ResearchGate

See discussions, stats, and author profiles for this publication at: https://www.researchgate.net/publication/5590898

Determination of Conformational Preferences of Dipeptides Using Vibrational Spectroscopy

ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · APRIL 2008

Impact Factor: 3.3 · DOI: 10.1021/jp7096313 · Source: PubMed

CITATIONS
45

READS
25

3 AUTHORS, INCLUDING:

Joze Grdadolnik
National Institute of Chemistry
56 PUBLICATIONS **1,263** CITATIONS
SEE PROFILE

Simona Golič Grdadolnik
National Institute of Chemistry
110 PUBLICATIONS **1,370** CITATIONS
SEE PROFILE

Available from: Joze Grdadolnik
Retrieved on: 26 January 2016

Determination of Conformational Preferences of Dipeptides Using Vibrational Spectroscopy

Jože Grdadolnik,* Simona Golič Grdadolnik, and Franc Avbelj*

National Institute of Chemistry, Hajdrihova 19, SI 1000 Ljubljana, Slovenia

Received: October 2, 2007; In Final Form: November 27, 2007

The NMR coupling constants ($^3J(H_N, H_\alpha)$) of dipeptides indicate that the backbone conformational preferences vary strikingly among dipeptides. These preferences are similar to those of residues in small peptides, denatured proteins, and the coil regions of native proteins. Detailed characterization of the conformational preferences of dipeptides is therefore of fundamental importance for understanding protein structure and folding. Here, we studied the conformational preferences of 13 dipeptides using infrared and Raman spectroscopy. The main advantage of vibrational spectroscopy over NMR spectroscopy is in its much shorter time scale, which enables the determination of the conformational preferences of short-lived states. Accuracy of structure determination using vibrational spectroscopy depends critically on identification of the vibrational parameters that are sensitive to changes in conformation. We show that the frequencies of the amide I band and the A_{12} ratio of the amide I components of dipeptides correlate with the $^3J(H_N, H_\alpha)$. These two infrared vibrational parameters are thus analogous to $^3J(H_N, H_\alpha)$, indicators for the preference for the dihedral angle φ. We also show that the intensities of the components of the amide III bands in infrared spectra and the intensities of the skeletal vibrations in Raman spectra are indicators of populations of the P_{II}, β, and α_R conformations. The results show that alanine dipeptide adopts predominantly a P_{II} conformation. The population of the β conformation increases in valine dipeptides. The populations of the α_R conformation are generally small. These data are in accord with the electrostatic screening model of conformational preferences.

Introduction

Recently, it has been shown that the conformational preferences for the dihedral angle φ of dipeptides (amino acids blocked by acetyl and *N*-methyl groups)[1] are similar to those of residues in small peptides,[2−6] in denatured proteins,[7−9] and in the coil regions of native proteins.[10,11] Determination of the conformational preferences (population distributions) of dipeptides in aqueous solution by experimental methods is therefore of fundamental importance for understanding protein structure and folding. Data on the conformational preferences of dipeptides are primarily based on NMR coupling constants; however, these data are due to long time scales that are characteristic of NMR (≈1 ms) averaged over all available states, which makes determination of the preferences by NMR very difficult. To obtain more reliable conformational preferences of dipeptides, we need to use vibrational spectroscopy with short characteristic times ($\approx 10^{-14}$ s).

Accuracy of structure determination using vibrational spectroscopy depends critically on the selection of vibrational parameters that are sensitive to changes in conformation. Experimental and theoretical studies of polypeptides have shown that several infrared and Raman bands are sensitive to amino acid residue backbone conformations. The conformational sensitive bands in the spectra of polypeptides in water are amide I vibrations,[12−16] amide III vibrations,[12,16−21] NH stretching vibrations (amide A),[22,23] $C_\alpha D$ stretching vibrations,[24] and Raman skeletal vibrations.[25,26] The most frequently used are the amide I and amide III bands.

Conformational studies using vibrational spectroscopy have been focused on unblocked di- and tripeptides. In a series of papers, Schweitzer-Stenner and co-workers[6,20,27−33] have shown that tripeptides in aqueous solutions adopt predominantly P_{II} and β conformations. Population of the α_R conformation is small. The average dihedral angles φ and ψ were determined from polarized Raman, FT infrared, and CD spectra. Similar results were obtained by electron circular dichroism and ^{1}H NMR spectroscopy of AX and XA dipeptides in aqueous solutions.[34] Woutersen and Hamm employed two-dimensional coherent femtosecond IR spectroscopy to obtain the coupling between two amide I modes (A and E1) and the orientation of their transition dipole moments of the two peptide groups in trialanine.[35] They showed that the β and P_{II} conformations are the most probable conformations of trialanine in water. The α_R conformation is unstable. The distribution of the structures of alanine dipeptide in aqueous and carbon tetrachloride solutions has been studied by nonlinear infrared spectroscopic experiments and molecular dynamics simulations.[13] Dependence of the amide I frequency on hydrogen bonding, vibrational coupling, and solvent induced internal fluctuations has been obtained. It has been shown that the most stable conformations in water are α_R and P_{II}. The internally hydrogen bonded conformation (C_7) exists only in the low dielectric environment of the carbon tetrachloride solution. The preferred conformations of glycine and alanine dipeptides in aqueous solution have been studied by laser Raman spectroscopy and depolarized Rayleigh scattering.[36] Anisotropic perturbation theory has shown that the most probable is the C_7 conformation. Raman high-pressure studies of alanine and glycine dipeptides in aqueous solution have indicated that the most populated is the P_{II} conformation.[26,37] Populations of the α_R and C_{7eq} conformations have been found to be small. The enthalpy differences of alanine dipeptide between the P_{II} and the α_R (4.4 kJ/mol) and the C_{7eq} (12.2 kJ/mol) conformations has been determined. Several other experimental studies suggest

* Corresponding authors. E-mail: (J.G.) joze.grdadolnik@ki.si and (F.A.) franc.avbelj@ki.si.

10.1021/jp7096313 CCC: $40.75 © 2008 American Chemical Society
Published on Web 02/09/2008

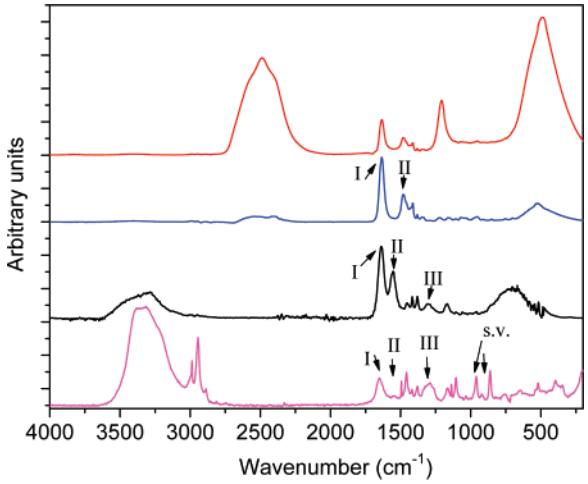

Figure 1. Calculated absorbance spectrum of 0.125 M alanine dipeptide in D_2O before (red line) and after (blue line) subtraction of the spectrum of pure D_2O, calculated absorbance spectrum of 0.063 M alanine dipeptide in H_2O after subtraction of the spectrum of pure H_2O (black line), and Raman spectrum of 0.5 M alanine dipeptide in H_2O (pink line). I−III indicate the positions of amide I, amide II, and amide III bands, respectively. (s.v. is an abbreviation for Raman skeletal vibrations.)

that the P_{II} conformation is the dominant conformation of alanine dipeptide[38−40] and of its analogue *N*-acetyl-L-alanine amide in aqueous solution.[41] It was found that tryptophan dipeptide in heavy water adopts conformations with the values of dihedral angles $\varphi = 160 \pm 10°$ and $\psi = 75 \pm 10°$.[42]

In this work, we studied the conformational preferences of 13 dipeptides in aqueous solution using infrared and Raman spectroscopy. The amide I bands in infrared spectra were analyzed in terms of two distinct amide modes. We used ATR spectroscopy, which improves the accuracy of measurements of diluted aqueous solutions. The ratios of band areas of two amide I components were correlated with the NMR coupling constants $^3J(H_N, H_\alpha)$.[1] The amide III region in the IR spectra and the skeletal modes near 900 cm^{-1} in the Raman spectra of alanine and valine dipeptides were used to determine population distribution of the most important backbone conformations.

Materials and Methods

Experimental Determination of Vibrational Spectra. Amino acids, blocked by CH_3-CO- and -NH-CH_3 groups (*N*-acetyl-amino acid-*N*′-methyl amides; dipeptides), were purchased from Bachem and Biosyn or synthesized from the corresponding *N*-acetyl-amino acid-methyl ester using methylamine at 0 °C. The samples were prepared in the range of a 50−1000 mM concentration in mQ H_2O and D_2O solutions. The infrared spectra were measured using PerkinElmer System 2000 and Bruker FTS-66 infrared spectrometers. Spectra were recorded in the range between 7000 and 370 cm^{-1}. Typically, 256 scans were averaged and apodized with a triangular function. All spectra of the 13 dipeptides were recorded at 30 °C using a diamond ATR cell equipped with a heated top plate and KRS-5 lenses. Temperature measurements were performed on the same ATR cell with a Specac temperature controller. To reduce the strong bands of diamond, backgrounds were collected for each recorded temperature up to 80 °C. The generalized two-dimensional correlation spectra (2-DGCS) were calculated using the procedure described by Noda and co-workers.[43,44] The subtraction of the spectrum of bulk water was achieved using Grams software. The subtraction factor was adjusted to a value at which the negative bands of the water stretching region

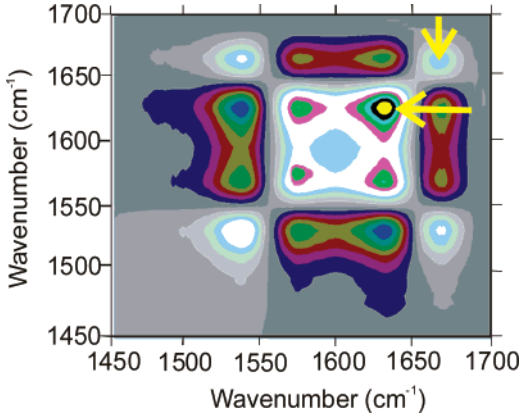

Figure 2. Synchronous spectrum of temperature measurement[50] (T = 30−80 °C) of 0.125 M alanine dipeptide in D_2O. Two diagonal peaks are observed in the amide I region, which indicate the presence of two temperature sensitive amide I components. The centers of patterns are pointed out with yellow arrows. Off-diagonal peaks show the correlation between two amide I components and bands in amide II region.0

appeared. The amide I band, amide III bands, and Raman skeletal vibrations were analyzed using the Grams band fitting procedure. The shape of the intrinsic amide I components was approximated by Voigt profile. The amide III and Raman skeletal vibrations were fitted by the sum of bands with a mixed Lorentzian and Guassian shape. Raman spectra were recorded using the PerkinElmer system 2000, equipped with a NIR Raman source with the excitation line at 106.4 nm and power between 400 and 750 mW. Typically, 2000 scans were averaged and apodized with a triangular function.

Calculation of the Optical Constants and Absorbance Spectrum. The ATR setup with one reflection allowed examination of the whole mid-infrared region without saturation of the water stretching band; however, an anomalous dispersion significantly altered the spectrum recorded in ATR mode. The frequency differences between the calculated and the original ATR bands were large, particularly for the strong bands (νOH of water shifts by 56 cm^{-1}, and the amide I band shifts by 9 cm^{-1}). The band shape is also modified in ATR mode; therefore, a pure absorption spectrum has to be calculated using optical constants. Optical constants and related absorption or ϵ'' spectra were calculated using the method proposed by Bertie and Lan[45] and Bertie and Eysel[46] in a Matlab environment. The missing part of spectra in the far-infrared region was substituted by a descent Gaussian function. To achieve a better description of the ATR optical setup, the spectrum of mQ H_2O was recorded before each ATR measurement. The precise optical constants of bulk water are known;[47,48] therefore, they can be used for calibration of the number of reflections. The number of internal reflections of an infrared beam for the diamond ATR cell is theoretically equal to 1; however, the distortions and imperfections of the optical path reduce the actual number of reflections to slightly below 1. In an aligned ATR diamond cell, the value appears to be between 0.89 and 0.97.

Results

Amide I Vibrations (1620−1680 cm^{-1}). Amide I vibrations are very sensitive to changes in conformation; therefore, they are the most useful vibrations in the infrared spectra of amino acids, peptides, and proteins. Sensitivity to structural changes is a consequence of a large contribution of the backbone C═O stretching to these vibrations.[12,14,16] The force constant of C═O stretching depends on the environment of C═O group and on the hydrogen bonding strength. Recent normal-mode analysis

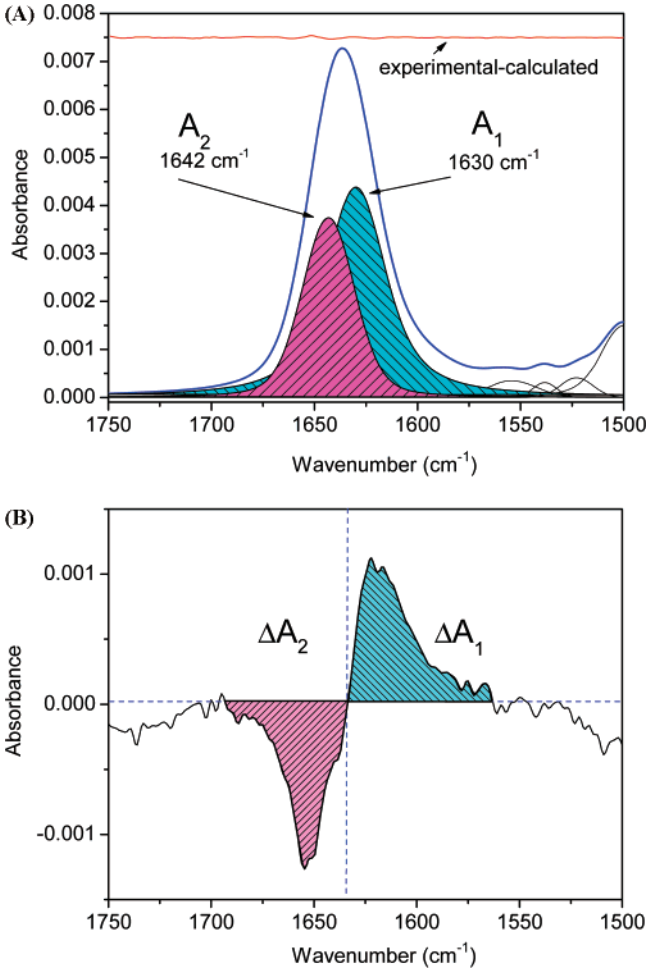

Figure 3. Decomposition of amide I region of alanine and valine dipeptides. (A) Band components in amide I region in the absorption spectrum of 0.063 M alanine dipeptide in D_2O. (B) Difference spectrum after subtraction between the spectra of alanine and the spectra of valine dipeptides. Difference spectra between the experimental and the calculated spectra are also shown (red line).

of alanine dipeptide in water[13] revealed two bands separated by almost 10 cm^{-1}, with different distributions of potential energy. The low-frequency amide I band is attributed to the vibration of the amino end C═O groups (54%), C−N stretching (10%), and C_α−C−N bending (7%). The high-frequency amide I band consists of the contributions of acetyl end C═O stretching (56%), C−N stretching (9%), and C_α−C−N bending (6%).

Figure 1 shows the calculated absorbance spectrum of 0.125 M alanine dipeptide in heavy water before and after subtraction of bulk water. For comparison, the calculated spectrum of the same dipeptide in mQ water after subtraction of water is shown. Bulk water is subtracted to the level that no negative bands appear in the region of OH (OD) stretching. The bending vibrations of the remaining H_2O molecules (1640 cm^{-1}) considerably modify the envelope of the amide I band; therefore, heavy water (bending frequency $\approx$ 1205 cm^{-1}) was used for the infrared studies of all dipeptides in this work.

Determination of the band components in the amide I region of spectra of dipeptides was hindered by the high symmetry of the amide I envelope (1620−1680 cm^{-1}, Figure 1). The common characteristic of all dipeptides studied in this work is smooth and symmetric amide I band with no traces of shoulder(s), which may be utilized for band decomposition. The approximate positions of the band components were resolved by calculating the appropriate 2-DGC spectrum[49] from the series of temperature-dependent IR spectra recorded in the temperature range between room temperature and 80 °C (Figure 2). The value of the *z*-coordinate in the synchronous spectrum corresponds to the temperature autocorrelation function of spectral intensity variations observed during the temperature measurement.[50] The synchronous spectrum shown in Figure 2 indeed reveals two correlated patterns in the amide I region of dipeptides. Thus, the major intensity change occurs at the lower and higher wavenumbers with respect to the peak maximum. In the case of the nonstructured amide I band, the expected diagonal peak would appear at the position of peak maximum. The centers of these patterns from Figure 2 (indicated with yellow arrows) were used as an initial guess for the frequencies of maxima of both components in the band fitting algorithm.

TABLE 1: Ratio A_{12}, Frequency of Amide I Maximum in Absorbance Spectrum, and $^3J(H_N, H_\alpha)$ from Ref 1 for 13 Dipeptides

amino acid	A_{12}	amide I (cm^{-1})	$^3J(H_N, H_\alpha)$ (Hz)
Gly	0.98	1639.6	5.85
His	1.14	1639.1	7.89
Trp	1.34	1635	6.91
Tyr	1.36	1638.3	7.13
Arg	1.43	1635	6.85
Ala	1.52	1636.2	6.06
Lys	1.74	1634.1	6.83
Leu	1.83	1634.7	6.83
Ile	1.86	1634.5	7.36
Val	2.11	1633.7	7.32
Thr	2.21	1638.8	7.35
Ser	2.22	1639.9	7.02
Met	2.54	1637.6	7.02

Decomposition of the amide I band of alanine dipeptide in D_2O is shown in Figure 3A. The components were obtained by fitting the experimental spectrum with Voigt functions (see Materials and Methods). The red line in Figure 3 represents the agreement between the experimental and the calculated spectra. It has been shown that the ratio of integrated areas of band components A_{12} is a sensitive indicator of the backbone conformations of peptides.[32] Ratio A_{12} is defined as

$$A_{12} = A_1/A_2 \quad (1)$$

where A_1 is the integrated area of the amide I band component at the lower wavenumber, and A_2 the integrated area of the amide I band component at the higher wavenumber. Because of the high symmetry of the amide I bands in the spectra of dipeptides, we used the fitting procedure for calculating A_{12} (eq 1) only for alanine dipeptide. For the other dipeptides, we employed another strategy. Instead of using the band fitting algorithm, we used the subtraction method. The spectrum of alanine dipeptide was subtracted from the spectra of other dipeptides. An example for valine dipeptide is shown in Figure 3B. The difference spectra were obtained by setting the subtraction factor to such a value that the intensity of the difference band was zero at the midpoint between the amide I maxima of alanine dipeptide. The ratios A_{12} for dipeptides were calculated by adding the areas of the two bands in the difference spectrum (ΔA_1 and ΔA_2) to the areas of the components already calculated for alanine dipeptide (eq 2). The ratio A_{12} of the amide I band depends on the type of side chain. The calculated ratios A_{12} of dipeptides are shown in Table 1

$$A_{12} = (\Delta A_1 + A_1)/(\Delta A_2 + A_2) \quad (2)$$

Figure 4A shows correlation between the ratio A_{12} and the NMR coupling constant $^3J(H_N, H_\alpha)$[1] of dipeptides in aqueous

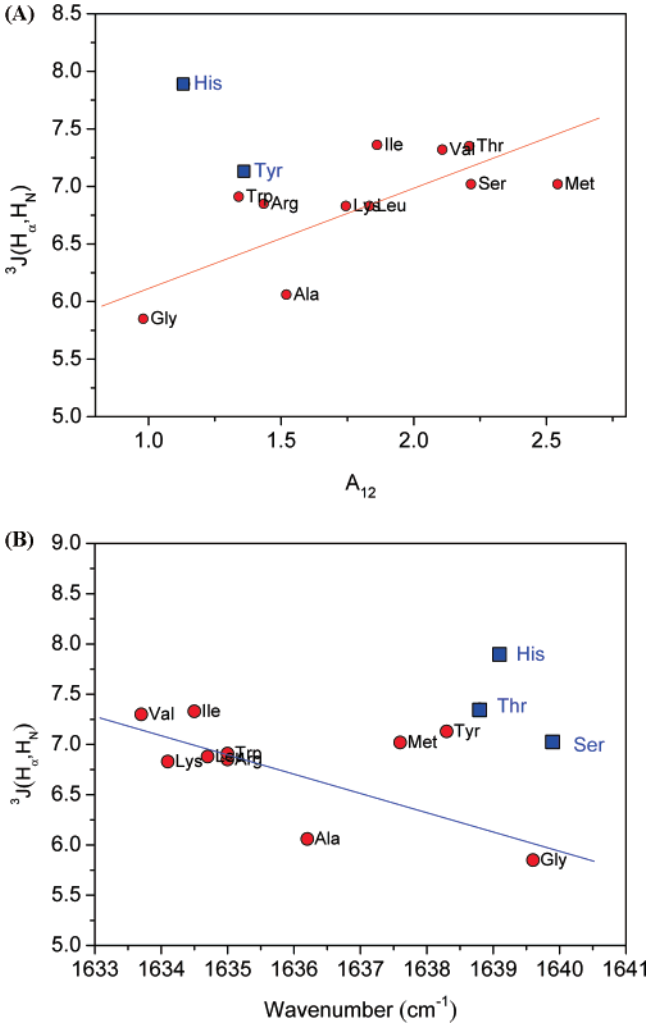

Figure 4. Correlations of the A_{12} ratio. (A) Correlation with the NMR coupling constant $^3J(H_N, H_\alpha)$. (B) Correlation with the frequency of amide I peak. Blue squares represent the outliers.

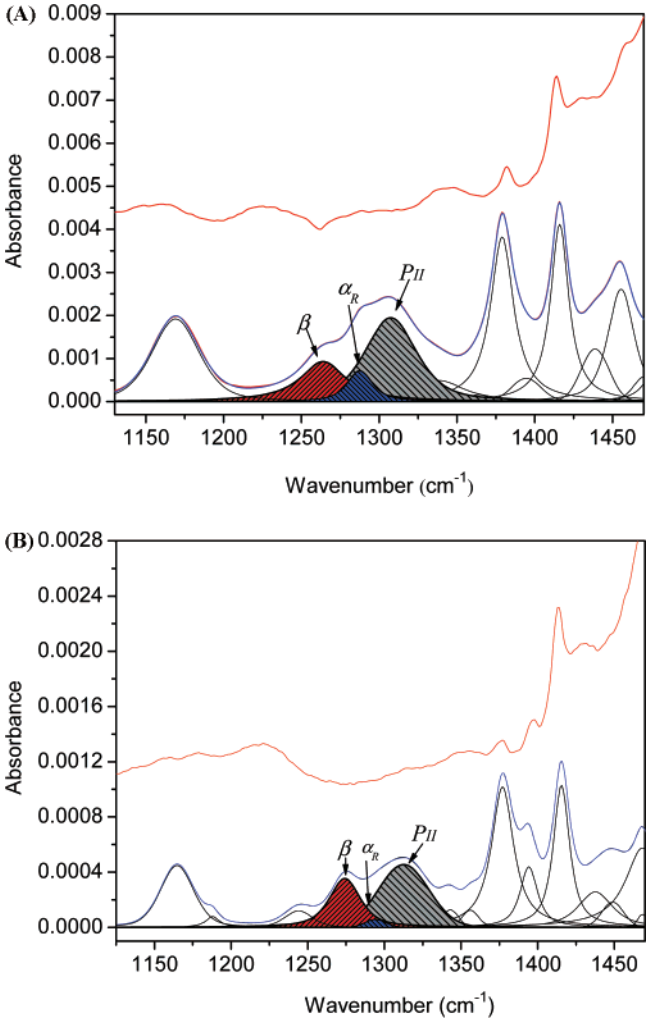

Figure 5. Decomposition of the amide III region of alanine (A) and valine (B) dipeptides. The bold lines represent the bands inside the amide III region. The spectra of dipeptides in D_2O solution are marked red.

solution. The chemical shifts are also commonly used in the determination of peptide and protein conformations. However, Avbelj and co-workers[51] have shown that the proton and ^{13}C chemical shifts of amino acid residues are reliable indicators of backbone structure only if a residue is buried in the protein interior. If a residue is exposed to solvent, then the chemical shifts are less sensitive to variations of the backbone structures. The coupling constant $^3J(H_N, H_\alpha)$ is an indicator of the conformational preferences of amino acid residues for the dihedral angle φ. The correlation coefficient for all 13 dipeptides is 0.65. If we exclude histidine, serine, and threonine dipeptides, the correlation coefficients increased to 0.76. This correlation indicates that the ratio A_{12} is an indicator of the conformational preferences for the dihedral angle φ. The interactions between side chain and main chain polar atoms may cause deviations from linearity of histidine, serine, and threonine dipeptides. The reason for the modest correlation is also the overlap of the vibrational bands in the amide I region and the vibrations of the side chains. These side chain vibrations are mainly due to ring C−C and C═C stretches.[52]

Figure 4B shows the correlation between the frequency of the amide I band and the NMR coupling constant $^3J(H_N, H_\alpha)$. The correlation coefficient for 10 dipeptides is 0.73. This correlation is significant because the frequency of the amide I band is the most accurate determinable vibrational spectral parameter of peptides in aqueous solution. The frequency of the amide I band depends on the intensities and positions of components, which are determined by the dipeptide conformation. The frequencies for 10 dipeptides also correlate with the A_{12} ratios with a high correlation coefficient (the correlation coefficient is 0.86, figure not shown). The outliers are methionine, threonine, and serine dipeptides. The side chain and main chain interactions may be responsible for the deviations from linearity of serine and threonine dipeptides. Moderate correlations can be observed also between ratio A_{12} and $^3J(H_N, H_\alpha)$ coupling constants of residues in small peptides, in denatured proteins, and in the coil regions of native proteins.

Amide III Vibrations (1240−1340 cm^{-1}). The amide III bands appear as moderately intensive band(s) in the region between 1320 and 1240 cm^{-1}. This vibration arises mainly due to the N−H in-plane bending coupled to some other peptide modes (C−N stretching, C−C stretching, and C−O in-plane bending). The sensitivity of the amide III band components for the change in conformation has been proven by numerous experimental and theoretical studies.[12,16−18,20,21,53] These studies show that the frequency of the amide III band depends not only on the dihedral angle φ but also on the angle ψ. This opens a possibility that this vibrational parameter can be used to distinguish the α_R from the other conformations, particularly from the P_{II} conformation, which has an almost equal value of the coupling constant $^3J(H_N, H_\alpha)$.

TABLE 2: Frequencies of Bands in Amide III Region and Raman Skeletal Vibrations[a]

	β	α_R	P_{II}
alanine (amide III)	1264 cm^{-1} (29%)	1287 cm^{-1} (11%)	1307 cm^{-1} (60%)
valine (amide III)	1274 cm^{-1} (36%)	1294 cm^{-1} (4%)	1312 cm^{-1} (60%)
alanine (skeletal vib.)	843 cm^{-1} (6%)	921 cm^{-1} (18%)	862 cm^{-1} (76%)
valine (skeletal vib.)	841 cm^{-1} (50%)	926 cm^{-1} (4%)	861 cm^{-1} (46%)
alanine (av)	17%	15%	68%
valine (av)	43%	4%	53%

[a] Calculated populations in water solution of alanine and valine dipeptides are given in parentheses. Averaged populations of β, α_R, and P_{II} conformers are also given.

Figure 5 shows the decomposition of the amide III vibrations of alanine and valine dipeptides. Several side chain vibrations may also have their characteristic vibrations in the amide III region. To detect these vibrations, we recorded the spectra of dipeptides in H_2O and D_2O. The amide III band components shift after the isotopic substitution to the region near 1000 cm^{-1}. The side chain vibrations are expected not to shift significantly. In this way, however, we succeeded in assigning unambiguously the amide III components only for a few dipeptides. The reason lies probably in the coupling of side chains with some protic vibrations.

The spectra of the amide III region of all dipeptides show three components: 1310, 1290, and 1260 cm^{-1}. The theoretical investigation of Mirkin and Krimm[19] predicts that the characteristic frequency of the amide III band of the α_R conformation is near 1290 cm^{-1}; therefore, we assigned this band to the α_R conformation. Analogous to other studies,[12,14,16] we assigned the low-frequency component to the β conformation and the high-frequency component to the P_{II} conformation. The extinction coefficients for the most important conformations of dipetides are not known. Assuming equal values of extinction coefficients for all three components, we can calculate the conformational populations from the band areas. The results of alanine and valine dipeptides in a water solution are shown in Table 2. The dominant conformation of both dipeptides is the P_{II} conformation. Other conformations are less populated.

The confidence limits of calculated populations can be estimated from the calculated and averaged populations from the two independent measurements. The estimated absolute error is less than $\pm 3\%$ for the α_R population. The scattering of the calculated results of the β and the P_{II} conformers from two independent calculations implies a relatively high absolute error of predicted populations. It is estimated to be $\pm 10\%$. However, the increase of the β population associated with the decrease of the P_{II} population in valine is still detectable and in accordance with the results of the amide I band analysis.

Raman Skeletal Vibrations (810−950 cm^{-1}). Theoretical and experimental studies of alanine dipeptide in aqueous solution show that the skeletal vibration in the Raman spectrum is sensitive to changes in conformation.[25] Ab initio calculations and normal coordinate analysis[25,26] show that this vibration is composed mainly of $C_\alpha-C$ stretching, CH_3 rocking, $C_\alpha-N$ stretching, and $N-C_\alpha-C$ bending modes. In the Raman spectrum of alanine dipeptide (Figure 6), three characteristic bands appear in the region between 810 and 950 cm^{-1}. The band near 920 cm^{-1} is assigned to the α_R conformation and the most intense one near 860 cm^{-1} to the P_{II} conformation.[25] The band at 843 cm^{-1} is attributed to the skeletal vibration of dipeptides in the β conformation.[25,26,37] The populations of alanine dipeptide in these conformations can be calculated from the band areas, assuming similar extinction coefficients for all three bands. Such calculations show that alanine dipeptide is predominantly in the P_{II} conformation (76%) (Table 2). The population of the α_R conformation is 18%, and the population of the β conformation is 6%. Distribution of populations of valine dipeptide is different. The population of the β conformation increases to 50%, but the population of the P_{II} conformation decreases to 46%. The population of the α_R conformation is small (4%). These populations are in accord with those obtained by analyzing the amide III region (Table 2). These probability distributions are also in accord with the trends shown by the NMR coupling constants $^3J(H_N, H_\alpha)$,[1] the ratios A_{12}, and the frequencies of the amide I band (Table 1).

Discussion

Relation between Results Obtained by NMR and Vibrational Spectroscopy. It has been suggested that the full range of intrinsic conformational preferences displayed by amino acid residues in proteins is already present in dipeptides.[1] This result is based on the NMR coupling constants $^3J(H_N, H_\alpha)$ of dipeptides, which correlate with the coupling constants of residues in small peptides, in denatured proteins, and in the coil regions of native proteins. The coupling constants $^3J(H_N, H_\alpha)$ are related to the preferences for the dihedral angle φ with the Karplus equation. The weakness of the NMR coupling constants is averaged over available states, which are not present in vibrational spectroscopy. Nevertheless, the results obtained by vibrational spectroscopy described in this work confirm the results obtained previously by NMR spectroscopy. The ratio A_{12} of the amide I band and the frequency of the amide I band correlate with the coupling constant $^3J(H_N, H_\alpha)$ of dipeptides, unfolded proteins, and residues in the coil region of native proteins. The conformational preferences displayed by residues in larger polypeptide chains are thus indeed already present in dipeptides. A consistent picture emerges from these experiments.

Assignment of Amide I, Amide III, and Raman Skeletal Vibrations. Accuracy of determining the conformational preferences of dipeptides by vibrational spectroscopy depends critically on the assignment of the vibrations that are sensitive to changes in conformation. The ratio A_{12} of the amide I band, the frequency of the amide I band, the relative intensities of the amide III bands, and the relative intensities of Raman skeletal vibrations give consistent conformational preferences of dipeptides. The correlations between the ratios A_{12} of the amide I band and the frequencies of the amide I band with the coupling constants $^3J(H_N, H_\alpha)$ of dipeptides in water (Figure 4) show that these two vibrational parameters are indicators of the preference for the dihedral angle φ. Correlation between the frequency of the amide I band and the coupling constant $^3J(H_N, H_\alpha)$ is very significant because it needs no additional spectral processing. A relatively good correlation was obtained also between the $^3J(H_N, H_\alpha)$ and the ratio A_{12}. Using subtraction instead of the unpredictable fitting algorithm gives more reliable ratios A_{12}. In assigning the three components of the amide III vibrations of dipeptides (1310, 1290, and 1260 cm^{-1}), we followed the conclusions published earlier.[12,16,17,19−21,53] The low-frequency component was assigned to the β conformation

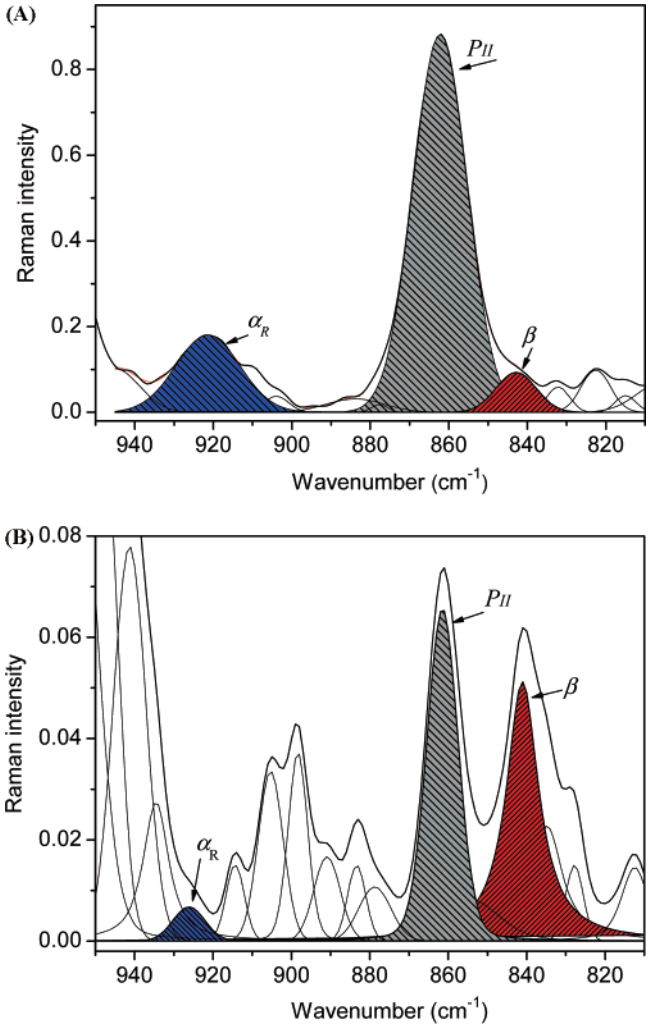

Figure 6. Raman skeletal vibration of alanine (A) and valine dipeptides (B) in H_2O solution.

and the high-frequency component to the P_{II} conformation. The component at 1290 cm^{-1} was assigned to the α_R conformation in accord with the theoretical investigation by Mirkin and Krimm.[19] Three characteristic bands appeared in the region between 810 and 950 cm^{-1} in the Raman spectra of dipeptides. The band near 920 cm^{-1} was assigned to the α_R conformation and the band near 860 cm^{-1} to the P_{II} conformation.[25] The band at 843 cm^{-1} was attributed to the skeletal vibration of dipeptides in the β conformation.[26,37]

As pointed out previously, the parameters based on the amide I vibrations detect the preferences for the dihedral angle φ. The values of the angle φ of the P_{II} ($\varphi \approx -65°$ and $\psi \approx 145°$) and α_R ($\varphi \approx -60°$ and $\psi \approx -40°$) conformations are very similar; therefore, these two parameters cannot distinguish between the P_{II} and the α_R conformations. However, the amide III vibration and Raman skeletal vibrations are able to detect the most important conformations of dipeptides, including the α_R conformation.

Conformational Preferences of Dipeptides. The experimental data presented previously show that alanine dipeptide populates predominantly the P_{II} conformation (Table 2). The population of the β conformation increases in other dipeptides and reaches the highest level in valine dipeptide, with approximately equal populations of the P_{II} and β conformations. The population of α_R is relatively small in all dipeptides. A large population of the P_{II} conformation of alanine dipeptide in aqueous solution is in accord with the results of many other experimental studies.[26,37−40,54] Note that only a few experimental studies suggest that alanine dipeptide in an aqueous solution adopts predominantly the α_R or the C_7 conformations.[13,36]

Relationship of Conformational Preferences of Dipeptides with Preferences in Larger Polypeptide Systems. Alanine residues adopt predominantly the P_{II} conformations also in small model peptides.[27,29,35,40,55−63] Moreover, the NMR experimental data show that residues in urea-denatured ubiquitin are predominantly in equilibrium between the β and the P_{II} conformations.[9] Very small populations of the α_R state are observed for the residues that correspond to the β-sheet in the native state of ubiquitin. Strong sequential $H^{\alpha}(i) - H^{N}(i + 1)$ NOEs, which indicate relatively small populations of the α_R conformation, have been observed throughout the sequences of all chemically denatured proteins.[64−69]

Physical Background of Conformational Preferences of Dipeptides. Physical reasons for conformational preferences of amino acid residues in dipeptides and polypeptides are controversial. Side chain conformational entropy,[70−74] hydrophobic interactions between side chains,[75,76] inter- and intraresidue steric interactions,[77−79] and screening of backbone electrostatic interactions by solvent[80−86] have all been suggested to cause the conformational preferences of residues in polypeptides; however, only the electrostatic screening model (ESM) is able to explain the conformational preferences of dipeptides.[1] Other models require molecular systems larger than dipeptides. Physical reasons for the stability of the P_{II} conformation are unclear. It has been suggested that intrinsic torsion of the dihedral angle φ,[86] bridging water molecules,[38−40] and steric repulsion[87] are responsible for the stability of the P_{II} conformation of alanine dipeptide.

Conclusion

We studied the conformational preferences of 13 dipeptides in aqueous solution using vibrational spectroscopy. The application of ATR spectroscopy and calculated optical constants improved the quality of the spectra. For determining the conformational preferences, we used the ratio A_{12} of the amide I band, the frequency of the amide I band, the relative intensities of the amide III components, and the relative intensities of the components of Raman skeletal vibration. For determination of the preferences for dihedral angle φ,we used the frequency of the amide I band and the ratio A_{12}. These two parameters correlate with the NMR coupling constants $^3J(H_N, H_\alpha)$. A new method for calculating areas of the amide I components was developed. The band fitting procedure was applied only for alanine dipeptide. The spectra of other dipeptides were analyzed using subtraction. The amide III vibrations in infrared spectra and skeletal vibrations in Raman spectra were used to obtain populations of the most important backbone conformations. The data show that alanine dipeptide populates predominantly the P_{II} conformation. The population of the β conformation increased in other dipeptides and reached the highest level in valine dipeptide, with approximately equal populations of the P_{II} and β conformations. Populations of the α_R conformation in alanine and valine dipeptides were small.

Acknowledgment. We thank S. Zagorc for technical assistance and B. Mohar and M. Manseend for their help with the synthesis of the dipeptides. This work was supported by a grant from the Ministry of Higher Education, Science, and Technology of Slovenia.

References and Notes

(1) Avbelj, F.; Golič Grdadolnik, S.; Grdadolnik, J.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2006**, *103*, 1272.

(2) Bundi, A.; Wuthrich, K. *Biopolymers* **1979**, *18*, 285.
(3) Merutka, G.; Dyson, H. J.; Wright, P. E. *J. Biomol. NMR* **1995**, *5*, 14.
(4) Plaxco, K. W.; Morton, C. J.; Grimshaw, S. B.; Jones, J. A.; Campbell, M. P. I. D.; Dobson, C. M. *J. Biomol. NMR* **1997**, *10*, 221.
(5) Schwarzinger, S.; Kroon, G. J. A.; Foss, T. R.; Wright, P. E.; Dyson, H. J. *J. Biomol. NMR* **2000**, *18*, 43.
(6) Eker, F.; Griebenov, K.; Cao, X.; Nafie, L. A.; Schweitzer-Stenner, R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10054.
(7) Penkett, C. J.; Redfield, C.; Dodd, I.; Hubbard, J.; McBay, D. L.; Mossakowska, D. E.; Smith, R. A. G.; Dobson, C. M.; Smith, L. J. *J. Mol. Biol.* **1997**, *274*, 152.
(8) Peti, W.; Henning, M.; Smith, L. J.; Schwalbe, H. *J. Am. Chem. Soc.* **2000**, *122*, 12017.
(9) Avbelj, F.; Golič Grdadolnik, S. *Protein Sci.* **2007**, *16*, 273.
(10) Serrano, L. *J. Mol. Biol.* **1995**, *254*, 322.
(11) Smith, L. J.; Bolin, B. A.; Schwalbe, H.; MacArthur, M. W.; Thornton, J. M.; Dobson, C. M. *J. Mol. Biol.* **1996**, *255*, 494.
(12) Bandekar, J. *Biochim. Biophys. Acta* **1992**, *1120*, 123.
(13) Gnanakaran, S.; Hochstrasser, R. M. *J. Am. Chem. Soc.* **2001**, *123*, 12886.
(14) Krimm, S.; Bandekar, J. *Adv. Protein Chem.* **1986**, *38*, 181.
(15) Wang, J.; Hochstrasser, R. H. *J. Phys. Chem. B* **2006**, *110*, 3798.
(16) Watson, T. W.; Hirst, J. D. *J. Phys. Chem. A* **2002**, *106*, 7858.
(17) Fu, N. F.; DeOliviera, D. B.; Trumble, W. R.; Sarkar, H. K.; Singh, B. R. *Appl. Spectrosc.* **1994**, *48*, 143231441.
(18) Lord, A. C. *Appl. Spectrosc.* **1977**, *31*, 187.
(19) Mirkin, N. G.; Krimm, S. *J. Phys. Chem. A* **2002**, *106*, 3391.
(20) Schweitzer-Stenner, R.; Eker, F.; Huang, Q.; Griebenow, K.; Mroz, P. A.; Kozlowski, P. M. *J. Phys. Chem. B* **2002**, *106*, 4294.
(21) Watson, T. M.; Hirst, J. D. *Phys. Chem. Chem. Phys.* **2004**, *6*, 998.
(22) Burgess, A. W.; Scheraga, H. A. *Biopolymers* **1973**, *12*, 2177.
(23) Maxfield, F. R.; Leach, S. J.; Stimson, E. R.; Powers, S. P.; Scheraga, H. A. *Biopolymers* **1979**, *18*, 2507.
(24) Mirkin, N. G.; Krimm, S. *J. Phys. Chem. A* **2004**, *108*, 10923.
(25) Deng, Z.; Polavarapu, P. L.; Ford, S. J.; Hecht, L.; Barron, L. D.; Ewing, C. S.; Jalkanen, K. *J. Phys. Chem.* **1996**, *100*, 2025.
(26) Takekiyo, T.; Imai, T.; Kato, M.; Taniguchi, Y. *Biopolymers* **2004**, *73*, 283.
(27) Eker, F.; Griebenov, K.; Schweizer-Stenner, R. *J. Am. Chem. Soc.* **2003**, *125*, 8178.
(28) Eker, F.; Griebenow, K.; Cao, X.; Nafie, L. A.; Schweitzer-Stenner, R. *Biochemistry* **2004**, *43*, 613.
(29) Eker, F.; Cao, X.; Nafie, L.; Huang, Q.; Schweizer-Stenner, R. *J. Phys. Chem. B* **2003**, *107*, 358.
(30) Eker, F.; Cao, X.; Nafie, L.; Schweitzer-Stenner, R. *J. Am. Chem. Soc.* **2002**, *124*, 14330.
(31) Schweitzer-Stenner, R. *Biophys. J.* **2002**, *83*, 523.
(32) Schweitzer-Stenner, R. *J. Phys. Chem. B* **2004**, *108*, 16965.
(33) Schweitzer-Stenner, R.; Eker, F.; Perez, A.; Griebenov, K.; Cao, X.; Nafie, L. A. *Biopolymers* **2003**, *71*, 558.
(34) Hagarman, A.; Measey, T.; Doddasomayajula, R. S.; Dragomir, I.; Eker, F.; Griebenow, K.; Schweitzer-Stenner, R. *J. Phys. Chem. B* **2006**, *110*, 6979.
(35) Woutersen, S.; Hamm, P. *J. Phys. Chem. B* **2000**, *104*, 11316.
(36) Avignon, M.; Garrigou-Lagrange, C.; Bothorel, P. *Biopolymers* **1973**, *12*, 1651.
(37) Takekiyo, T.; Imai, T.; Kato, M.; Taniguchi, Y. *Biochim. Biophys. Acta* **2006**, *1764*, 355.
(38) Han, W.-G.; Jalkanen, K. J.; Elstner, M.; Suhai, S. *J. Phys. Chem. B* **1998**, *102*, 2587.
(39) Poon, C.-D.; Samulski, E. T. *J. Am. Chem. Soc.* **2000**, *122*, 5642.
(40) Weise, C. F.; Weisshaar, J. C. *J. Phys. Chem. B* **2003**, *107*, 3265.
(41) Nielsen, E. B.; Schellman, J. A. *Biopolymers* **1971**, *10*, 1559.
(42) Bagchi, S.; Kim, S. Y.; Charnley, A. K.; Smith, A. B., III; Hochstrasser, R. M. *J. Phys. Chem. B* **2007**, *111*, 3010.
(43) Noda, I. *J. Am. Chem. Soc.* **1989**, *111*, 8116.
(44) Noda, I.; Dowrey, A. E.; Marcott, C.; Story, G. M.; Ozaky, Y. *Appl. Spectrosc.* **2000**, *54*, 236.
(45) Bertie, J. E.; Lan, Z. *J. Chem. Phys.* **1996**, *105*, 8502.
(46) Bertie, J. E.; Eysel, H. H. *Appl. Spectrosc.* **1985**, *39*, 392.
(47) Bertie, J. E.; Ahmed, M. K.; Baluja, S. *J. Phys. Chem.* **1989**, *93*, 6660.
(48) Bertie, J. E.; Ahmed, M. K.; Eysel, H. H. *J. Phys. Chem.* **1989**, *93*, 2210.
(49) Ozaki, Y. 2-D correlation spectroscopy in vibrational spectroscopy. In *Handbook of Vibrational Spectroscopy*; Chalmers, J. M., Griffits, P. R., Eds.; John Wiley and Sons: New York, 2002; Vol. 3, p 2135.
(50) Noda, I. General theory of two-dimensional (2-D) analysis. In *Handbook of Vibrational Spectroscopy*; Chalmers, J. M., Griffits, P. R., Eds.; John Wiley and Sons: New York, 2002; Vol. 3, p 2123.
(51) Avbelj, F.; Kocjan, D.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 17394.
(52) Barth, A. *Prog. Biophys. Mol. Biol.* **2000**, *74*, 141.
(53) Myshakina, N. S.; Asher, S. A. *J. Phys. Chem. B* **2007**, *111*, 4271.
(54) Madison, V.; Kopple, K. D. *J. Am. Chem. Soc.* **1980**, *102*, 4855.
(55) Schweitzer-Stenner, R.; Eker, F.; Huang, Q.; Griebenow, K. *J. Am. Chem. Soc.* **2001**, *123*, 9628.
(56) Shi, Z.; Olson, C. A.; Rose, G. D.; Baldwin, R. L.; Kallenbach, N. R. *Proc. Natl. Acad. U.S.A.* **2002**, *99*, 9190.
(57) Woutersen, S.; Pfister, R.; Hamm, P.; Mu, Y.; Kosov, D. S.; Stock, G. *J. Chem. Phys.* **2002**, *117*, 6833.
(58) Ding, L.; Santini, P. A.; Shi, Z.; Kallenbach, N. R. *J. Am. Chem. Soc.* **2003**, *125*, 8092.
(59) Mehta, M. A.; Fry, E. A.; Eddy, M. T.; Dedeo, M. T.; Anagnost, A. E.; Long, J. R. *J. Phys. Chem. B* **2004**, *108*, 2777.
(60) Liu, Z.; Chen, K.; Ng, A.; Shi, Z.; Woody, R. W.; Kallenbach, N. R. *J. Am. Chem. Soc.* **2004**, *126*, 15141.
(61) Schweitzer-Stenner, R.; Eker, F.; Griebenow, K.; Cao, X.; Nafie, L. A. *J. Am. Chem. Soc.* **2004**, *126*, 2768.
(62) Chen, K.; Liu, Z.; Kallenbach, N. R. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 15352.
(63) Graf, J.; Nguen, P. H.; Stock, G.; Schwalbe, H. *J. Am. Chem. Soc.* **2007**, *129*, 1179.
(64) Frank, M. K.; Clore, G. M.; Gronenborn, A. M. *Protein Sci.* **1995**, *4*, 2605.
(65) Arcus, V. L.; Vuilleumier, S.; Freund, S. M. V.; Bycroft, M.; Fersht, A. R. *J. Mol. Biol.* **1995**, *254*, 305.
(66) Schwalbe, H.; Fiebig, K. M.; Buck, M.; Jones, J. A.; Grimshaw, S. B.; Spencer, A.; Glaser, S. J.; Smith, L. J.; Dobson, C. M. *Biochemistry* **1997**, *36*, 8977.
(67) Meekhof, A. E.; Freund, S. M. V. *J. Mol. Biol.* **1999**, *286*, 579.
(68) Bai, Y.; Chung, J.; Dyson, H. J.; Wright, P. E. *Protein Sci.* **2001**, *10*, 1056.
(69) Yao, J.; Chung, J.; Eliezer, D.; Wright, P. E.; Dyson, H. J. *Biochemistry* **2001**, *40*, 3561.
(70) Piela, L.; Nemethy, G.; Sheraga, H. A. *Biopolymers* **1987**, *26*, 1273.
(71) McGregor, M. J.; Islam, S. A.; Sternberg, M. J. E. *J. Mol. Biol.* **1987**, *198*, 295.
(72) Creamer, T. P.; Rose, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 5937.
(73) Creamer, T. P.; Rose, G. D. *Protein Sci.* **1995**, *4*, 1305.
(74) Srinivasan, R.; Rose, G. R. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 14258.
(75) Blaber, M.; Zhang, X.; Matthews, B. W. *Science (Washington, DC, U.S.)* **1993**, *260*, 1637.
(76) Blaber, M.; Zhang, X.; Lindstrom, J. D.; Pepiot, S. D.; Basse, W. A.; Mathews, B. W. *J. Mol. Biol.* **1994**, *235*, 600.
(77) Yun, R. H.; Hermans, J. *Protein Eng.* **1991**, *4*, 761.
(78) Hermans, J.; Anderson, A. G.; Yun, R. H. *Biochemistry* **1992**, *31*, 5646.
(79) Street, A. G.; Mayo, S. L. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 9074.
(80) Avbelj, F. *J. Mol. Biol.* **2000**, *300*, 1335.
(81) Avbelj, F.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 1309.
(82) Avbelj, F.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2004**, *101*, 10967.
(83) Avbelj, F.; Fele, L. *J. Mol. Biol.* **1998**, *279*, 665.
(84) Avbelj, F.; Luo, P.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10786.
(85) Avbelj, F.; Moult, J. *Biochemistry* **1995**, *34*, 755.
(86) Avbelj, F.; Baldwin, R. L. *Proc. Natl. Acad. Sci. U.S.A.* **2003**, *100*, 5742.
(87) Pappu, R. V.; Srinivasan, R.; Rose, G. D. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 12565.