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Hydrogen Bond Strength and β-Sheet Propensities: The Role of a Side Chain Blocking Effect

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ABSTRACT **Amino acid side chains can enhance peptide group hydrogen bond strength in protein structures by obstructing the competing hydrogen bond to solvent in the unfolded state. Available data indicate that the steric blocking effect contributes an average of 0.5 kJ per residue to protein hydrogen bond strength and accounts for the intrinsic β-sheet propensities of the amino acids. In available data for helical models, the contribution to α-helix propensities is obscured especially by large context-dependent effects. These issues are all related by a common side chain–dependent steric clash which disfavors peptide to water H-bond formation, peptide to catalyst complexation in hydrogen exchange reactions (Bai et al., Proteins 17:75–86, 1993), and peptide to peptide H-bonding in the helical main chain conformation (Creamer and Rose, Proc. Natl. Acad. Sci. U.S.A. 89:5937–5941, 1992) but not in β-strands.**

Key words: protein structure prediction, protein stability, hydrogen bond, β-sheet, amino acid propensity, steric effect, hydrogen exchange

INTRODUCTION

Recent progress in the chemistry of protein hydrogen exchange reveals that amino acid side chains can sterically interfere with formation of the hydrogen exchange encounter complex between their neighboring peptide groups and solvent molecules.[1] If side chains similarly interfere with peptide to solvent hydrogen bonding in the random coil, this may act to increase the strength of hydrogen bonds in structured proteins. A direct test of this side chain blocking hypothesis is made possible by the availability of data for the contribution of the various amino acids to structural stability in a constant host background. Kim and Berg[2] have measured the stability of a β-sheet–containing zinc finger protein when a given residue position is substituted. Other workers[3–8] have tested the (de)stabilizing effects of the various amino acids in a variety of α-helical peptide hosts. This paper compares the amino acid–dependent stability of α and β structures with side chain blocking effects derived from hydrogen exchange results.

ANALYSIS

Steric Blocking of the Peptide Group

A quantitative measure of side chain blocking can be obtained from hydrogen exchange (HX) results for amino acid dipeptide models,[1] illustrated in Figure 1. The V-shaped curves for log(rate) against pD in Figure 1 reflect catalysis of the peptide group hydrogen exchange reaction by OH^- ion (right limb) and H^+ ion (left limb). Relative to alanine (dashed curve), the apolar side chains in Figure 1a sterically block both acid and base catalysis more or less equally and move the V-shaped curve to slower rates. Side chains that have some polar character can in addition exercise an inductive effect. They withdraw electron density from the peptide group, thus promote the base catalyzed reaction and slow acid catalysis, moving the curve to the left. For example, Figure 1b shows that threonine exerts an inductive effect (left shift) equal to that of serine and in addition a blocking effect (down shift) just over half that of valine. From data like this, we wish to derive a measure of the blocking effect alone.

Equation (1) defines a side chain–dependent steric blocking factor in terms of the effect of any amino acid side chain (Xaa) on the acid and base-catalyzed HX rate constants of its peptide group NH [k_A(Xaa) and k_B(Xaa), respectively], expressed relative to the rate constants for alanine [k_A(Ala) and k_B(Ala)]:

$$\Delta\log k_{min}(\text{Xaa}) = \{\log[k_A(\text{Xaa})/k_A(\text{Ala})] + \log[k_B(\text{Xaa})/k_B(\text{Ala})]\}/2 = (A + B)/2. \quad (1)$$

The term k_{min} is the minimum HX rate for the amino acid Xaa (see Fig. 1). The parameter $\Delta\log k_{min}$ relates solely to the steric blocking effect, which slows the acid and base-catalyzed rates essentially equally (Fig. 1a). The side chain inductive effect is eliminated because it contributes oppositely (minus

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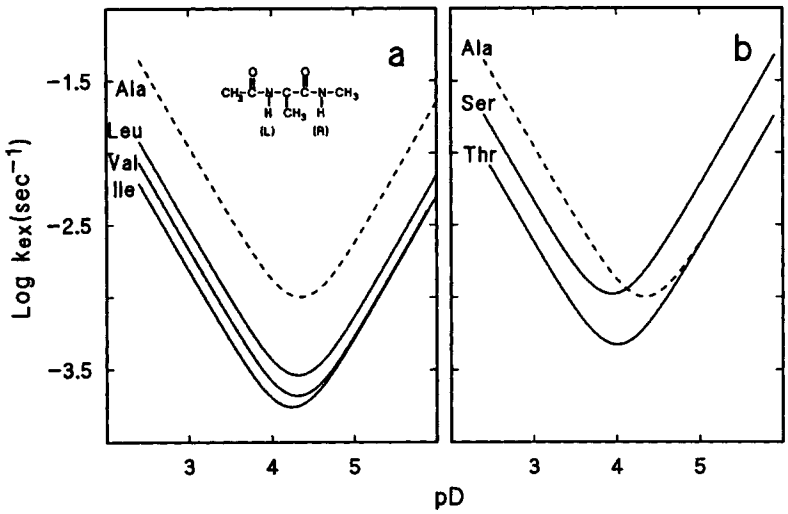


Fig. 1. Side chain effects on HX rate profiles measured in the dipeptide model shown. **(a)** Apolar residues exercise a steric blocking effect, indicated by a decrease in k_{min}. **(b)** Polar side chains exercise an inductive effect (left shift) as well as a blocking effect. The curves shown are for the left peptide group.[1] A small contribution due to a water-catalyzed reaction is omitted for clarity.

and plus) to log k_A and log k_B (see Fig. 1b). The A and B values are given by Bai et al.[1] for all the naturally occurring amino acids. The logarithmic form used allows the HX blocking factor to be expressed directly in free energy terms (relative to alanine; $\Delta\Delta G^{\ddagger}(HX) = -2.3RT\ \Delta\log\ k_{min}$) to facilitate comparisons with thermodynamically measured α-helix and β-sheet propensities. For example, the replacement of alanine by valine (Fig. 1a) slows both the acid and the base HX rates of the left peptide NH by 5-fold, leading to a decrease in $\log(k_{min})$ by 0.7 units, due to steric blocking that raises the HX activation energy by 3.8 kJ.

β-Sheet Stabilization

Figure 2a compares β-sheet propensities[2] with HX blocking factors.[1] The thermodynamic β-sheet propensities used were derived by Kim and Berg[2] from the effects of amino acid substitutions on the stability of a β-sheet–containing protein. Almost all of the variance from the regression line in Figure 2a is due to the apparent misplacement of cysteine and phenylalanine along the propensity axis. It is hard to see why the β-sheet propensity of cysteine should differ so strikingly from that of serine and why phenylalanine should be so different from tyrosine. If cysteine and phenylalanine are omitted from the analysis, the correlation coefficient is 0.96. When the blocking effect on the left peptide is averaged with the much smaller effect on the right peptide (2 to 4 times smaller in energy, due to its distance from the blocking side chain), the correlation coefficient changes from 0.96 to 0.95 (with Cys and Phe excluded). The slope of the regression line in Figure 2a is negative (−5) because increased blocking of the peptide to solvent interaction ($\Delta\Delta G^{\ddagger}_{HX}$ more positive) favors β-strand formation ($\Delta\Delta G^{\circ}$ more negative). The large slope found indicates that HX blocking energy increases 5-fold more rapidly than H-bond blocking energy. This presumably reflects the fact that the solvent target in the HX encounter complex, including a charged OH^- or H^+ ion together with its hydration shells, is considerably larger than the single water molecule involved in a peptide to solvent hydrogen bond.

In the peptide used by Kim and Berg,[2] the backbone NH and CO of the experimentally substituted residue face out to solvent as illustrated in Figure 2b, thus hydrogen bond to water rather than within the β structure itself. This by no means changes the thermodynamic picture considered here. When the main chain adopts a β conformation, the side chain to main chain steric clash is relieved (see Fig. 4). This is true whether the peptide group H-bonds are formed internally to other β-strands or externally to water. The fact of H-bonding to water in the model peptide probably helps to account for the clean correlation observed in Figure 2a by avoiding the introduction of variance due to context and other effects. However this situation may affect the $\Delta\Delta G^{\circ}$ values measured by Kim and Berg[2] and therefore the precise calibration of the side chain effect on hydrogen bond energy.

α-Helix Stabilization

Does steric blocking also contribute to α-helix stability? Figure 3a compares the side chain blocking effect with a set of α-helix propensities measured at residue 32 in the barnase protein.[7] A small correlation coefficient of 0.55 is found between side

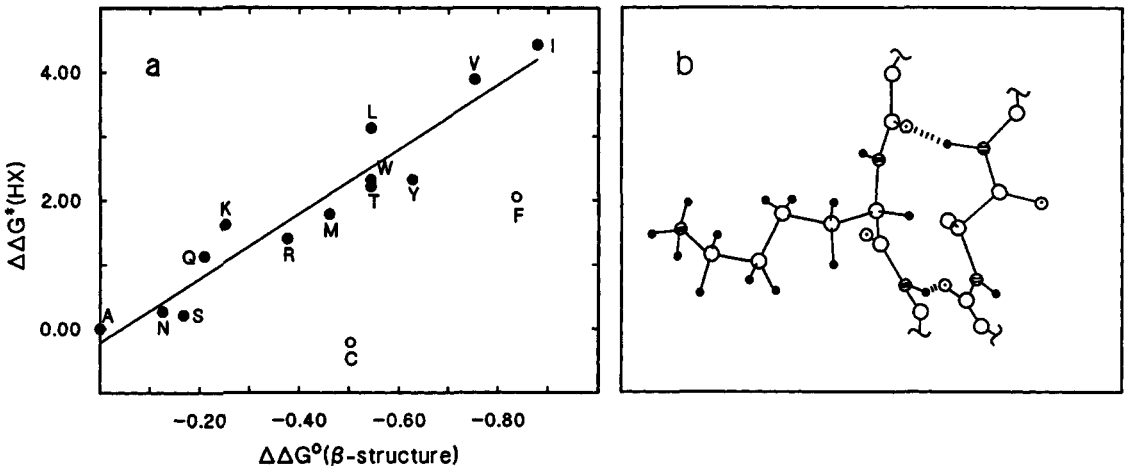


Fig. 2. **(a)** The correlation between β-sheet stabilization energy (propensity) and HX activation energy due to side chain blocking (in kJ/mol). The side chain-dependent increase in stability of a β-sheet model was measured by Kim and Berg.[2] The increase in HX activation energy due to side chain blocking of the left peptide group was derived according to Eq. (1) (see text). Glycine and proline which have singular conformational properties were omitted here and by Kim and Berg.[2] Histidine, aspartic acid, and glutamic acid are also omitted; they change their titration state through the HX region measured[1] so that their steric blocking factors are poorly determined. Cysteine and phenylalanine are discussed in the text. Amino acid residues are identified by the one letter code. **(b)** A fragment of the β-sheet structure studied by Kim and Berg.[2] The amino acid experimentally substituted is shown as lysine. Its neighboring peptide groups form H-bonds to water and to the antiparallel chain shown to the right. C atoms are drawn as open circles, N are striped, O are dotted, and H are black.

chain blocking and this set of α-helix propensities. An even smaller correlation ($r = 0.2$) is found with propensity data obtained from similar substitutions in T4 lysozyme,[8] which in turn correlates poorly with the barnase propensities ($r = 0.6$). The possible contribution of side chain blocking is obscured by context-dependent interactions among side chain atoms,[3-8] by the 4-fold larger range of α-helix propensities generated by differential side chain entropy,[9] and by the fact that these steric effects are anticorrelated with hydrogen bond promotion.

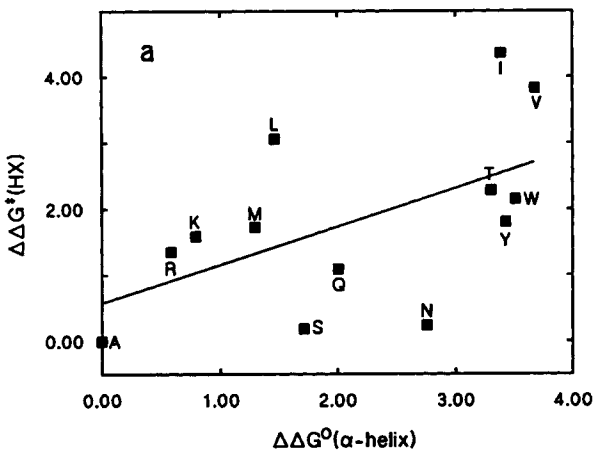
These confounding effects are illustrated in Figure 3b which compares α-helix propensities computed by Creamer and Rose[9] with propensities measured in six different host–guest systems.[3-8] Creamer and Rose[9] computed the side chain contribution to α-helix propensities in terms of the loss of configurational entropy experienced by different amino acid side chains when the main chain is changed from random coil to helix. The experimental data sets in Figure 3b individually correlate well with the computed values, indicating that side chain entropy loss does contribute importantly to loss of helix propensity. The disagreements among the measured sets of α-helix propensities[3-8] focus attention on the additional and potentially major role of context effects, i.e., on interactions between the experimentally varied side chain and the side chains in the particular host–guest system studied.

DISCUSSION

One knows that specific amino acids tend to promote the formation of α-helix or β-sheet in proteins,[10,11] but the physicochemical bases of these propensities are unclear. Progress in the chemistry of protein hydrogen exchange (HX) has revealed a previously unsuspected side chain steric blocking effect[1] that can slow HX rates as much as an order of magnitude by interfering with the HX encounter complex between the peptide group and solvent molecules.[2] The steric blocking hypothesis tested here considers the possibility that side chains may similarly interfere with peptide to solvent hydrogen bonding and thus increase the stability of intraprotein hydrogen bonding.

The test shown in Figure 2a indicates that the side chain blocking effect measured by hydrogen exchange accounts for essentially all of the amino acid-dependent variance in β-sheet stability. Figure 2a also evaluates the side chain–dependent blocking of peptide to water H-bonding in energy terms. The average side chain–dependent difference of 0.5 kJ of free energy per mol residue will be sizable when multiplied by many residues, thus can be important for protein structural stability.

The solvent blocking effect must contribute to hydrogen bond strength in α-helix as well as in β-sheet. However this effect accounts for only a small part of the amino acid–dependent variance in helix stability (Fig. 3). Thermodynamically, the contribution of side chain–solvent blocking to α-helix stability is overwhelmed by a counteracting effect, a steric clash between the side chain and upstream H-bonding peptide groups, which has been evaluated in terms of the resulting loss in side chain configurational entropy.[9] Statistically, in various host–



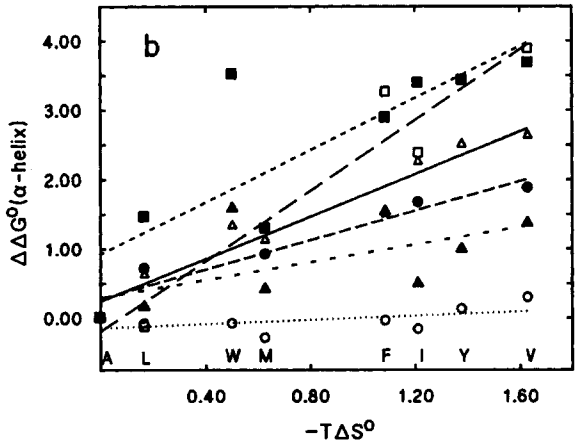


Fig. 3. **(a)** The correlation between a set of alpha helix propensities and HX blocking energy (relative to alanine). Helix propensities were measured in terms of stability changes in barnase imposed by mutational substitutions at Ala-32.[7] The same 13 residues as in Figure 2a are shown. The correlation coefficient is 0.55; the possible blocking effect on H-bond strength (0.9 kJ range, from Fig. 1a) is obscured by the much larger contributions of side chain entropy loss and context interactions (4 kJ range). **(b)** Decrease in α-helix propensity (in kJ/mol) measured in various host–guest systems compared with computed side chain entropy loss between the random and α-conformations. The sizeable differences between data sets emphasize the major role of context effects. Relative entropy loss was computed by Creamer and Rose[9] for the eight residues indicated by their one-letter code. Relative α-helix propensities were measured by Sueki et al.[3] in poly[(hydroxybutyl)glutamine] (○), by Padmanabhan et al.[4] in lysine-solubilized polyalanine (□), by Lyu et al.[5] in glutamyl-lysyl copolymers (●), by O'Neil and DeGrado[6] in a synthetic coiled coil (△), by Horovitz et al.[7] in barnase (■), and by Balber et al.[8] in T4 lysozyme (▲).

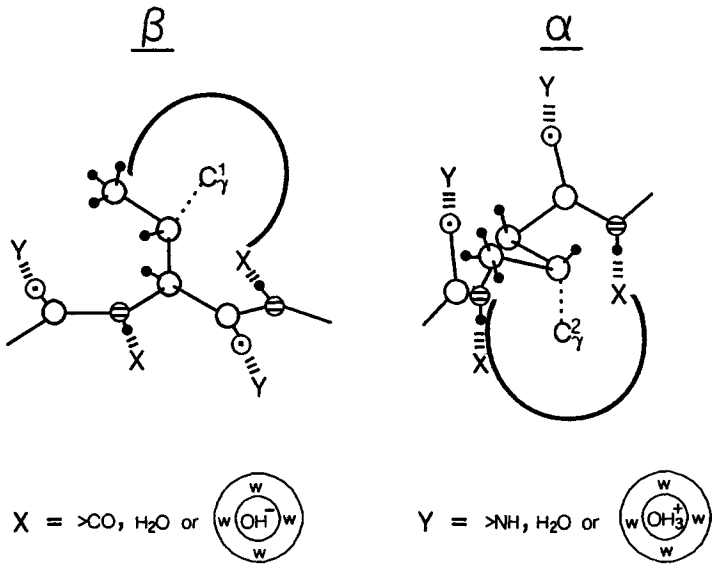


Fig. 4. The side chain-peptide group steric blocking effect. Structured proteins and random chain polypeptides can exist with main chain Φ,Ψ dihedral angles in the β-strand (left) or α-helical (right) conformation. When the main chain has the helix conformation, the methyl groups of the valine side chain (shown as an example) in two of the three χ_1 configurations of the side chain library[12] sterically interfere with species that interact with the peptide group. Much less interference occurs when the main chain adopts the β-strand conformation. The extent of the nonbonded, hard sphere, van der Waals surface of one of the valine methyls is indicated. The approximate relative sizes of the interacting species labeled X and Y are suggested, including the main chain carbonyl and NH, a water molecule, and the HX catalysts (OH^- or H^+ ion plus associated water molecules). The solvated OH^- and H_3O^+ ions are much larger, and softer, than the valine methyls. Atom types are coded as in Figure 2b.

guest systems that have been studied,[3-8] the solvent blocking effect is obscured by diverse side chain to side chain context effects as well as by the side chain to main chain entropy effect.[9] Therefore one finds only a small correlation with helix propensity (Fig. 3). In the β model of Kim and Berg considered here (Fig. 2b), the fact that the peptide group of the experimentally varied residue bonds to water in both the unfolded and the structured states helps to ensure that context-dependent effects due to the neighboring polypeptide chain are absent. Also the side chain entropy penalty[9] is largely absent in a β-strand (Fig. 4). The absence of these additional side chain-dependent factors in the β-sheet model allows a cleaner test for the existence of a side chain blocking effect on hydrogen bond strength and helps to account for the excellent correlation found in Figure 2a.

It is remarkable that *intrinsic amino acid propensities* for α and β structures and also the HX slowing factors all depend on a destabilizing side chain blocking effect at the same peptide group position. (We use the term *intrinsic propensity* to distinguish the residue-specific effects dealt with here from additional effects—due to position, pattern, and context—that also contribute to secondary structure determination.) The common spatial effect is illustrated in Figure 4. Side chain-induced slowing of the hydrogen exchange reaction reflects interference with the HX encounter complex. Intrinsic β-sheet propensities are produced by side chain interference with peptide group H-bonding to water in the random coil and not in the β conformation. Intrinsic α-helix propensity[9] is enhanced by the solvent blocking effect via H-bond promotion but is opposed by the energetically larger side chain interference with the helix H-bond acceptor. Thus blocking side chains favor β structure formation and disfavor helix formation. In all these cases there remains some ambiguity concerning the relative contributions of the peptide group NH and carbonyl.

In summary, the β structure stabilization energies measured by Kim and Berg[3] can be understood in terms of a side chain-dependent destabilization of peptide group to water hydrogen bonding in the unfolded chain. The steric blocking effect can contribute an enthalpic stabilization to intraprotein hydrogen bonds in the range of 0 to 0.9 kJ/mol of side chains relative to alanine. This effect appears to account for the intrinsic β-sheet propensities of most of the naturally occurring amino acids (not proline and glycine). An analogous side chain-dependent steric clash at the same spatial position inhibits helix formation and determines intrinsic α-helix (anti-)propensities.[9]

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