# Effects of Amino Acid $\phi,\psi$ Propensities and Secondary Structure Interactions in Modulating Hα Chemical Shifts in Peptide and Protein $\beta$-Sheet

**Gary J. Sharman,[†] Samuel R. Griffiths-Jones,[‡] Muriel Jourdan,[‡] and Mark S. Searle*[,‡]**

*Contribution from AstraZeneca, Silk Road Business Park, Macclesfield, Cheshire SK10 2NA, UK, and Department of Chemistry, University of Nottingham, University Park, Nottingham NG7 2RD, UK*

*Received July 5, 2001*

**Abstract:** Hα chemical shifts are often used as indicators of secondary structure formation in protein structural analysis and peptide folding studies. On the basis of NMR analysis of model $\beta$-sheet and α-helical peptides, together with a statistical analysis of protein structures for which NMR data are available, we show that although the gross pattern of Hα chemical shifts reflects backbone torsion angles, longer range effects from distant amino acids are the dominant factor determining experimental chemical shifts in $\beta$-sheets of peptides and proteins. These show context-dependent variations that aid structural assignment and highlight anomalous shifts that may be of structural significance and provide insights into $\beta$-sheet stability.

## Introduction

The Hα chemical shift of amino acids provides a simple and widely used indicator of secondary structure in peptides and proteins. Dalgarno et al.[1] were the first to notice a clear relationship between the "secondary structure shift", that is the difference between the observed chemical shift and the random coil value ($\delta_{H\alpha}{}^{obs} - \delta_{H\alpha}{}^{rc} = \Delta\delta_{H\alpha}$),[2] and secondary structure context (α-helix or $\beta$-sheet). Jimenez et al.[3] later demonstrated downfield chemical shifts in $\beta$-sheets and upfield shifts in α-helices and turns. A number of subsequent studies have confirmed this trend, and the ever-increasing amount of protein structural data improves the observed correlation.[4,5] Dalgarno's original analysis[1] attributed the observed shift changes to variations in backbone $\psi$ angle, but later Osapay and Case suggested an overriding influence from the $\phi$ angle.[6,7] More recently, the consensus of opinion is that both $\phi$ and $\psi$ contribute to observed chemical shifts.[8] A number of studies have also concluded that the Hα chemical shift is largely determined by local backbone restriction, with a contribution from aromatic ring currents.[9−11] It has been suggested that inaccuracies in predicted shifts, where only these two effects are taken into account, are largely a consequence of inaccuracies in protein coordinates.

The work of Wishart and Sykes shows a correlation between $\phi$ angle and observed shifts in a database of over 70 proteins.[10] However, they report significant deviations from their model such that backbone angles are only able to account for the gross pattern of upfield shifts in α-helices and downfield shifts in $\beta$-sheets. Williamson and Asakura also report a weak correlation,[12] even after taking into consideration the contribution from ring current effects of neighboring residues. It seems that there are other factors which are important in determining observed secondary structure shifts, particularly on the more subtle level of why some residues in some contexts give bigger or smaller downfield shifts in the $\beta$-sheet. These anomolies may be of structural significance and provide insights into $\beta$-sheet structure and stability particularly with regard to folding studies with model peptides where conformational dynamics are a significant factor in the interpretation of experimental data. We illustrate a number of examples of context-dependent effects on Hα chemical shifts in model peptide systems (both α-helix and $\beta$-sheet) that cannot be accounted for entirely by backbone $\phi$ angle effects and conformational preferences ($\phi,\psi$ propensities) in the "random coil". We present a statistical analysis of Hα chemical shift data for protein structures determined by NMR which support our conclusions that although the gross pattern of Hα shifts reflects backbone torsion angles, longer range effects, particularly in $\beta$-sheets, are the dominant contribution with clear context-dependent effects relating to the position of a residue on an edge strand (hydrogen bonded versus non-hydrogen bonded) or central strand of a $\beta$-sheet.

## Materials and Methods

**Database Analysis.** A database of 100 proteins has been constructed for which structural information has been deposited in the Protein Data Bank, and for which NMR chemical shift data are available in the

* Corresponding author: (e-mail) mark.searle@nottingham.ac.uk; (phone) 0115 951 3567; (fax) 0115 951 3564.
[†] AstraZeneca.
[‡] University of Nottingham.

(1) Dalgarno, D. C.; Levine, B. A.; Williams, R. J. P. *Biosci. Rep.* **1983**, *3*, 443−452.
(2) Bundi, A.; Wuthrich, K. *Biopolymers* **1979**, *18*, 285−297. Wuthrich, K. *NMR of proteins and Nucleic acids*; Wiley: New York, 1986.
(3) Jimenez, M. A.; Nieto, J. L.; Herranz, J.; Rico, M.; Santoro, J. *FEBS Lett.* **1987**, *221*, 320−324.
(4) Szilagyi, L. *Prog. NMR. Spectrosc.* **1995**, *27*, 325−443.
(5) Szilagyi, L.; Jardetzky, O. *J. Magn. Reson.* **1989**, *83*, 441−449.
(6) Osapay, K.; Case, D. A. *J. Am. Chem. Soc.* **1991**, *113*, 9436−9444.
(7) Osapay, K.; Case, D. A. *J. Biomol. NMR* **1994**, *4*, 215−230.
(8) Beger, R. D.; Bolton, P. H. *J. Biomol. NMR* **1997**, *10*, 129−42. Wishart, D. S.; Nip, A. M. *Biochem. Cell Biol.* **1998**, *76*, 153−163.
(9) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *J. Mol. Biol.* **1991**, *222* (2), 311−333.
(10) Wishart, D. S.; Sykes, B. D.; Richards, F. M. *Biochemistry* **1992**, *31* (6), 1647−1651.
(11) Williamson, M. P.; Asakura, T.; Nakamura, E.; Demura, M. *J. Biomol. NMR* **1992**, *2*, 83−98.
(12) Williamson, M. P.; Asakura, T. *J. Magn. Reson.* **1993**, *101*, 63−71.

10.1021/ja0116369 CCC: $20.00 
Published on Web 11/16/2001

BioMagResBank. The PDB and corresponding BMRB accession codes of the relevant proteins are the following: 1ahl (bmr374), 1atx (bmr80), 1auu (bmr4095), 1aw3 (bmr4131), 1axh (bmr4233), 1ayj (bmr4071), 1azm (bmr4022), 1bbn (bmr4094), 1bds (bmr480), 1bgs (bmr975), 1bla (bmr4091), 1bmi (bmr4102), 1bpi (bmr48), 1bym (bmr4183), 1caa (bmr1991), 1cbh (bmr192), 1cdl (bmr4056), 1cfe (bmr4301), 1cgf (bmr4064), 1cho (bmr4068), 1clb (bmr327), 1clh (bmr4037), 1ctd (bmr3118), 1dhm (bmr4035), 1dis (bmr3524), 1dtk (bmr66), 1ego (bmr2151), 1eot (bmr4155), 1epg (bmr1674), 1fxa (bmr447), 1glc (bmr1624), 1gpr (bmr1663), 1hcc (bmr1479), 1hdn (bmr29), 1hip (bmr2219), 1hng (bmr4109), 1hoe (bmr1816), 1hrq (bmr2999), 1hsa (bmr3078), 1hst (bmr30), 1hue (bmr4047), 1kst (bmr2856), 1kum (bmr4011), 1lfo (bmr4098), 1msp (bmr4242), 1ncs (bmr4024), 1nrb (bmr4043), 1ntx (bmr132), 1osp (bmr4076), 1par (bmr395), 1pdc (bmr1474), 1pes (bmr4048), 1pfl (bmr4082), 1pk2 (bmr349), 1ppf (bmr44), 1put (bmr2278), 1rbj (bmr443), 1rcf (bmr1580), 1rsy (bmr4039), 1srn (bmr682), 1sxl (bmr4085), 1tta (bmr2476), 1ttg (bmr2281), 1tur (bmr42), 1tym (bmr555), 1ubq (bmr68), 1vtx (bmr4234), 1yha (bmr2039), 1yua (bmr4045), 1zer (bmr2030), 2bbi (bmr1495), 2bop (bmr4087), 2bus (bmr53), 2cbh (bmr196), 2crd (bmr114), 2ech (bmr2204), 2hip (bmr4042), 2i1b (bmr1062), 2igg (bmr1639), 2lzm (bmr915), 2pcb (bmr274), 2plh (bmr55), 2rn2 (bmr1657), 2sh1 (bmr275), 2sob (bmr4010), 2tgf (bmr246), 3b5c (bmr294), 3ci2 (bmr1870), 3cys (bmr2208), 3il8 (bmr280), 3mef (bmr4296), 3pat (bmr144), 451c (bmr759), 4hck (bmr4122), 4ins (bmr554), 4tgf (bmr162), 4trx (bmr257), 5rxn (bmr4050), 6i1b (bmr434), 9pcy (bmr169).

We selected for all residues in antiparallel β-sheets according to the Kabsch and Sander algorithm[13] using STRIDE[14] and classified the β-sheet environment of the Hα protons according to hydrogen-bonding patterns. Those residues whose immediate neighbors have both amide NH and the carbonyl group involved in hydrogen bonds (as determined by STRIDE) have Hα protons facing in toward an opposing strand. Of these inward facing Hα protons, those belonging to residues whose own amide NH and carbonyl groups are hydrogen bonded to a residue on an opposing strand are classified as being on the central strand of a multistranded sheet ($H^C$), whereas those not involved in hydrogen bonds are found on an edge strand ($H^E$). Residues in a β-sheet whose NH and carbonyl group are hydrogen bonded, but not those of the immediate neighbors, are classified as pointing outward ($H_{out}$). These classifications are further discussed below and as illustrated in Figure 3. In total a database of 2062 chemical shifts of Hα protons in antiparallel β-sheets was constructed and the statistical significance of the context-dependent results checked by analysis of variance (ANOVA). For completeness, we also constructed databases of chemical shifts for residues in α-helices and in regions of no defined secondary structure. These consisted of 2764 and 4435 residues, respectively. Δδ values were calculated by using the random coil values of Wuthrich[2] measured from tetrapeptides.

Ideal β-sheet geometries were constructed by using MacroModel[15] with $\phi$ angles of −139° and $\psi$ angles of 135°. The strands were aligned with hydrogen bonding NH and CO coplanar and an interstrand N−O distance of 2.6 Å. Interstrand separation, horizontal and vertical displacements, and hydrogen-bonding angle (defined as the angle between NH and CO vectors) were then altered in turn and the dependence on secondary structure shifts measured. The dependence of shift on $\phi$ was also tested on a single β-strand. Shift calculations on all structures were performed with SHIFTS.[16]

**NMR Methods.** All NMR data on the model peptides were collected on a Bruker DRX500 spectrometer, using standard experimental protocols. In phase-sensitive DQF-COSY, TOCSY, NOESY, and ROESY experiments, 1024 or 2048 data points were collected in f2 and 400−600 points in f1. Quadrature detection in f1 was achieved by using TPPI, and solvent suppression employed either presaturation or the WATERGATE solvent suppression sequence. TOCSY experiments employed a spin locking field of 5 kHz, and ROESY 2 kHz. Data were processed on a Silicon Graphics Indy Workstation with Bruker XWINNMR software. Typically, a sine-squared window function shifted by $\pi/4-\pi/2$ was applied in both dimensions, with zero-filling in f1 to 1k points.

## Results and Discussion

**Effects of Local $\phi,\psi$ Angle Preferences on Hα Chemical Shifts.** While imprecision in protein crystal structure coordinates is likely to contribute to differences between calculated and observed chemical shifts, here we have investigated the possible contribution of other factors using both a statistical analysis of a large body of deposited protein NMR data and experimental analysis of model systems. One such factor that has been suggested is that Δδ values are residue specific. A number of studies have established that the backbone angles $\phi$ and $\psi$ in an unstructured polypeptide are highly residue specific,[17,18] largely as a consequence of steric constraints imposed by the nature of the side chain. It follows therefore, from the dependence of chemical shifts on $\phi$ and $\psi$, that we would expect residue $\phi,\psi$ preferences in the "random coil" to be reflected in the observed shift changes with secondary structure formation. For example, residues such as Lys, Ala, and Leu have been shown to favor α-helical regions of Ramachandran space in the random coil, whereas Val and Ile have a high intrinsic β-sheet propensity.[17,18] We can therefore conclude that the random coil shifts of residues such as Lys will be biased toward α-helical values, and β-like residues will show random coil shifts closer to that in β-sheets. The backbone angle model for secondary structure shifts would therefore predict that Val in an α-helix will undergo a much larger upfield shift than Lys. Such a correlation was demonstrated in an α-helical peptide,[19] although much poorer correlation has been observed in β-sheets.[20]

To examine this further, Hα chemical shifts in a number of model peptide systems have been analyzed. α-Helical peptides U(21−35) (sequence DTIENVKAKIQDKEG, derived from residues 21−35 of bovine ubiquitin) and α18 (a rationally designed helical peptide, sequence SRSDELAKLLRLLQDKEG) show different degrees of folded conformation in aqueous solution, and both demonstrate stabilization of the helical conformation in aqueous methanol or trifluoroethanol. The changes in Hα chemical shifts with respect to random coil reference values are plotted in Figure 1a,b. While Val26 and Ile30 of peptide U(21−35) show large upfield shifts in agreement with the above model, Lys27 (a residue with high α-helical propensity) also shows a similar behavior. In the designed helical peptide, residues such as Ala, Lys, and Leu undergo some of the largest upfield secondary structure shifts observed. It therefore seems that there is no clear-cut correlation between intrinsic $\phi,\psi$ backbone preferences and secondary structure shifts in α-helices based on these two model systems. A similar analysis of chemical shift perturbations in a β-hairpin model system derived from native ubiquitin, but with a mutated turn sequence (MQIFVKNPDGTITLEV), leads to a similar conclusion. This hairpin has been shown previously to be significantly folded in water, but with a different β-strand alignment to that

(13) Kabsch, W.; Sander, C. *Biopolymers* **1983**, *22* (12), 2577−2637.
(14) Frishman, D.; Argos, P. *Protein Struct. Funct. Genet.* **1995**, *23*, 566−579.
(15) Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comput. Chem.* **1990**, *11*, 440−467.
(16) Sitkoff, D.; Case, D. A. *J. Am. Chem. Soc.* **1997**, *119* (50), 12262−12273.
(17) Swindells, M. B.; Macarthur, M. W.; Thornton, J. M. *Nat. Struct. Biol.* **1995**, *2*, 596−603.
(18) Griffiths-Jones, S. R.; Maynard, A. J.; Sharman, G. J.; Searle, M. S. *J. Mol. Biol.* **1998**, *284*, 1597−1609.
(19) Bolin, K. A.; Pitkeathly, M.; Miranker, A.; Smith, L. J.; Dobson, C. M. *J. Mol. Biol.* **1996**, *261*, 443−453.
(20) Serrano, L. *J. Mol. Biol.* **1995**, *254*, 322−333.

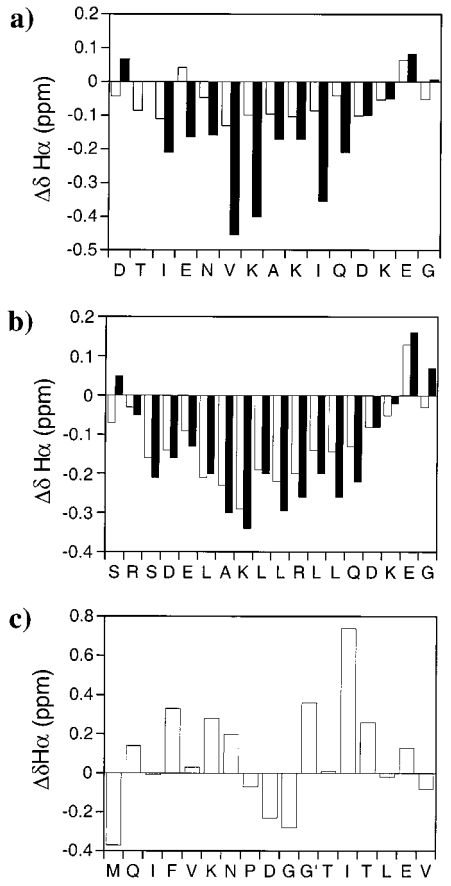


**Figure 1.** Secondary structure shifts for model α-helical peptides (a) U(21−35) from ubiquitin, (b) designed peptide α18, and (c) β-hairpin peptide U(1−16) derived from ubiquitin but containing the TLTGK→NPDG turn mutation. Open bars show data in water, while black bars show data in 40% TFE; all data are at 278 K.

of the native hairpin.[21] The data in Figure 1c show the expected profile for a β-hairpin, with downfield shifts in the strands (positive $\Delta\delta_{H\alpha}$ values) and upfield shifts in the turn. However, again we see no obvious correlation between secondary structure shift and $\phi,\psi$ propensity. Residues previously identified as having high β-propensity, such as Ile, Phe, and Val, do not appear to have significantly smaller secondary structure shifts than residues with low β-propensity, such as Glu, Leu, and Lys. These data do not fit well with the $\phi,\psi$ preference model described above.

Additionally, we have obtained a wealth of NMR data on the designed β-hairpin peptide β16 (sequence KKYTVSINGK-KITVSI) and the single-stranded peptide β8 (sequence GK-KITVSI). Studies involving this family of peptides have been described in some detail previously.[22] Coupling constant analysis and intensities of interresidue NOEs show that many residues in the single-stranded β8 prefer $\phi,\psi$ angles corresponding to a β-sheet conformation in solution, even in the absence of a second β-strand. However, while chemical shifts for Hα's in the strands of the hairpin β16 are significantly downfield with respect to reference random coil shifts, those in the single-stranded β8, which lacks the cross-strand interactions characteristic of the hairpin, are small (<0.1 ppm).[22] Several related studies involving β-strand peptides derived from autonomously folding hairpin sequences have also demonstrated this β-sheet propensity in isolated fragments with only small deviations in chemical shift observed.[21,23] These data suggest that an extended conformation with β-sheet backbone $\phi$ and $\psi$ angles is not sufficient to confer large Hα chemical shift changes, and that significant secondary structure shifts in β-strands are only observed in the presence of an opposing strand. Although it is unlikely that an isolated single strand maintains a rigid extended conformation, NOE and coupling constant data show that the time-averaged conformation is weighted toward β-sheet $\phi$ and $\psi$ angles. However, it has already been noted that chemical shifts are quite sensitive to small changes in backbone torsion angles[6,7,12] (also see below), such that backbone dynamics may be much more effective at reducing $\phi$ and $\psi$ effects on Hα chemical shifts.

**Database Analysis of Secondary Structure Shifts in α-Helices and β-Sheet.** The above examples demonstrate at best only a weak correlation between residue $\phi,\psi$ preferences and secondary structure shifts in specific α-helical and β-sheet model systems. The potential problem with specific examples is that context-dependent effects may obscure overall trends. We have therefore examined the distribution of secondary structure shifts in a large number of high-resolution protein structures for which NMR chemical shift data have been deposited (see Methods). Such a statistical analysis should average out any context-dependent effects. As a measure of intrinsic $\phi,\psi$ preferences we have used a β-propensity value ($P_\beta$) as described previously[18] that is calculated based on the relative population of α- and β-conformational space in a database of protein structures.

In Figure 2 we plot the average secondary structure shift observed for each residue in α-helices and β-sheets versus $P_\beta$. On the basis of the above model that secondary structure shifts are determined by backbone $\phi,\psi$ angles, we would expect to see a correlation between secondary structure shift and $P_\beta$, with residues with high $P_\beta$ values associated with large shift changes in α-helices and small shift changes in β-sheets. The correlation for α-helices is rather poor ($R = 0.64$; $R = 0.67$ if Gly is excluded due to its unique $\phi,\psi$ distribution in unstructured peptides), but some residues with bulky, β-branched side chains (Phe, Tyr, Ile, and Val) that have high β-propensities do appear to experience the largest changes in chemical shift, in accordance with the above model. However, residues with high helical propensity (low $P_\beta$ values) show much more variable secondary structure shifts. In the case of β-sheets, the correlation between $P_\beta$ values and average shifts is very poor ($R = 0.25$; this increases to 0.35 if Gly is excluded). The data suggest that $\phi,\psi$ preferences may play some role in determining $\Delta\delta$ values, but that the magnitude of the shift, particularly in the case of β-sheets, is dependent on a number of other factors.

In conclusion, data from model peptide systems that fold to form α-helical and β-sheet (β-hairpin) conformations, together with data from the statistical analysis of residues in elements of secondary structure in proteins, fail to demonstrate a convincing correlation between Hα chemical shift changes in secondary structure formation and any intrinsic conformational preference ($\phi,\psi$ propensities) of residues in the random coil state. The correlation is weak for helical residues,[10,12] but essentially absent for residues in the β-sheet.[20] Subsequently, we have examined context-dependent effects to explain the absence of such a correlation for β-sheet residues.

(21) Searle, M. S.; Williams, D. H.; Packman, L. *Nat. Struct. Biol.* **1995**, *2*, 999−1006. Jourdan, M.; Griffiths-Jones, S. R.; Searle, M. S. *Eur. J. Biochem.* **2000**, *267*, 3539−3548.

(22) Maynard, A. J.; Sharman, G. J.; Searle, M. S. *J. Am. Chem. Soc.* **1998**, *120*, 1996−2007. Griffiths-Jones, S. R.; Maynard, A. J.; Searle, M. S. *J. Mol. Biol.* **1999**, *292*, 1051−1069. Searle, M. S.; Griffiths-Jones, S. R.; Skinner-Smith, H. *J. Am. Chem. Soc.* **1999**, *121*, 11615−11620.

(23) Zerella, R.; Evans, P. A.; Ionides, J. M. C.; Packman, L. C.; Trotter, B. W.; Mackay, J. P.; Williams, D. H. *Protein Sci.* **1999**, *8*, 1320−1331.

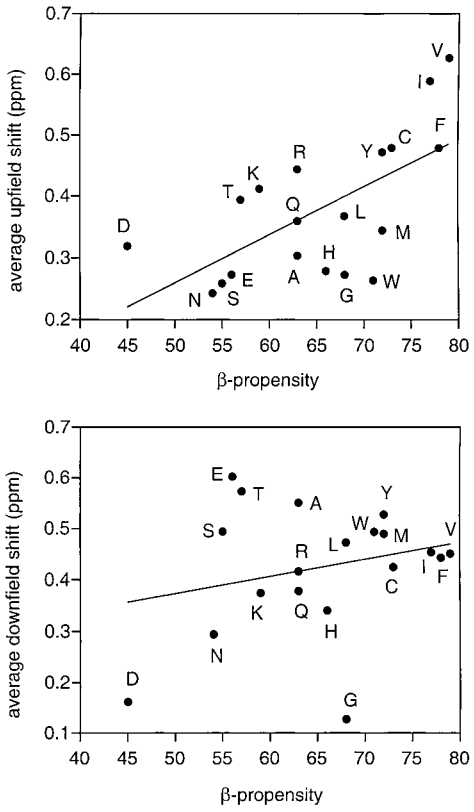


**Figure 2.** Plots of mean secondary structure shift versus residue $\beta$-propensity ($P_\beta$) (determined from $\phi,\psi$ distributions of residues in the coil regions of protein structures[18]) in (top) α-helices and (bottom) $\beta$-sheets

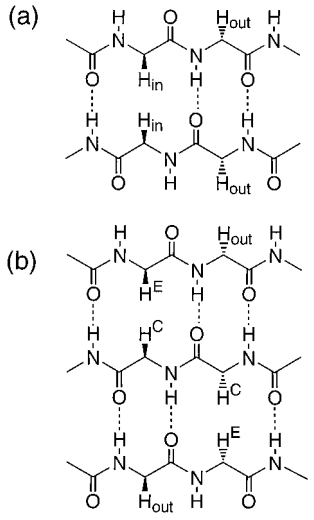


**Figure 3.** Context and nomenclature for Hα's in protein $\beta$-sheets: (a) $\beta$-hairpin and (b) multistranded antiparallel $\beta$-sheet.

**Context Dependence of Hα-Chemical Shifts in $\beta$-Sheets.** While examining experimental data for a number of $\beta$-hairpin peptide systems, it became clear that although there was a poor correlation between shift and secondary structure propensities, context-dependent effects are apparent. There is a clear alternation of large and small shift changes along the strand. This is readily correlated to the fact that there are two distinct sites in an $\beta$-hairpin as each strand has a hydrogen-bonded and a non-hydrogen-bonded edge (Figure 3a). The consequence of this is that Hα protons may experience two very different environments facing either inward ($H_{in}$) toward another $\beta$-strand or outward ($H_{out}$) into the solvent. The result is an *i,i+2* periodicity in the magnitude of the Hα chemical shift changes along the sequence. It would appear that the proximity to the opposite strand leads to a much larger chemical shift change for $H_{in}$ than $H_{out}$, and clearly this difference cannot be explained purely in terms of differences in $\phi$ and $\psi$ angles. This effect is particularly pronounced when the population of the folded state is high, either intrinsically or when enhanced by the effects of cosolvents, such as methanol. We have previously described the folding and stability of a family of peptides based on the 16-mer sequence KKYTV<u>X</u>INGKKITV<u>X</u>I which illustrate these context-dependent effects, as do other model systems. The data for the hairpin (X = S) in water and 50% methanol are shown in Figure 4a.[22] Data for the analogue with stabilizing Lys/Glu salt bridges (<u>X</u> = E), which is appreciably more folded in water, are shown in Figure 4b. Hα shift changes for the native ubiquitin hairpin U(1−17) in water and 30% methanol are illustrated in Figure 4c from the work of Zerella et al.[23] Alcoholic cosolvents are well-known to stabilize peptide secondary structure in partially folded peptides by promoting intramolecular hydrogen bonding, facilitated through poorer solvation of amide groups in the unfolded state.[24] The increase in population of the folded state is synonymous with an increase in the magnitude of the downfield shift, and results in more pronounced *i,i+2* periodicity (see Figure 4). Other examples of $\beta$-hairpin peptides and cyclic analogues exhibit similar effects.[25−28] It is important to note that the effects of the cosolvent are due to changes in the population of the folded state and not due to changes in solvent composition as evident in Figure 4b where the already significantly folded hairpin undergoes only small additional changes in population induced by cosolvent. Control experiments on unstructured peptides in 50% (v/v) aqueous methanol show that solvent-induced perturbations to chemical shifts are modest compared to those induced by secondary structure formation.[27]

The *i,i+2* periodicity described above has been predicted by theoretical calculations by Osapay and Case.[6,7] By considering the electrostatic and anisotropic effects of the carbonyl group, they showed that a larger shift change would be expected for residues in $\beta$-sheets whose Hα faces an opposing strand. This paper has been widely cited as demonstrating that Hα shifts are dependent on $\phi$, but the part of the paper related to structural shifts seems to have been largely ignored. Yet the authors predict that a large proportion of the Hα shift change could be due to long-range secondary structure interactions. These theoretical predictions were illustrated with chemical shift data from a number of proteins with multistranded sheets, where the central strands have no outward facing Hα's. As expected, these did not demonstrate the *i,i+2* periodicity. Thus, we reiterate and extend some of these earlier predictions and show, based on experimental data, that backbone angle effects on the magnitude of secondary structure shifts are modulated by other factors. The following sections extend the database model described above in an effort to determine the relative contributions of these factors to chemical shift changes.

(24) Nelson, J. W.; Kallenbach, N. R. *Biophys. J.* **1987**, *51*, 2, 555. Sonnichsen, F. D.; Van Eyk, J. E.; Hodges, R. S.; Sykes, B. D. *Biochemistry* **1992**, *31* (37), 8790−8798. Storrs, R. W.; Truckses, D.; Wemmer, D. E. *Biopolymers* **1992**, *32* (12), 1695−1702.

(25) Schenck, H. L.; Gellman, S. H. *J. Am. Chem. Soc.* **1998**, *120*, 4869−4870.

(26) Searle, M. S.; Zerella, R.; Williams, D. H.; Packman, L. C. *Prot. Eng.* **1996**, *9*, 559−565.

(27) Sharman, G. J.; Searle, M. S. *J. Am. Chem. Soc.* **1998**, *120*, 5291−5300.

(28) Ramirez-Alvarado, M.; Blanco, F. J.; Serrano, L. *Nat. Struct. Biol.* **1996**, *3*, 604−612.

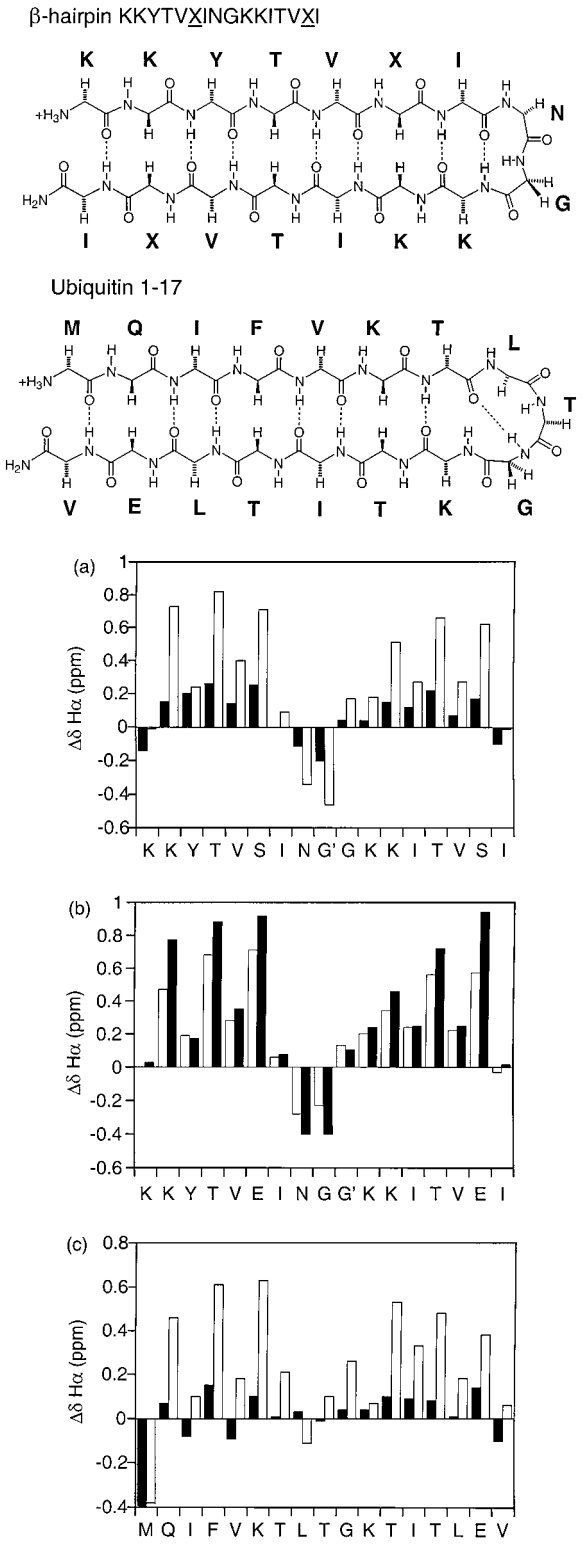


**Figure 4.** (Top) backbone alignment of $\beta$-hairpin peptide KKYTV-XINGKKITVXI and U(1−17), with side chains removed for clarity; (bottom) Hα chemical shift deviations from random coil values ($\Delta\delta_{H\alpha}$) for (a) $\beta$-hairpin peptide KKYTVXINGKKITVXI (X = S) in water and 50% (v/v) aqueous methanol solution, (b) the analogous $\beta$-hairpin (X = E) stabilized by Lys/Glu salt bridges in water and 50% aqueous methanol, and (c) the native hairpin sequence of ubiquitin U(1−17) in water and 30% methanol (data from Zerella et al.[23]).

**Statistical Analysis of Context-Dependent Effects in Protein $\beta$-Sheets.** Statistical analyses of protein structure from the Protein Data Bank have been used by a number of groups to correlate experimental NMR parameters and structural information.[10,17−20,29] The use of a large sample size should average out any anomalies due to specific perturbations such as ring current effects. Here, we have used a database of 100 proteins for which both NMR chemical shifts and structural information are known (see Methods). We have selected for residues in antiparallel $\beta$-sheets, for comparison with the model systems described above, and have classified Hα protons based on hydrogen-bonding patterns. Hα's are divided into those facing outward ($H_{out}$) and those pointing in toward an opposing strand ($H_{in}$). The inward facing protons are further subdivided into those on a Central strand of a multistrand sheet ($H^C$) and those on an Edge strand ($H^E$). These classifications are summarized in Figure 3b. In total, we generated a database containing 2062 chemical shifts of residues in antiparallel $\beta$-sheets, and average shifts for each of the classes of Hα protons were calculated. These results are summarized in Table 1. The data show significant differences in average shifts between protons in the different $\beta$-sheet environments. Inward and outward facing protons show differences of ∼0.3 ppm, and inward facing protons are further differentiated when considering the location on either edge or central strands. All differences from the average value over all environments are found to be statistically significant by analysis of variance.

This analysis shows that there is a major contribution to the chemical shift of Hα protons in $\beta$-sheets that cannot be attributed to local backbone conformation. We can use the above data to qualitatively estimate the relative contributions of each of the components to downfield shift and these data would suggest that the cross-strand contribution to downfield shifts is the most significant. The average downfield shift observed for $H_{out}$, which is not juxtaposed to an opposing strand, appears to arise mainly from the backbone $\phi$-angle contribution, through the relative orientation of the carbonyl group of the preceding residue, and leads to a downfield shift of only 0.21 ppm. This effect is also present for the inward facing protons ($H^E$ and $H^C$), but other

**Table 1.** Average Secondary Structure Chemical Shifts and Standard Deviations for Hα Protons in Different Environments in Antiparallel $\beta$-Sheets

| residue context[a] | no. of residues | mean shift (ppm) | standard dev (ppm) |
|---|---|---|---|
| $H_{all}$ | 2062 | 0.42 | 0.51 |
| $H_{in}$ | 1443 | 0.51 | 0.53 |
| $H_{out}$ | 619 | 0.21 | 0.38 |
| $H^C$ | 749 | 0.59 | 0.50 |
| $H^E$ | 694 | 0.42 | 0.56 |

[a] See Figure 3 for nomenclature; $H_{all}$ is the average over all data, while $H_{in}$ is the average of $H^C$ and $H^E$.

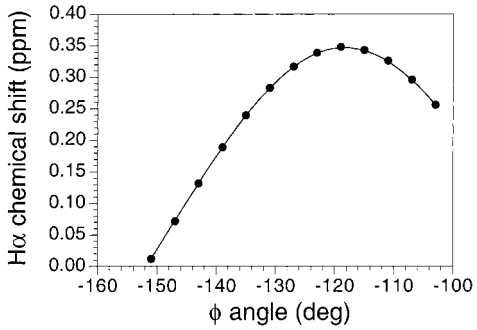


**Figure 5.** The effect of $\phi$ angle on calculated Hα secondary structure shift within a single $\beta$-strand calculated with the program SHIFTS.[16]

(29) Smith, L. J.; Fiebig, K. M.; Schwalbe, H.; Dobson, C. M. *Folding Des.* **1996**, *1*, R95−R106.

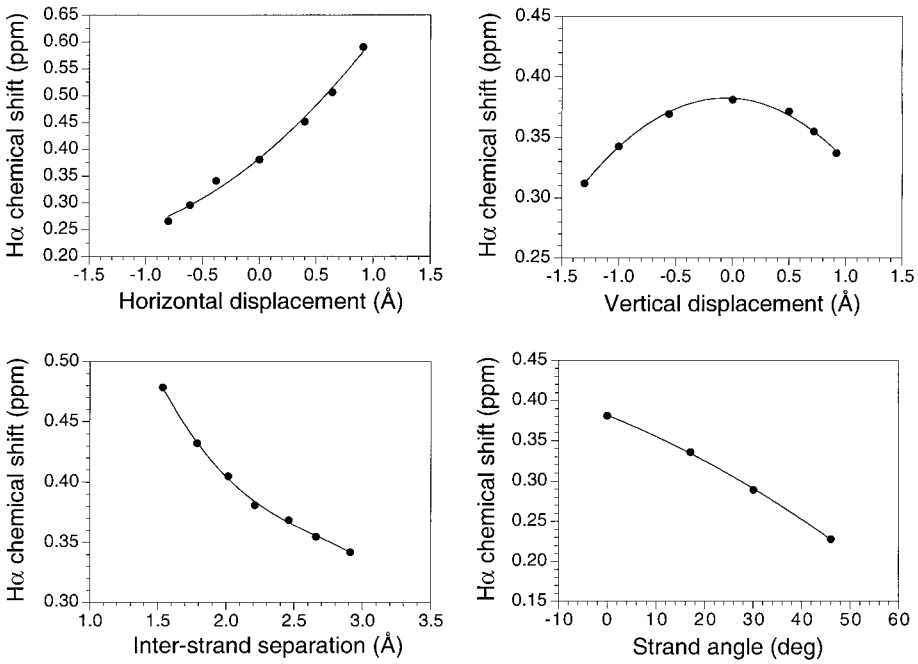


**Figure 6.** Variation in secondary structure shifts for inward facing Hα ($H_{in}$) with structural changes from ideal β-sheet geometry within a realistic range of values compatible with cross β-strand interactions: horizontal displacement (positive and negative in Å), vertical displacement (positive and negative in Å), interstrand distance (Å), and interstrand hydrogen bonding angle (θ, degrees).

effects contribute an additional 0.21 or 0.38 ppm on average for edge and central strands, respectively.

**Influence of Backbone Conformation and *β*-Strand Geometry.** Despite the clear context-dependent effects on Hα chemical shifts evident in the above analysis of β-sheet residues, the standard deviations from these mean values are large (see Table 1). If we consider outward facing protons ($H_{out}$) in isolation, it is clear that the standard deviation from the mean value is much reduced (0.21 ± 0.39 ppm) and very similar to that observed for the α-helix (−0.37 ± 0.36 ppm). But for $H^E$ and $H^C$, the scatter of values remains fairly large (one standard deviation from the mean, ±0.55 and ±0.52 ppm, respectively). Thus, even for residues within these specific structural contexts, variations are large. We can discount the possibility that the large standard deviations reflect a strong dependence on residue type, because when we examine the mean structural shift and standard deviation for particular amino acids (where the sample number is large enough to retain statistical significance) we see that they all exhibit the same trends of large standard deviations (between 0.39 and 0.70 ppm) for all inward facing Hα's, falling to somewhere between 0.23 and 0.45 ppm for outward facing α-protons. Thus, the large standard deviation for inward facing protons probably reflects the fact that the observed secondary structure shifts are strongly influenced by the effects of the opposing strand. It is clear from inspection of any β-sheet that the position, in both distance and angle, of the carbonyl groups on the opposing strand is highly variable.

To examine how variations in backbone conformation and β-strand geometry can contribute to the observed context-dependent variations in Hα shifts noted above, we constructed a number of β-sheet structures with differences in β-sheet geometry (horizontal and vertical displacement, interstrand separation, and strand angle) and investigated φ-effects within an isolated single β-strand. Within a single β-strand we observe a significant dependence of Hα shift on φ angle (Figure 5). The maximum shift does not occur at the ideal β-sheet angle of −139°, but at around −120°. These data suggest that the φ angle alone is able to contribute a maximum of around 0.35 ppm to the downfield shift but that this value may fall close to zero over a relatively small range of φ values (Figure 5) demonstrating some sensitivity to overall backbone conformation, as previously indicated,[6,7,12] although the maximum calculated shift contribution is relatively modest. The optimum φ value of −139° gives a predicted downfield shift of ∼0.20 ppm in very good agreement with the above statistical analysis of residues on the edge strand ($H_{out}$) of protein β-sheets. This is also consistent with the β-hairpin data shown in Figure 4 for the peptides that are significantly folded in water or aqueous methanol, suggesting that $H_{out}$ values are largely a consequence of φ-effects.

Examining the effects of interstrand geometry (Figure 6), we also observe a dependence on strand separation such that shorter hydrogen-bonding distances result in increased secondary structure shift, as a consequence of closer Hα proximity to the carbonyl group of the opposing strand. Only relatively weak dependence of the Hα shift is observed for the parameters of vertical displacement and interstrand angle, and both only serve to decrease the observed shift on deviation from ideal β-sheet geometries. A significant change in shift is observed with horizontal displacement along the plane of the sheet, which results in a decrease in secondary structure shift, whereas movement in the positive direction results in a large increase in shift. We attribute this to the fact that moving the strand in the positive direction brings the inward facing Hα into closer proximity to the opposing carbonyl groups. Thus, where we observe a statistically significant difference between $H^E$ and $H^C$ (see Table 1), we do not rule out subtle differences in conformation between an edge strand and a central strand of β-sheet arising from possible differences in steric restraints and strand twisting. Using the SHIFTS program to calculate context-dependent effects on chemical shifts using our database of structures, we are able to reproduce the relative order for $H_{out}$, $H^E$, and $H^C$ (0.11, 0.19, and 0.28 ppm, respectively), though the agreement with the experimental data is only qualitative. In contrast, with an idealized β-sheet model, SHIFTS does not distinguish between $H^E$ and $H^C$, showing that there are real

conformational differences between edge and central strands in a protein $\beta$-sheet that are not manifested in model $\beta$-sheets. The geometry-dependent variations between strands summarized in the data in Figure 6 appear to account for the large standard deviations shown for $H^C$ and $H^E$, and for the difference between these two environments. Consistent with this model, $H_{out}$, which is not directly under the influence of an opposing strand, has a significantly smaller standard deviation. These data support our conclusions that longer range electrostatic interactions dominate over local $\phi,\psi$ effects in dictating observed secondary structure shifts.

Even though backbone torsion angles appear to determine the gross pattern of shifts (upfield for $\alpha$-helices and downfield for $\beta$-sheets), it is evident that these are not generally the most important factor for the determination of the magnitude of shift changes *within* $\beta$-sheets. This observation also suggests why there has been little success in correlating $\phi,\psi$ propensities of amino acids in $\beta$-sheets with their chemical shift changes. The effects are masked by larger context-dependent components of the secondary structure shift relating to relative $\beta$-strand geometry, orientation, separation, and location on the edge of a sheet or as a central strand. We have shown that experimental observations in simple model $\beta$-hairpin peptide systems can be rationalized on the basis of statistics determined from a large body of protein NMR data, where context-dependent effects are useful in structural assignment for identifying the $\beta$-sheet hydrogen-bonding register.

**Acknowledgment.** We thank the EPSRC (UK) and Roche Discovery (Welwyn, UK) for financial support to S.R.G.-J. and the European Community for a Marie Curie Fellowship to M.J.

JA0116369