temperatures between 5 to 45 °C and pH values between 6.5 and 8.0. Pf1 phage (ASLA Biotech Ltd., Riga, Latvia) was prepared in 10 mM potassium phosphate, 0.02% NaN_3, 10% D_2O, pH 7.6 at a concentration of 24 mg/ml. A modest decrease in ^2H quadrupolar splitting with increasing pressure up to 1000 bar was observed, which remained essentially unchanged from 1000 to 2500 bar (Fig. 1a). The decrease in ^2H splitting with elevated pressure could arise from a number of sources including a reduction in the degree of Pf1 alignment and changes in the properties of Pf1 hydration water at high pressure (Zweckstetter and Bax 2001).

The liquid crystalline phase of d(GpG) provides an alternative negatively charged alignment medium and can be used around and below neutral pH and at temperatures up to 40 °C (Lorieau et al. 2008). Two samples of d(GpG) (Rasayan Inc., Encinitas, CA) were prepared at concentrations of 14.7 and 9.3 mg/ml in 10 mM imidazole, 0.02% NaN_3, 10% D_2O at pH 7.2, containing 23 mM and 15 mM KCl, respectively. In contrast to Pf1 phage, pressure caused a significant increase in the residual ^2H quadrupolar splitting (Figs. 1c and 1d). Indeed, the lower concentration sample displayed isotropic behavior at ambient pressure that was replaced by an effective alignment at higher pressures. It should be noted that, although d(GpG) and Pf1 phage have similar shape and charge, their long axes are aligned differently with Pf1 phage aligned parallel and with d(GpG) orthogonal to the applied magnetic field (Lorieau et al. 2008). This difference in the alignment, in turn, results in different orientations of the hydration water relative to the axes of the alignment media. This may be the origin of the different pressure response of these two alignment media.

Mixtures of *n*-Alkyl-poly (ethylene glycol) and *n*-alkyl alcohol or glucopone and *n*-hexanol also form dilute liquid crystalline phases in aqueous solution (Ruckert and Otting 2000). They are uncharged, insensitive to pH, have little sensitivity to salt and can be used at temperatures ranging up to ~40 °C. A 5% (w/w) C12E5/*n*-hexanol mixture with a C12E5/*n*-hexanol molar ratio (r) of 0.87 was prepared in 10 mM imidazole, 100 mM KCl, 20 mM $CaCl_2$, 0.02% NaN_3, 10% D_2O at pH 6.5 (Ruckert and Otting 2000). ^2H spectra collected from 1 bar to 2.5 kbar at 30 °C indicate that this alignment medium has only a modest sensitivity to elevated pressure (Fig. 1b).

DMPC/DHPC bicelles are perhaps the most popular liquid crystalline alignment medium (Tjandra and Bax 1997). Unfortunately, as might be expected for a large assembly of lipids stabilized by weak interactions, the bicelle-induced alignment showed acute sensitivity to elevated pressure. The effective alignment was abolished as the pressure was increased to 500 bar (Fig. 2). Collagen gel was another alignment medium that we tested. Although it appeared to be pressure resistant, in our hands, the quality of the alignment seemed to be largely compromised by the difficulty in preparing homogeneous samples using commercial rat-tail collagen. Hence, its applicability for high-pressure studies was not investigated in detail.

To further illustrate the utility of d(GpG) and C12E5/*n*-hexanol as suitable weak alignment media for high-pressure studies of proteins, ^1H-^{15}N RDCs of β- cyclodextrin bound *E.coli* MBP and human ubiquitin were measured at elevated pressures (Fig. 3). Comparing the experimental RDCs with those back calculated from a structural model is often used to quantify the quality of the model and *vice versa* (Bax and Grishaev 2005; Valafar and Prestegard 2004). We use this approach to assess the structural distortions of ubiquitin due to pressure perturbation. ^1H-^{15}N RDCs collected at 1, 1200 and 2500 bar are plotted against those back calculated from the ambient-pressure ubiquitin solution structure (PDB 1D3Z) (Cornilescu et al. 1998) (Fig. 4 and Table S2). They correspond to Q factors (Cornilescu et al. 1998) of 0.09, 0.13 and 0.18 respectively, indicating that the agreement between the observed RDCs and the ambient structure decreases as the pressure increases. At ambient pressure, as previously noted (Cornilescu et al. 1998), the largest deviations between the observed and the calculated RDCs are associated with residues that are located in or next to the flexible loop regions (Fig. 5a), underlining the sensitivity of RDC to motion. At elevated pressures, residues sandwiched between the α-helix and β-sheet in the structural core of the protein display the largest deviations (Figs. 5b and 5c). These regions of ubiquitin correspond to those previously highlighted as pressure-induced structural changes in a solution structure determined using classical NMR restraints at 3 kbar (Kitahara et al. 2005).

In conclusion, we have examined five widely used alignment media for their suitability for solution NMR spectroscopy under elevated pressure. Pf1 phage, d(GpG) and C12E5/*n*-hexanol are largely pressure tolerant. The alignment of these three alignment media is stable under pressure for at least 12 hours, as seen by the unchanged ^2H splitting observed at 2.5 kbar overnight. The ^2H lineshape is unaffected by high pressure indicating that the homogeneity of the weak alignment is preserved. They cover a wide range of experimental conditions and sample characteristics and thus should be generally useful for high-pressure NMR studies of proteins and nucleic acids. Although pressure-induced RDC change can be used to identify regions of pressure sensitivity within a protein molecule, whether these changes are due to changes in protein structure or dynamics needs to be carefully examined. In addition, the application of a significant perturbation such as high hydrostatic pressure also requires consideration of the potential for a change in the character of the interaction with the alignment medium. Nevertheless, with these qualifications in mind, the addition of residual dipolar couplings to the library of restraints that are available for characterization of macromolecular structure at elevated pressure is anticipated to have the same impact on the

quality of structural models that can be obtained as they have for structures determined at ambient pressure (Bax and Grishaev 2005).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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References

Akasaka K. Probing conformational fluctuation of proteins by pressure perturbation. Chem Rev. 2006; 106:1814–1835. [PubMed: 16683756]

Barbieri R, Bertini I, Cavallaro G, Lee YM, Luchinat C, Rosato A. Paramagnetically induced residual dipolar couplings for solution structure determination of lanthanide binding proteins. J Am Chem Soc. 2002; 124:5581–5587. doi: [PubMed: 11996601]

Bax A, Grishaev A. Weak alignment NMR: a hawk-eyed view of biomolecular structure. Curr Opin Struc Biol. 2005; 15:563–570. doi:

Cornilescu G, Marquardt JL, Ottiger M, Bax A. Validation of protein structure from anisotropic carbonyl chemical shifts in a dilute liquid crystalline phase. J Am Chem Soc. 1998; 120:6836–6837. doi:

Fu YN, Kasinath V, Moorman VR, Nucci NV, Hilser VJ, Wand AJ. Coupled Motion in Proteins Revealed by Pressure Perturbation. J Am Chem Soc. 2012; 134:8543–8550. doi: [PubMed: 22452540]

Fuentes EJ, Wand AJ. Local stability and dynamics of apocytochrome b(562) examined by the dependence of hydrogen exchange on hydrostatic pressure. Biochemistry. 1998; 37:9877–9883. doi: [PubMed: 9665691]

Hansen MR, Mueller L, Pardi A. Tunable alignment of macromolecules by filamentous phage yields dipolar coupling interactions. Nat Struct Biol. 1998; 5:1065–1074. doi: [PubMed: 9846877]

Kamatari YO, Kitahara R, Yamada H, Yokoyama S, Akasaka K. High-pressure NMR spectroscopy for characterizing folding intermediates and denatured states of proteins. Methods. 2004; 34:133–143. doi: [PubMed: 15283922]

Kitahara R, Yokoyama S, Akasaka K. NMR snapshots of a fluctuating protein structure: Ubiquitin at 30 bar-3 kbar. J Mol Biol. 2005; 347:277–285. doi: [PubMed: 15740740]

Lange OF, Lakomek NA, Fares C, Schroder GF, Walter KFA, Becker S, Meiler J, Grubmuller H, Griesinger C, de Groot BL. Recognition dynamics up to microseconds revealed from an RDC-derived ubiquitin ensemble in solution. Science. 2008; 320:1471–1475. doi: [PubMed: 18556554]

Lorieau J, Yao LS, Bax A. Liquid crystalline phase of G-tetrad DNA for NMR study of detergent-solubilized proteins. J Am Chem Soc. 2008; 130:7536–7537. doi: [PubMed: 18498162]

Ma JH, Goldberg GI, Tjandra N. Weak Alignment of Biomacromolecules in Collagen Gels: An Alternative Way to Yield Residual Dipolar Couplings for NMR Measurements. J Am Chem Soc. 2008; 130:16148–16149. doi: [PubMed: 18998689]

Meirovitch E, Lee D, Walter KFA, Griesinger C. Standard tensorial analysis of local ordering in proteins from residual dipolar couplings. J Phys Chem B. 2012; 116:6106–6117. [PubMed: 22512459]

Ottiger M, Bax A. Bicelle-based liquid crystals for NMR-measurement of dipolar couplings at acidic and basic pH values. J Biomol NMR. 1999; 13:187–191. doi: [PubMed: 10070759]

Prestegard JH, Al-Hashimi HM, Tolman JR. NMR structures of biomolecules using field oriented media and residual dipolar couplings. Q Rev Biophys. 2000; 33:371–424. doi: [PubMed: 11233409]

Prestegard JH, Bougault CM, Kishore AI. Residual dipolar couplings in structure determination of biomolecules. Chem Rev. 2004; 104:3519–3540. doi: [PubMed: 15303825]

Prestegard JH, Kishore AI. Partial alignment of biomolecules: an aid to NMR characterization. Curr Opin Chem Biol. 2001; 5:584–590. doi: [PubMed: 11578934]

Roche J, Caro JA, Norberto DR, Barthe P, Roumestand C, Schlessman JL, Garcia AE, Garcia-Moreno BE, Royer CA. Cavities determine the pressure unfolding of proteins. Proc Natl Acad Sci USA. 2012; 109:6945–6950. [PubMed: 22496593]

Ruckert M, Otting G. Alignment of biological macromolecules in novel nonionic liquid crystalline media for NMR experiments. J Am Chem Soc. 2000; 122:7793–7797. doi:

Tjandra N, Bax A. Direct measurement of distances and angles in biomolecules by NMR in a dilute liquid crystalline medium. Science. 1997; 278:1111–1114. doi: [PubMed: 9353189]

Tjandra N, Omichinski JG, Gronenborn AM, Clore GM, Bax A. Use of dipolar H-1-N-15 and H-1-C-13 couplings in the structure determination of magnetically oriented macromolecules in solution. Nat Struct Biol. 1997; 4:732–738. doi: [PubMed: 9303001]

Tolman JR, Flanagan JM, Kennedy MA, Prestegard JH. Nuclear Magnetic Dipole Interactions in Field-Oriented Proteins - Information for Structure Determination in Solution. Proc Natl Acad Sci USA. 1995; 92:9279–9283. doi: [PubMed: 7568117]

Tolman JR, Ruan K. NMR residual dipolar couplings as probes of biomolecular dynamics. Chem Rev. 2006; 106:1720–1736. [PubMed: 16683751]

Valafar H, Prestegard JH. REDCAT: a residual dipolar coupling analysis tool. J Magn Reson. 2004; 167:228–241. doi: [PubMed: 15040978]

Weber G, Drickamer HG. The effect of high-pressure upon proteins and other biomolecules. Q Rev Biophys. 1983; 16:89–112. [PubMed: 6348832]

Zweckstetter M, Bax A. Characterization of molecular alignment in aqueous suspensions of Pf1 bacteriophage. J Biomol NMR. 2001; 20:365–377. doi: [PubMed: 11563559]

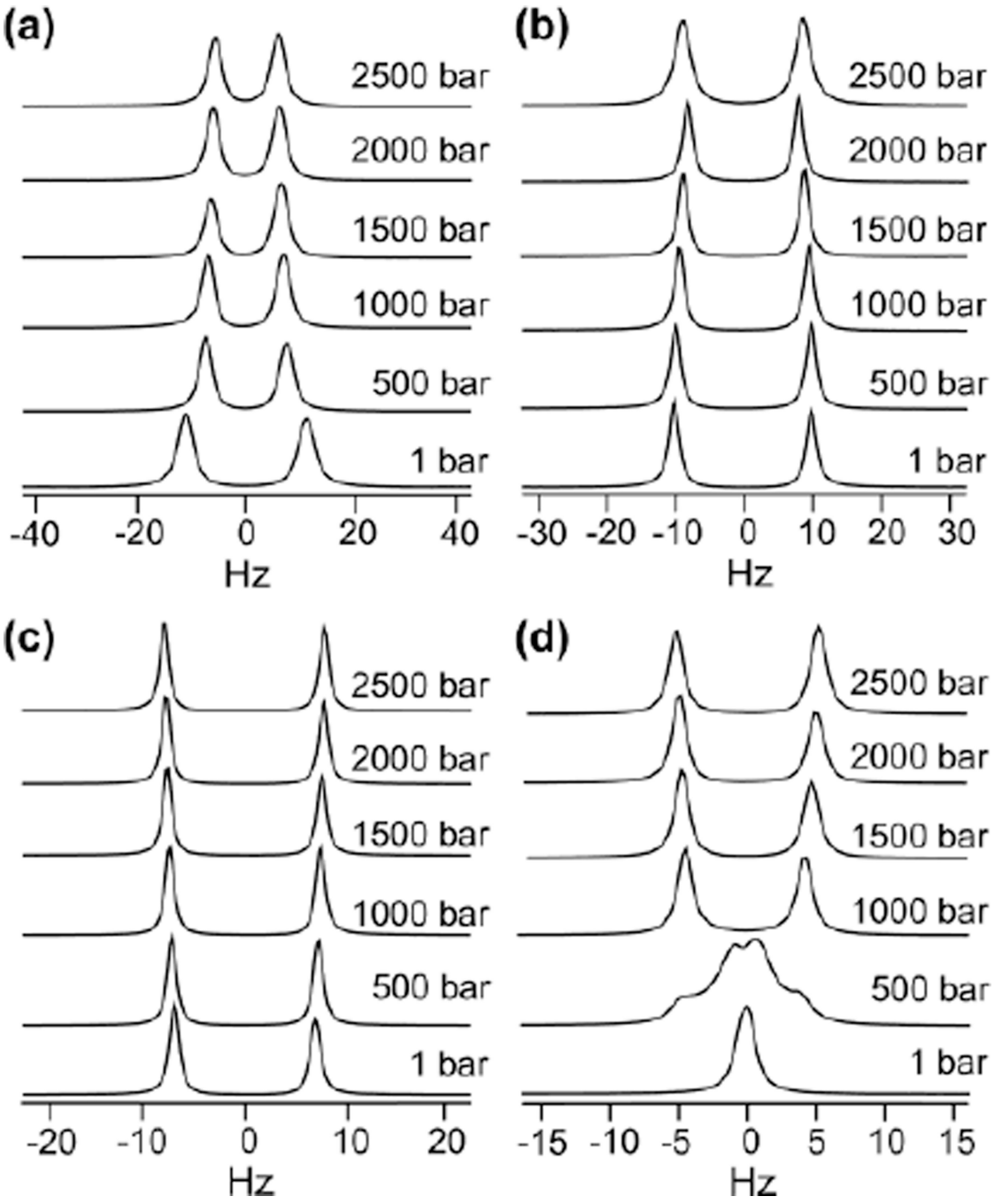

Fig. 1.

Pressure response of the effective alignment of Pf1 phage, C12E5/*n*-hexanol and d(GpG). **a** One dimensional ^{2}H spectra of a solution of 24 mg/ml Pf1 phage at 25 °C under various pressures. **b** ^{2}H spectra of a solution of 5% (w/w) C12E5/*n*-hexanol (r = 0.87) at 30 °C under various pressures. **c** and **d** ^{2}H spectra of solutions of 14.7 mg/ml and 9.3 mg/ml d(GpG) at 37 °C under various pressures, respectively. Spectra were obtained using a 3.0 mm i.d. high-pressure NMR cell rated to 2.5 kbar and an Xtreme 60 high-pressure generator (Daedalus Innovations, Aston, Pennsylvania). Spectra were acquired with a Bruker Avance III 600 MHz NMR spectrometer equipped with a triple resonance cryoprobe

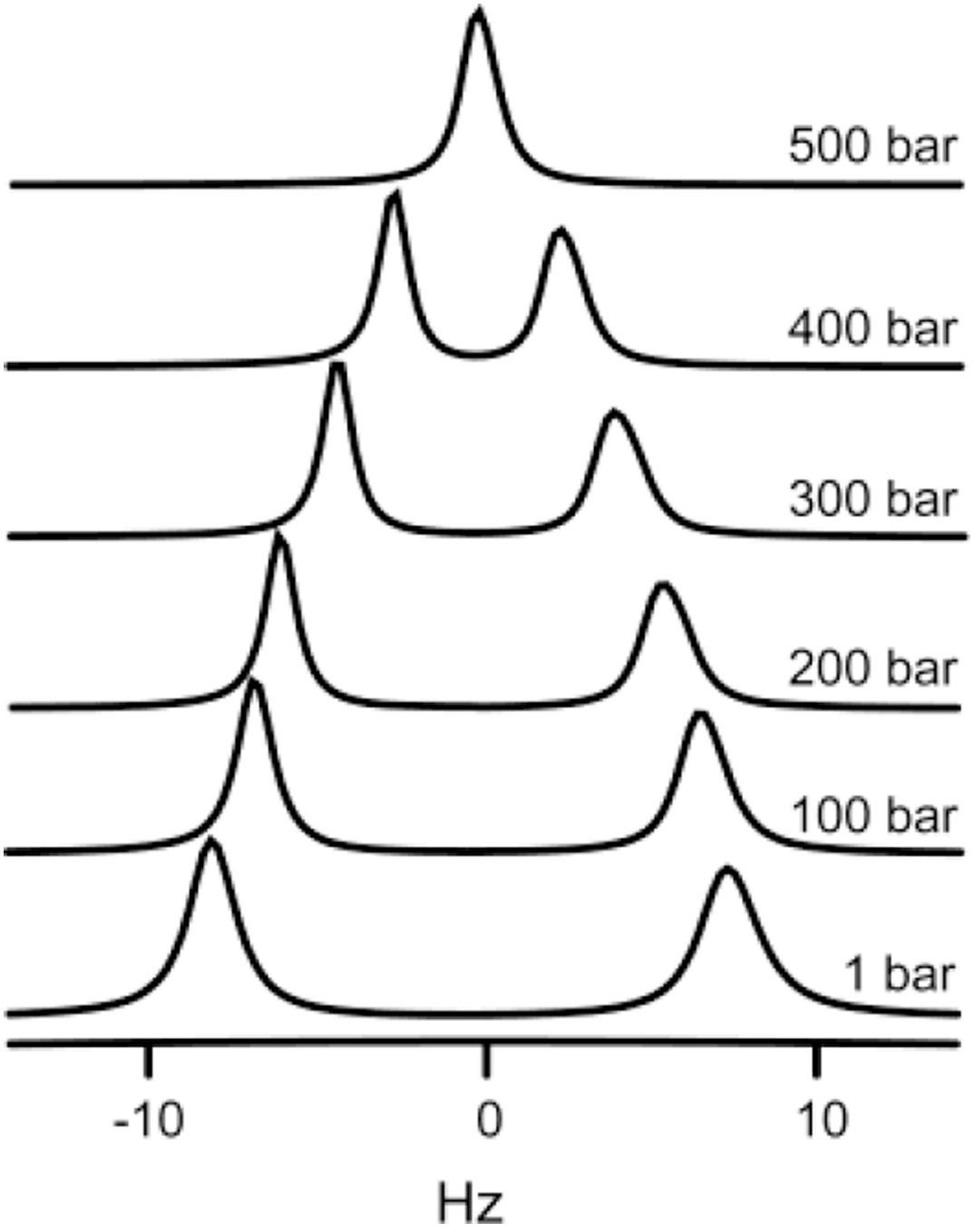

Fig. 2.

Sensitivity of DMPC/DHPC bicelles induced partial alignment to elevated pressure. Series one dimensional ^{2}H spectra were collected at 37 °C on a 13.3% bicelle solution with a DMPC/DHPC molar ratio of 3:1 in 10 mM imidazole, 0.02% NaN_3, 10% D_2O at pH 7.15

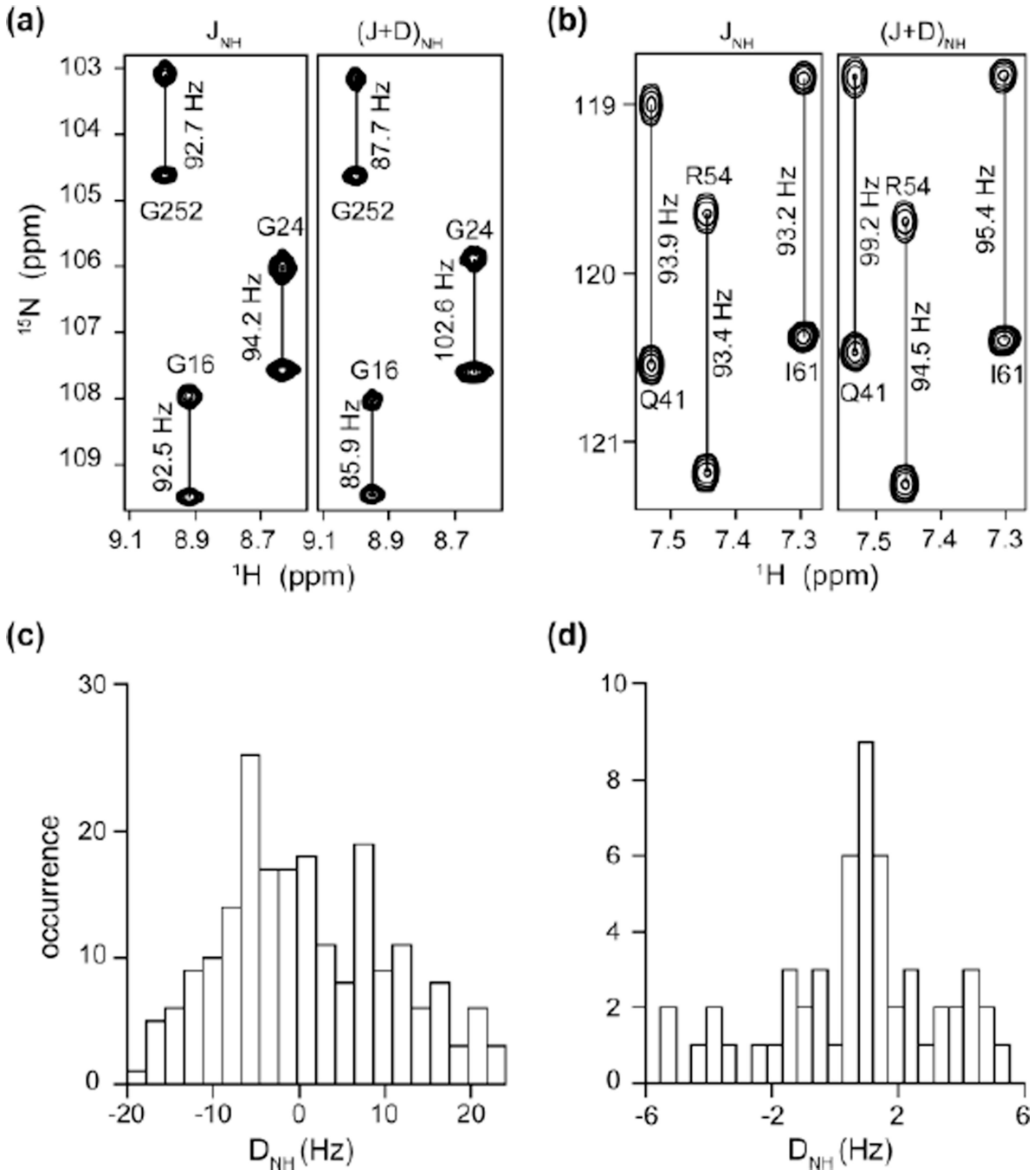

Fig. 3.

Measurement of ^{1}H-^{15}N RDCs at 2.5 kbar of **a** the MBP:β- cyclodextrin complex in d(GpG) (9.3 mg/ml) at pH 7.2 and 37 °C and **b** ubiquitin in 5% (w/w) C12E5/*n*-hexanol (r = 0.87) at pH 6.5 and 24 °C. Shown are selected expansions of ^{1}H-^{15}N IPAP HSQCs collected on the unaligned and partially aligned proteins. Distribution of RDCs at 2.5 kbar of **c** the MBP:β-cyclodextrin complex and **d** ubiquitin are also shown

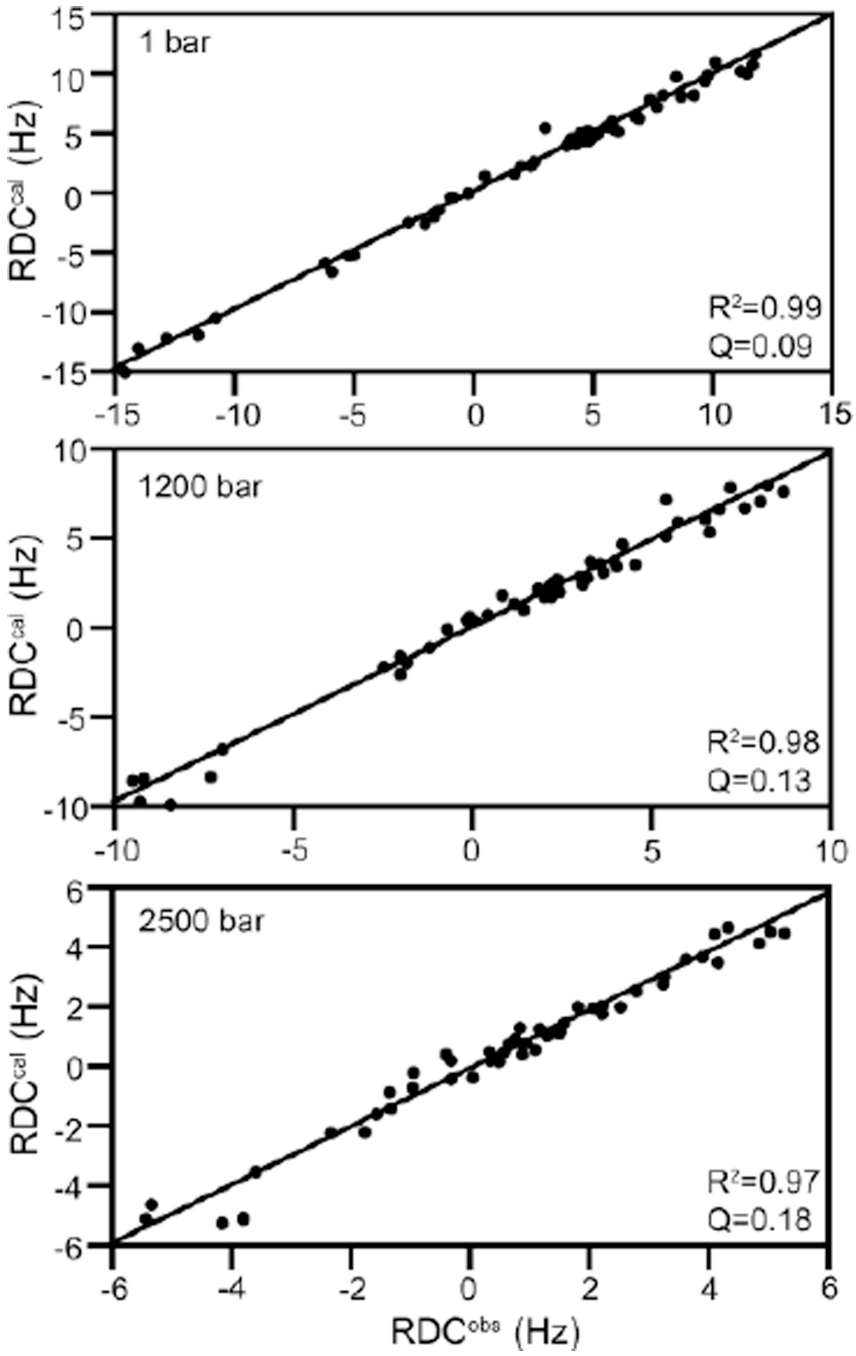

Fig. 4.

Correspondence between ^{1}H-^{15}N RDCs of ubiquitin measured at various pressures with those predicted by the reference structure (1D3Z) (Cornilescu et al. 1998) obtained at 1, 1200 and 2500 bar. Highly disordered residues 72–76 were excluded from the analysis

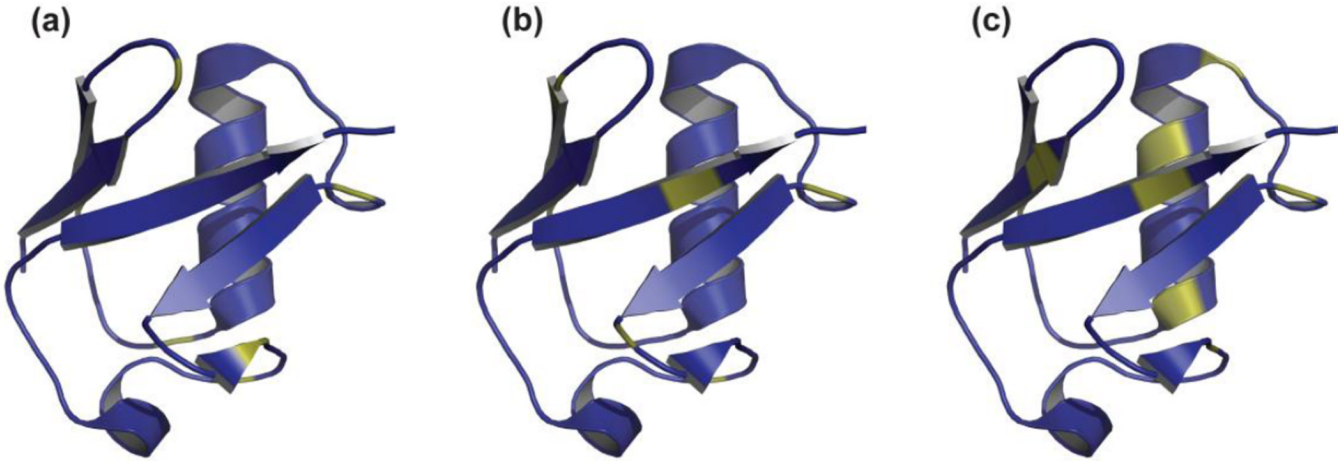

Fig. 5.

Pressure-induced structural change in ubiquitin detected by ^{1}H-^{15}N RDCs. The sites that show the largest deviations between the observed RDCs and those calculated using the ambient pressure solution structure (1D3Z) (Cornilescu et al. 1998) are highlighted in yellow for **a** 1, **b** 1200 and **c** 2500 bar