FOR THE RECORD

H-bonding mediates polarization of peptide groups in folded proteins

NENAD JURANIĆ, SLOBODAN MACURA, AND FRANKLYN G. PRENDERGAST

Department of Biochemistry and Molecular Biology, Mayo Graduate School, Mayo Clinic and Foundation, Rochester, Minnesota 55905, USA

(RECEIVED April 7, 2003; FINAL REVISION June 4, 2003; ACCEPTED June 18, 2003)

Abstract

The carbon-nitrogen J-couplings in the hydrogen bonding chains of proteins show that H-bonding mediates peptide-group polarization, which results in the general reduction of peptide-group polarity of folded proteins in solution. The net effect is to make large regions of protein secondary structure, especially β -sheets, intrinsically more hydrophobic, contributing thereby to overall stability of the tertiary structure.

Keywords: Protein H-bond networks; peptide-group polarizations; NMR spin-spin coupling

Supplemental material: See www.proteinscience.org

The geometry of protein backbone H-bond networks has been considered to be determined mainly by the polarity of the peptide groups. The peptide-group dipole alignment (Liwo et al. 1993; Pillardy et al. 2001) results in formation of extended H-bond chains (HB chains). Because of the partial double-bond character of the peptide bond, the peptide groups in HB chains are prone to polarization by H-bonding (Scheiner and Wang 1993; Milner-White 1997). The high-resolution crystal structures of small model compounds (Jeffrey et al. 1980) and of proteins (Esposito et al. 2000) suggest structural perturbation of the peptide group consequent on such polarization (Juranić et al. 2002). Although the structural perturbations are at the resolution limit of protein crystal structures, the electronic polarizations of peptide groups are readily observed in solution by NMR spectroscopy (Juranić et al. 1995).

Direct observation of the protein-backbone HB chains and their polarizations in solution is enabled by the combined use of the nuclear spin-spin couplings across the H-bonds ($^hJ_{NC'}$; Cordier and Grzesiek 1999; Cornilescu et al.

1999), and through the peptide bonds ($^1J_{NC'}$; Delaglio et al. 1991; Juranić et al. 1995, 1996, 2002; Juranić and Macura 2001). Here we present new properties of protein H-bond networks that emerged from analysis of the spin-spin couplings in the HB chains of human ubiquitin, carp parvalbumin (*holo*-CPV), and rat intestinal fatty acid binding protein (*apo*-IFABP).

Results

We have detected (Juranić and Macura 2001; Juranić et al. 2002) the HB chains by the connected sequence of the nitrogen-carbon spin-spin couplings within peptide-bonds ($^1J_{NC'}$) and across H-bonds ($^hJ_{NC'}$) at the protein backbone (Fig. 1).

Generally, the inner regions of protein secondary structure with extended HB chains have lower $^1J_{NC'}$ and higher $^hJ_{NC'}$ couplings than peripheral regions exposed to water (Fig. 2). The main determinant for elevated $^1J_{NC'}$ couplings (>15 Hz) appears to be water exposure of the backbone carbonyl oxygen. Molecular dynamics simulations of ubiquitin with explicit water showed that an elevated peptide bond coupling ($^1J_{NC'} \sim 16$ Hz) is associated with the short H-bond distances between water molecules and the carbonyl oxygen (Juranić et al. 1996). The H-bonding of car-

Reprint requests to: Nenad Juranić, Department of Biochemistry and Molecular Biology, Mayo Graduate School, Mayo Clinic and Foundation, Rochester, MN 55905, USA; e-mail: juranic.nenad@mayo.edu.

Article and publication are at <http://www.proteinscience.org/cgi/doi/10.1110/ps.03127103>.

Figure 1. Carbon-nitrogen J-couplings in HB chains of protein backbone. HB chain connects several strands in protein secondary structure (red box) and are detected in solution by the sequence of J-couplings: $-^1J_{NC'(-1)}-h^3J_{NC'(-1)}-^1J_{NC'}-h^3J_{NC'}-^1J_{NC'(+1)}-$. The HB chains of protein backbone ([left, up] ubiquitin, 1ubq.pdb; Vijay-Kumar et al. 1987; [right] apo-IFABP, 1ifc.pdb; Scapin et al. 1992) exhibit a range of the J-coupling values (color coded, numerical data were published elsewhere; Juranić et al. 2002).

bonyl oxygen to water requires a pronounced curvature of the protein backbone, as found in reverse turns and α -helices. Accordingly, the highest $^1J_{NC'}$ couplings (>16 Hz) are observed in reverse turns of the studied proteins (Juranić et al. 1995, 1996). In α -helices, elevated $^1J_{NC'}$ couplings are observed for the side of the helix that is exposed to water (Fig. 2). Analogous behavior is observed for all helices in parvalbumin and ubiquitin (Supplemental Material, Fig. S1). These two proteins have well-defined hydrophobic cores to which the orientation of the helices could be related. Similar evidence of the H-bonding between water and backbone carbonyl groups in the exposed regions of the

helices has been reported in an IR study of helical peptides (Manas et al. 2000).

The $^1J_{NC'}$ couplings of unfolded proteins are of great interest for the proper assessment of how peptide group polarization is related to protein folding. However, their measurement is hampered by spectral overlap and we therefore lack these data. We have determined the $^1J_{NC'}$ couplings in a series of dipeptides (Supplemental Material, Table S1) and found large (~17 Hz) and almost uniform couplings. The large couplings are consistent with pronounced polarization of peptide groups due to water solvation. The uniformity of the values suggests that a short range effect (neighboring side chains) may not cause much dispersion of $^1J_{NC'}$ couplings in unfolded proteins.

Figure 2. α Helix of carp-parvalbumin (*holo*-CPV, 4cpv.pdb; Kumar et al. 1990) exposed to water and hydrophobic core. Peptide groups whose carbonyl oxygen is exposed to water have the highest $^1J_{NC'}$ couplings and lowest $h^3J_{NC'}$ ([blank box] below detection, <0.05 Hz; color coding same as in Figure 1). The couplings oscillate along the peptide sequence (gray thick line), but vary monotonously in the HB chains (black thin lines).

Discussion

Proton donation to the carbonyl oxygen via H-bonding promotes a more polar form of the peptide bond (Jeffrey et al. 1980; Scheiner and Wang 1993; Juranić et al. 2002). In proteins, the polarization generally depends on the water accessibility of the backbone carbonyl oxygen, because water molecules are much better proton donors (Eberhardt and Raines 1994) than NH groups (Fig. 1). High $^1J_{NC'}$ coupling constants (>15 Hz) characterize peptide groups structurally shifted toward the “polar” form, whereas low $^1J_{NC'}$, often combined with high $h^3J_{NC'}$, indicates peptide groups that are shifted toward the “nonpolar” form (Fig. 3).

In helices, a number of the backbone carbonyl oxygens are exposed and, hence, easily attacked by solvent water. The respective peptide groups are polarized via H-bonding

Figure 3. The J-couplings reflect electronic structure of HB chains. Polarization of peptide group strongly affects the $^1J_{NC'}$ -coupling. The extreme values for model compounds in the fully "polar" (acetamide hydrochloride in TFAA; Juranić et al. 2002) and fully "nonpolar" (twisted amides in organic solvents; Yamada 1996) forms are 21 and 4 Hz, respectively. Indicated dipole moments (magenta arrows) correspond to gaseous (non-polar) and to aqueous (polar) *N*-methyl-acetamide (Tanner et al. 1994). The same electronic charge distribution causing high $^1J_{NC'}$ diminishes $^hJ_{NC'}$ coupling due to the competition for *s* electron density between the peptide bond and H-bond couplings (Juranić and Macura 2001; Juranić et al. 2002). The low $^1J_{NC'}$ -combined with high $^hJ_{NC'}$ indicates H-bond between nonpolar peptide groups.

to water, and polarization may propagate along the HB chains of the helix. This will increase the hydrophilicity of the protein backbone and potentially destabilize the internal H-bonds of the helix. A recent molecular mechanics study of protein folding strongly connects the backbone desolvation to stabilization of backbone H-bonds (Fernandez et al. 2003). In contrast to helices, the extended plates of β -sheets have the backbone peptide groups in a close to coplanar arrangement and carbonyl-oxygen-to-water H-bonding is sterically restricted. Therefore, HB chains shift toward the nonpolar form evident in mainly low $^1J_{NC'}$ and high $^hJ_{NC'}$. The protein backbone of the β -sheet structures is thus intrinsically more hydrophobic and resistant to solvent influence. This same principle would contribute to irreversibility of the β -sheets structure formation, which appears to be a key factor in the protein misfolding (Soto 2003).

The peptide group polarizations that emerged from the analysis of the spin-spin couplings in the HB chains point to stabilization of protein H-bond networks upon increased hydrophobicity of the protein backbone. These agree with the finding that protein backbone desolvation stabilizes backbone H-bonds (Fernandez et al. 2003). Protein backbone solvation has been considered as the major factor in determining propensities of amino acids in the secondary structure elements of protein. The propensities of helices were related to stabilizing energetic of the water-backbone H-bonds (Luo and Baldwin 1999; Avbelj et al. 2000). However, β propensities were found less related to stabilization

by the backbone solvation than to hydrophobicity (Avbelj and Baldwin 2002). A complexity of the H-bond involvement in protein-fold stabilization probably arises from the fact that in the process of protein folding, the H-bonds between water and the protein backbone are replaced (apparently) by energetically unfavorable backbone H-bonds. Compensation of that energy loss may be quite complex and may involve cooperativity of H-bonding. Recent calculations have found that the energy per H-bond in an "infinite" HB chain is more than twice that of an isolated H-bond between two peptide groups (Ireta et al. 2003). From our analysis, it is likely that the compensation comes also via adjustment of peptide group polarity to that of the surroundings. Lower polarity (less charge separation) in a nonpolar environment is energetically beneficial. This inference touches on an early, unexpected result (Roseman 1988) that the peptide group of *N*-methylacetamide is energetically insensitive to the transfer from water to CCl_4 . That finding is consistent with peptide group polarity adjustment to solvent polarity; that is, $^1J_{NC'}$ couplings of peptide groups change from ~16 to ~13 Hz from polar to nonpolar solvent (Juranić et al. 1995).

Acknowledgments

The publication costs of this article were defrayed in part by payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

References

- Avbelj, F. and Baldwin, R.L. 2002. Role of backbone solvation in determining thermodynamic β propensities of the amino acids. *Proc. Natl. Acad. Sci.* **99**: 1309–1313.
- Avbelj, F., Luo, P.Z., and Baldwin, R.L. 2000. Energetics of the interaction between water and the helical peptide group and its role in determining helix propensities. *Proc. Natl. Acad. Sci.* **97**: 10786–10791.
- Cordier, F. and Grzesiek, S. 1999. Direct observation of hydrogen bonds in proteins by residues (3h)J(NC') scalar couplings. *J. Am. Chem. Soc.* **121**: 1601–1602.
- Comilescu, G., Hu, J.S., and Bax, A. 1999. Identification of the hydrogen bonding network in a protein by scalar couplings. *J. Am. Chem. Soc.* **121**: 2949–2950.
- Delaglio, F., Torchia, D., and Bax, A. 1991. Measurements of 15N-13C J couplings in staphylococcal nuclease. *J. Biomol. NMR* **1**: 439–446.
- Eberhardt, E.S. and Raines, R.T. 1994. Amide-amide and amide-water hydrogen-bonds—Implications for protein-folding and stability. *J. Am. Chem. Soc.* **116**: 2149–2150.
- Esposito, L., Vitagliano, L., Zagari, A., and Mazzarella, L. 2000. Experimental evidence for the correlation of bond distances in peptide groups detected in ultrahigh-resolution protein structures. *Protein Eng.* **13**: 825–828.
- Fernandez, A., Kardos, J., and Goto, Y. 2003. Protein folding: Could hydrophobic collapse be coupled with hydrogen-bond formation? *FEBS Lett.* **536**: 187–192.
- Ireta, J., Neugebauer, J., Scheffler, M., Rojo, A., and Galvan, M. 2003. Density functional theory study of the cooperativity of hydrogen bonds in finite and infinite α -helices. *J. Phys. Chem. B* **107**: 1432–1437.
- Jeffrey, G.A., Ruble, J.R., McMullan, R.K., DeFrees, D.J., Binkley, J.S., and Pople, J.A. 1980. Neutron diffraction at 23 K and *ab initio* molecular-orbital studies of the molecular structure of acetamide. *Acta Crystallogr. B* **36**: 2292–2299.
- Juranić, N. and Macura, S. 2001. Correlations among (1)J(NC') and (h3)J(NC')

- coupling constants in the hydrogen-bonding network of human ubiquitin. *J. Am. Chem. Soc.* **123**: 4099–4100.
- Juranić, N., Ilich, P.K., and Macura, S. 1995. Hydrogen-bonding networks in proteins as revealed by the amide (1)J(NC') coupling-constant. *J. Am. Chem. Soc.* **117**: 405–410.
- Juranić, N., Likic, V.A., Prendergast, F.G., and Macura, S. 1996. Protein-solvent hydrogen bonding studied by NMR (1)J(NC') coupling constant determination and molecular dynamics simulations. *J. Am. Chem. Soc.* **118**: 7859–7860.
- Juranić, N., Moncrieffe, M.C., Likic, V.A., Prendergast, F.G., and Macura, S. 2002. Structural dependencies of h3JNC' scalar coupling in protein H-bond chains. *J. Am. Chem. Soc.* **124**: 14221–14226.
- Kumar, V.D., Lee, L., and Edwards, B.F. 1990. Refined crystal structure of calcium-liganded carp parvalbumin 4.25 at 1.5 Å resolution. *Biochemistry* **29**: 1404–1412.
- Liwo, A., Pincus, M.R., Wawak, R.J., Rackovsky, S., and Scheraga, H.A. 1993. Calculation of protein backbone geometry from α -carbon coordinates based on peptide-group dipole alignment. *Protein Sci.* **2**: 1697–1714.
- Luo, P.Z. and Baldwin, R.L. 1999. Interaction between water and polar groups of the helix backbone: An important determinant of helix propensities. *Proc. Natl. Acad. Sci.* **96**: 4930–4935.
- Manas, E.S., Getahun, Z., Wright, W.W., DeGrado, W.F., and Vanderkooi, J.M. 2000. Infrared spectra of amide groups in α -helical proteins: Evidence for hydrogen bonding between helices and water. *J. Am. Chem. Soc.* **122**: 9883–9890.
- Milner-White, E.J. 1997. The partial charge of the nitrogen atom in peptide bonds. *Protein Sci.* **6**: 2477–2482.
- Pillardy, A., Czaplewski, C., Liwo, A., Lee, J., Ripoll, D.R., Kazmierkiewicz, R., Oldziej, S., Wedemeyer, W.J., Gibson, K.D., Arnautova, Y.A., et al. 2001. Recent improvements in prediction of protein structure by global optimization of a potential energy function. *Proc. Natl. Acad. Sci.* **98**: 2329–2333.
- Roseman, M.A. 1988. Hydrophobicity of the peptide C=O and H-N hydrogen-bonded groups. *J. Mol. Biol.* **201**: 621–623.
- Scapin, G., Gordon, J.I., and Sacchettini, J.C. 1992. Refinement of the structure of recombinant rat intestinal fatty acid-binding apoprotein at 1.2 Å resolution. *J. Biol. Chem.* **267**: 4253–4269.
- Scheiner, S. and Wang, L. 1993. Hydrogen-bonding and proton transfers of the amide group. *J. Am. Chem. Soc.* **115**: 1958–1963.
- Soto, C. 2003. Unfolding the role of protein misfolding in neurodegenerative diseases. *Nat. Rev. Neurosci.* **4**: 49–60.
- Tannor, D.J., Marten, B., Murphy, R., Friesner, R.A., Sitkoff, D., Nicholls, A., Ringnalda, M., Goddard, W.A., and Honig, B. 1994. Accurate first principles calculation of molecular charge distributions and solvation energies from ab initio quantum mechanics and continuum dielectric theory. *J. Am. Chem. Soc.* **116**: 11875–11882.
- Vijay-Kumar, S., Bugg, C.E., and Cook, W.J. 1987. Structure of ubiquitin refined at 1.8 Å resolution. *J. Mol. Biol.* **194**: 531–544.
- Yamada, S. 1996. Effects of C(O)-N bond rotation on the C-13, N-15, and O-17 NMR chemical shifts, and infrared carbonyl absorption in a series of twisted amides. *J. Org. Chem.* **61**: 941–946.