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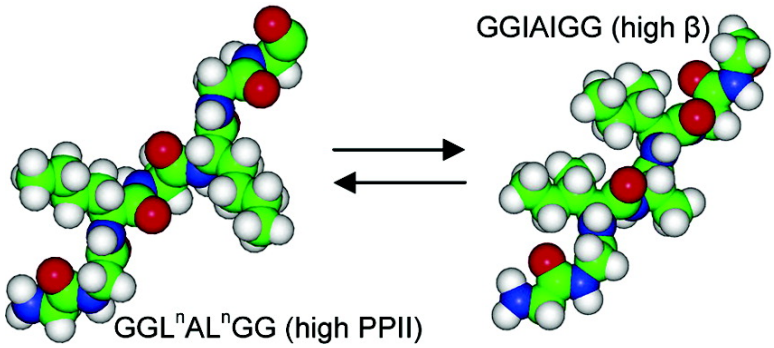

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Neighbor Effect on PPII Conformation in Alanine Peptides

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Unfolded polypeptides have been demonstrated to have significant local structure[1−8] while obeying overall random coil chain statistics.[9,10] In particular the backbone polyproline II (PPII) ($\Phi = -75°$, $\Psi = +145°$) conformation is present in unfolded proteins[8] and short peptides[7] as well as coil libraries from the PDB.[11,12] Temperature studies show that PPII conformation is in equilibrium with β structure.[12−16] The propensity for a given residue to favor PPII structure is influenced by its side chain, with alanine among the strongest while isoleucine is weak.[17] As well, the neighboring $i - 1$ or $i + 1$ residues influence the conformation of the residue i: a residue in the denatured state of a protein is shifted to a more negative Φ angle by neighboring aromatic or β-branched side chains (FHITVWY).[18,19] Baldwin and Avbelj recently surveyed the coil library and calculated the electrostatic solvation free energy (ESF) for PPII and β structures. They interpret the neighbor effect as due to shielding from water by the $i \pm 1$ side chain so that branched neighbors favor β structure over PPII.[15]

NMR and CD studies on model alanine peptides, $X_2A_7O_2$ and G_2AG_2 and similar oligomers of alanine have confirmed that PPII is dominant.[13,14] Recently, we found that the Ala residues in the peptide AcGGAA*AGGNH2 (AAA) (* represents ^{15}N label) are in PPII. The conformation of Ala* is not affected by flanking alanines from 0 to 80 °C.[20] Thus, the PPII conformation of Ala is noncooperative, and the Flory isolated pair theory holds in the PPII region.[21] To investigate the origin of the neighbor effect due to branched side chains, we synthesized the series of peptides $AcGGL^nA^*L^nGGNH_2$ (L^nAL^n, L^n represents norleucine), $AcGGIA^*AGGNH_2$ (IAA), and $AcGGIA^*IGGNH_2$ (IAI) as described previously.[20] The norleucine peptide L^nAL^n serves as a negative control for neighbor effect from β-branched side chains. The isoleucine peptides IAA and IAI are expected to show moderate and strong effects. Here the side-chain shielding explanation is confirmed by NMR spectroscopy.

The temperature dependence of the far-UV CD spectra of L^nAL^n, IAA, and IAI are consistent with a PPII-β transition as observed in AAA peptide.[20] The positive 215-nm band for PPII conformation[22] is the weakest for IAI peptide at all temperatures, consistent with lowest amount of PPII (Figure 1). The two-state behavior is indicated in each case by an isodichroic point near 203 nm in all peptides. The conformation of the central Ala can be studied in detail by NMR by means of the ^{15}N label.

The NMR $^3J_{\alpha N}$ coupling constant is related to Φ angle by parametrized Karplus equation.[23,24] ^{15}N labeling the central Ala affords more accurate measurement of $^3J_{\alpha N}$ temperature profile.[20] Standard $^3J_{\alpha N}$ values for PPII are around 5.5 Hz (J_{PPII})[13] and for β are 8−10 Hz ($J_\beta \approx 9$ Hz).[25] At 20 °C the PPII% results, estimated from $(J_\beta - J_T)/(J_\beta - J_{PPII})$, are 91% and 84% for AA*A and IA*I, respectively. The central alanines in IA*A and IA*I show higher $^3J_{\alpha N}$ values than AA*A especially at high temperatures (Figure 2), corresponding to more negative Φ angles. $L^nA^*L^n$ shows similar

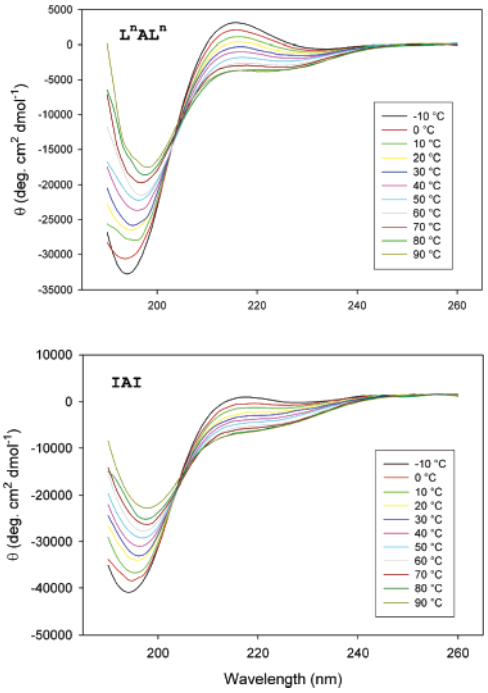

Figure 1. Far-UV CD temperature spectra of the peptide L^nAL^n and IAI. The measurements and instrument are as described previously.[20]

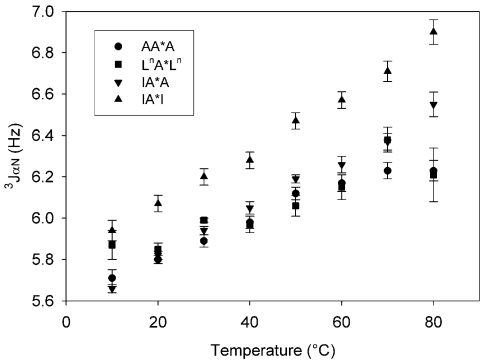

Figure 2. Temperature profiles of $^3J_{\alpha N}$ coupling constant of the central alanine in peptides of this series. The one-dimensional (1D) NMR high-resolution measurements have been described previously.[20]

$^3J_{\alpha N}$ values to AA*A at most temperatures. The conformation of Ala in $L^nA^*L^n$ is affected, but not as strong as those for IAA or IAI. If we apply a two-state transition analysis to Ala* we calculate apparent free energy differences from PPII to β (ΔG) using the equation $\Delta G = -RT \ln[(J_T - J_{PPII})/(J_\beta - J_T)]$, where R is the gas constant, T is the temperature, J_T is the measured Ala $^3J_{\alpha N}$ at temperature T. The $\Delta\Delta G$ values decrease in the presence of Ile's. At 20 °C the $\Delta\Delta G$ ($\Delta\Delta G = \Delta G_{IAI} - \Delta G_{AAA}$) of IAI is -0.4 ± 0.1 kcal/mol, and that of IAA is close to zero. At 80 °C the

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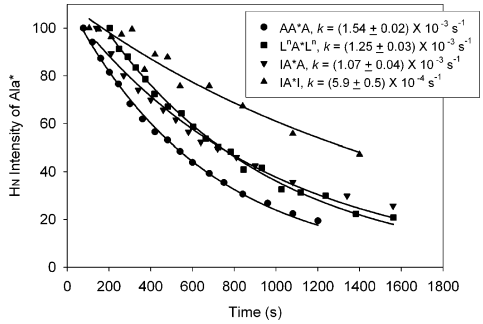

Figure 3. HX rate measurements of the central alanine HN in the peptide series. Each peptide (~1 mg) was dissolved in 600 μL of 20 mM potassium phosphate buffer at pH 3.6 and lyophilized. 99% D_2O was added to the dry powder, and spectra were recorded after loading into a preshimmed Bruker Avance 500 spectrometer. Temperature was 20 °C both inside and outside the probe. The dead time is ca. 2 min. The 1D HMQC followed by a 3919 WATERGATE pulse sequence was used to monitor spectra. All FIDs were processed identically in XWINNMR 3.5. Exponential fits to the data points give the first-order rate constants.

differences are larger: $\Delta\Delta G$ of IAI is -0.6 ± 0.06 kcal/mol and that of IAA is -0.3 ± 0.06 kcal/mol.

One reasonable explanation is that neighboring Ile is destabilizing the PPII structure of the vicinal Ala. At 80 °C, for example, the effect of two Ile's is about twice that of one. ESF calculations show that Ile gives 0.1 kcal/mol less than Ala-like small residues, and the average value for large branched residues is 0.21 kcal/mol (see Figure 5 of ref 15). Our $\Delta\Delta G$ values agree with the ESF predictions, e.g. at 20 °C $\Delta\Delta G$ ranging from 0 (IAA) to 0.2 (IAI, 0.4/2) kcal/mol per residue. Thus, solvation effects exerted by neighboring Ile side-chain shielding contribute to the destabilization of Ala PPII structure.

Since ESF calculations correlate well with hydrogen exchange (HX) protection factors,[15,26] we examined the HX rates in our model peptides. The slowest HX rate is observed for IAI while that of AAA is the fastest (Figure 3). The observed rate constant values are consistent with predicted protection factors (http://hx2.med.upenn.edu/download.html). At 20 °C, there is little difference in $^3J_{\alpha N}$ between AA*A and $L^nA^*L^n$ (Figure 2), while the 20%-fold slower HX rate of $L^nA^*L^n$ indicates that the flexible aliphatic side chain blocks catalysis (Figure 3). Neighboring Ile's have a stronger effect: the HX rate decreases 1.4-fold for a single Ile and 2.6-fold for two Ile's.

The experimental observation of neighbor effect in Ala PPII peptides has several implications. First the shielding of water by the Ile side chain decreases Ala PPII structure components and makes Ala more β as judged by $^3J_{\alpha N}$ coupling constants, consistent with the coil library survey results.[11,12,27] The effect is not seen in the control unbranched norleucine side chain. The free energy changes are consistent with ESF calculations,[15] suggesting that this semiempirical approach is applicable for small free energy differences. Second the results bear on attempts to define a PPII propensity scale for the amino acids. While developing such a scale in an all-Ala background minimizes neighbor effects, prediction of a protein unfolded structure will require including neighbor effect. While Ala residues show little cooperativity, this may not be true for other side chains. Neighbor effects may not be adequately sampled in existing coil libraries. Finally, the results apply also to helix propensity scales as well. The norleucine side chain, for example, stabilizes α-helix more than alanine,[28,29] while exerting only a minor effect, if any, on PPII. We conclude that the difference in propensity must apply to the native α-helical state.

Mounting evidence suggests that local determinants of folding strongly influence folding behavior,[30] so that deciphering the intrinsic structural propensity of each amino acid within a limited neighboring sequence context may prove useful and practical. The roles of locally determined backbone conformational preferences in the unfolded state need to be determined, since they influence early events and guide the subsequent events in folding a given sequence. Any propensity scale for α helix or β strand or turns must address the role of residual structure in the unfolded state. The results in this study demonstrate that the scale is dependent on sequence context.

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