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Interaction between water and polar groups of the helix backbone: An important determinant of helix propensities

PEIZHI LUO* AND ROBERT L. BALDWIN†

Biochemistry Department, Beckman Center, Stanford University Medical Center, Stanford, CA 94305-5307

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ABSTRACT **We report an enthalpic factor involved in determining helix propensities of nonpolar amino acids. Thermal unfolding curves of the five 13-residue peptides, Ac-KA_4XA_4KGY-NH_2 (X = Ala, Leu, Ile, Val, Gly), have been measured by using CD in water/trifluoroethanol (TFE) mixtures. The peptide helix contents show that the rank order of helix propensities changes with temperature: although Ala has the highest helix propensity at 0°C in all TFE concentrations, it is lower than Leu, Ile, and Val at 50°C in 20% TFE. This change is attributed to shielding by nonpolar side chains of the interaction between water and polar groups in the helix backbone for the following reasons. (*i*) Helix content is directly related to helix propensity for these designed peptides because side-chain–side-chain interactions are absent. (*ii*) The change in rank order with temperature is enthalpic in origin: in water, the apparent enthalpy of helix formation calculated from the thermal unfolding curves varies widely among the five peptides and has the same rank order as the helix propensities at 0°C. The rank order does not result from burial of nonpolar surface area because the calculated heat capacity change (ΔCp) on helix formation is opposite in sign from the expected ΔCp. (*iii*) A nonpolar side chain can exclude water from interacting with helix polar groups, according to calculations of water-accessible surface area, and the polar interaction between water and peptide polar groups is entirely enthalpic, as shown by amide transfer data.**

There are basic reasons for believing that a still unknown factor, in addition to side-chain entropy, is important in determining the values of the helix propensities of the nonpolar amino acids. Only nonpolar residues are considered because polar and charged amino acids present a more complex case. The first reason is that, although the rank order is the same in both systems, the relative helix propensities are quantitatively different in alanine-based peptides (1) and in peptide sequences from protein helices (2, 3). The helix propensity of alanine is 35 times greater than that of glycine in alanine-based peptides (1) but only six times greater in an RNase T1 peptide helix (3) or in RNase T1 itself (3), or in a compilation of data for 323 peptides taken from the literature and analyzed by the algorithm AGADIR (2). The second reason is that, although helix propensities of the nonpolar amino acids are highly correlated with the loss of side-chain conformational entropy that occurs upon helix formation (4–6), nonetheless this effect accounts only for one-third of the free energy differences arising from the helix propensities of the nonpolar amino acids (1).

Consequently, we undertook a search for an unknown factor that plays a dominant role in determining the values of helix propensities. Our procedure and its rationale are as follows. First, there are good reasons for believing that the context dependence of the helix propensities arises from the immediate neighboring four residues on either side of the test amino acid. This inference follows from the observation (3) that the helix propensities are quantitatively the same when measured at a solvent-exposed position in a given sequence placed either in a protein or in a peptide helix. There are two different ways in which neighboring side chains might affect the helix propensity of a test amino acid: by a direct side-chain–side-chain interaction or by an indirect mechanism in which the test amino acid and its neighbors make competing interactions with the helix backbone. Direct interactions, such as H-bonds between side chains, salt bridges, and nonpolar interactions, are known to influence helix stability, and they can be measured quantitatively in alanine-based peptides (7). Also, they can be estimated by the statistical fitting procedure used to construct AGADIR (2). Although these specific side-chain interactions affect helix stability, they can be determined separately from helix propensities.

Indirect interactions, in which the test amino acid and its neighbors make competing interactions with the helix backbone, can be tested by the following procedure. The basic assumption is that such interactions should affect the enthalpy of helix formation. We study a set of five peptides with the sequence Ac-KA_4XA_4KGY-NH_2 (X = Ala, Leu, Ile, Val, Gly). There are three reasons for choosing this sequence. The first is that the test amino acid has only alanine neighbors for four residues on either side, so that the test amino acid contacts only alanine. The second reason is that, because the peptide is short, its helix content depends sensitively on the choice of the test amino acid. The third is that these peptides are just long enough to form a moderately strong helix in water without forming aggregates (data not shown). The thermal unfolding curves of the peptide helices are measured by CD at a series of trifluoroethanol (TFE) concentrations (0–50% vol/vol), as described (8). These data are used to determine the helix propensities of the test amino acids, relative to a reference (Ala), to find out whether they vary with temperature in a manner that cannot be explained by changes in side-chain entropy. By repeating the experiment at different TFE concentrations, we can increase the helix contents of the five peptides, especially at high temperatures where the extent of helix formation in water is low. The TFE results then can be extrapolated back to water to give reliable helix propensities at high temperatures. The temperature dependence exhibited in the thermal unfolding curves of alanine-based peptides is known to change drastically with increasing TFE concentration; this behavior was investigated and explained in a previous study (8).

In experiments with peptide helices, the helix propensity is defined as the helix propagation parameter of helix-coil theory (9–11). We use the Lifson-Roig theory (10) here, whose helix propagation parameter is denoted by *w*; it is related to the propagation parameters of Zimm-Bragg theory (9) through a

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Abbreviations: TFE, trifluoroethanol; ASA, accessible surface area.

*Present address: Xencor, 2585 Nina Street, Pasadena, CA 91107.
†To whom reprint requests should be addressed. e-mail: bbaldwin@cmgm.stanford.edu.

relation given by Qian and Schellman (11). The corresponding standard free energy is $\Delta G = -RT \ln [w/(1+v)]$, where v^2 is the helix nucleation constant of Lifson-Roig theory ($v = 0.036$; ref. 1). Because w is an equilibrium constant, it varies with temperature according to its value of ΔH, the standard enthalpy of helix formation. If w depends only on side-chain entropy for nonpolar amino acids besides Gly and Pro, then Ala, Leu, Ile, and Val all should have the same value of ΔH, and $\Delta\Delta G/T$ (the difference between $\Delta G/T$ for the test amino acid and the reference) should be independent of temperature.

Although helix propensity itself can be determined in experiments with alanine-based peptides (1), only the ratio of w_X/w_R or the free energy difference $\Delta G_X - \Delta G_R$ (r = reference amino acid) can be determined in experiments with natural sequence peptides such as the RNase T1 peptide (3), because these helices are stabilized by unknown side-chain interactions. We follow both procedures here: we determine the actual helix propensities by using Schellman's heteropolymer algorithm (12), and we also determine the helix propensities relative to a reference amino acid by using the homopolymer approximation, as in the RNase T1 study (3). The two procedures are found to give equivalent results. We showed earlier (8) that helix-coil theory remains valid for peptide helices in TFE-water mixtures. The test is whether a single set of parameters fits the thermal unfolding curves of a set of repeating sequence peptides with varying chain lengths. This test is satisfied, and only the helix propensity and its associated enthalpy need be allowed to change with TFE molarity (8).

MATERIALS AND METHODS

Peptide Synthesis. Peptides were synthesized by the solid-phase method using fluorenylmethoxycarbonyl (Fmoc) chemistry as described (8). Pentafluorophenyl esters of Fmoc-protected amino acids were used for all coupling reactions except for Ala, where the free acid of Fmoc-Ala and the *in situ* activation reagent 2-(1H-benzotriazole-1-yl)-1,1,3,3,-tetramethyluronium tetrafluoroborate (TBTU) were used. The peptides were purified by a preparative C18 reverse-phase column; the peptides were >95% pure by analytical C18 reverse-phase column chromatography, and the identity was confirmed by mass spectrometry.

CD Measurements. CD measurements were made as in ref. 8 on an Aviv 62 DS spectropolarimeter equipped with a Peltier temperature control unit. The ellipticity was calibrated with (+)-10-camphorsulfonic acid. The concentration of each peptide stock solution was determined by measuring tyrosine absorbance in 6 M guanidine hydrochloride solutions with 20 mM phosphate buffer at pH 6.5 ($\varepsilon_{275} = 1{,}450\ M^{-1}\cdot cm^{-1}$). CD samples contain 20–50 μM peptide in 2 ml of TFE/water mixtures. The thermal transition data were recorded every 2°C or 5°C in heating but every 10°C or 20°C in cooling with an equilibrium time of *ca.* 6–8 min.

Data Analysis. To obtain the helix propensity values at 0°C (ΔG) and the average enthalpy change for helix formation ($<\Delta H>$), the thermal unfolding curves were fitted to modified Lifson-Roig helix-coil theory (1), which includes N-capping. The N-cap values, the helix propensity values for all residues except the test residue, and the helix nucleation parameter were taken from ref. 1, except that the helix propensity value found here for Ala was used in fitting the data for the Leu, Ile, Val, and Gly peptides. To find $<\Delta H>$, the partial homopolymer approximation was used: an average helix propensity $<w>$ was computed for all residues except the C-terminal Gly

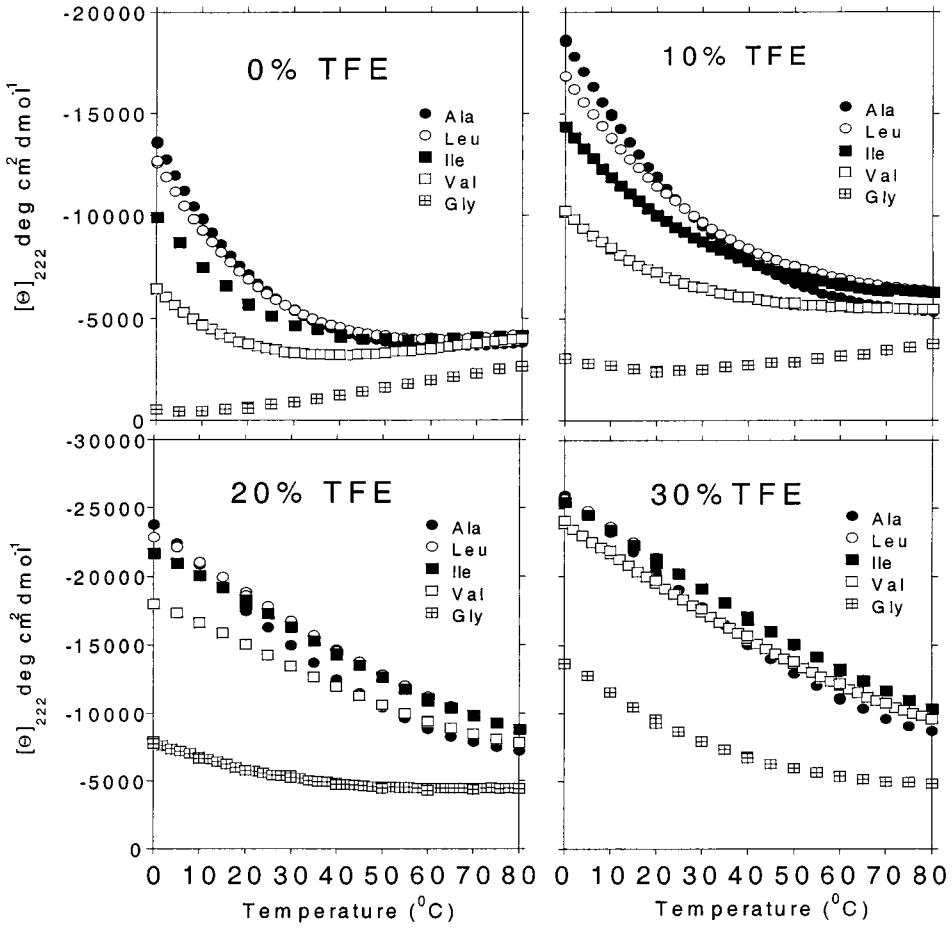

FIG. 1. The thermal unfolding curves of Ac-KA$_4$XA$_4$KGY-NH$_2$ (X = Ala, Leu, Ile, Val, Gly) from 0 to 80°C at 0%, 10%, 20%, and 30% TFE (0.1 M NaCl, 1 mM each of sodium phosphate, sodium borate, and sodium citrate, pH 7.0). Note the crossover between the Ala peptide curve and certain other curves, depending on the temperature and TFE concentration.

and Tyr, whose values in water were taken from ref. 1. The helix propensity ratios relative to Ala (ΔΔG) were found by applying the partial homopolymer approximation to all residues except the test residue and the C-terminal Gly and Tyr, and then solving for ΔΔG from the helix contents of the Ala peptide (used as a reference) and the test peptide (see ref. 3). The ellipticity values for the complete helix and coil and their temperature dependences were taken from our earlier work (8). The NONLIN software package (13) was used for nonlinear least-squares fitting.

RESULTS AND DISCUSSION

Thermal Unfolding Curves at Different TFE Concentrations. The thermal unfolding curves of the five peptides are shown in Fig. 1 for 0%, 10%, 20%, and 30% TFE. The Gly peptide at 0% TFE shows an unfolding curve close to that of a random coil peptide: it is nearly linear and tilts upward with increasing temperature. At 10% TFE and 0°C, the order of decreasing helix content is A, L, I, V, G but above 30°C the order of A and L reverses. At 10% TFE the helix contents of all peptides are higher, even the Gly peptide shows some helix formation, and above 50°C the order becomes L, I, A, V, G. At 20% TFE and 50°C, even the Val peptide has a higher helix content than the Ala peptide and the rank order of helix propensities becomes L, I, V, A, G. These results show that the rank order of helix propensities depends on temperature, and the Ala peptide presumably has a larger enthalpy change upon helix formation than the other peptides; this effect is observed more readily at higher TFE concentrations.

Fitting Results to Helix-Coil Theory. We follow the same fitting procedure here as before (8), and in addition, we solve directly for the ratio of helix propensities w_X/w_A (or for the corresponding free energy difference $\Delta\Delta G_{XA}$) by the same procedure used in the RNase T1 experiments (3).

The results of fitting the curves are shown in Fig. 2. Fig. 2*A* gives the helix propensity of each amino acid (expressed as ΔG) versus [TFE] at 0°C. Fig. 2*B* gives the helix propensity ratio relative to alanine (expressed as ΔΔG) versus [TFE], and Fig. 2*C* gives the average enthalpy change per residue, $<\Delta H>$, for helix formation versus [TFE]. For completeness, the values of $\Delta<\Delta H>$, the helix enthalpy difference per residue between the Ala peptide and another peptide, also are given. The alanine results, both for ΔG and $<\Delta H>$, are close to those found earlier (8) with a set of repeating–sequence peptides. The values of ΔΔG in Fig. 2*B* agree within error with the results of subtracting ΔG_X from ΔG_A (shown in Fig. 2*A*); the ΔΔG values shown in Fig. 2*B* can be compared directly with those from natural sequence peptides (3).

The results shown in Fig. 2 can be used to make several points. (*i*) As expected from the changes in relative helix propensities with temperature, there are differences among the five peptides in the apparent enthalpy of helix formation. These differences are surprisingly large, and there is an apparent enthalpy difference between every two peptides. (*ii*) Not only do the values of ΔΔG change with [TFE], so do the values of $\Delta<\Delta H>$. The correlation suggests that the changes in relative helix propensities are largely enthalpic in origin. At high [TFE] (>4 M), the values of $<\Delta H>$ approach a common value for all test residues, including Gly, which is a well-known helix breaker in water. (*iii*) The results for "all-backbone" alanine helices, which were known earlier, are deceptively simple. They have been interpreted with the aid of model compound data (8) to indicate that the peptide H-bond

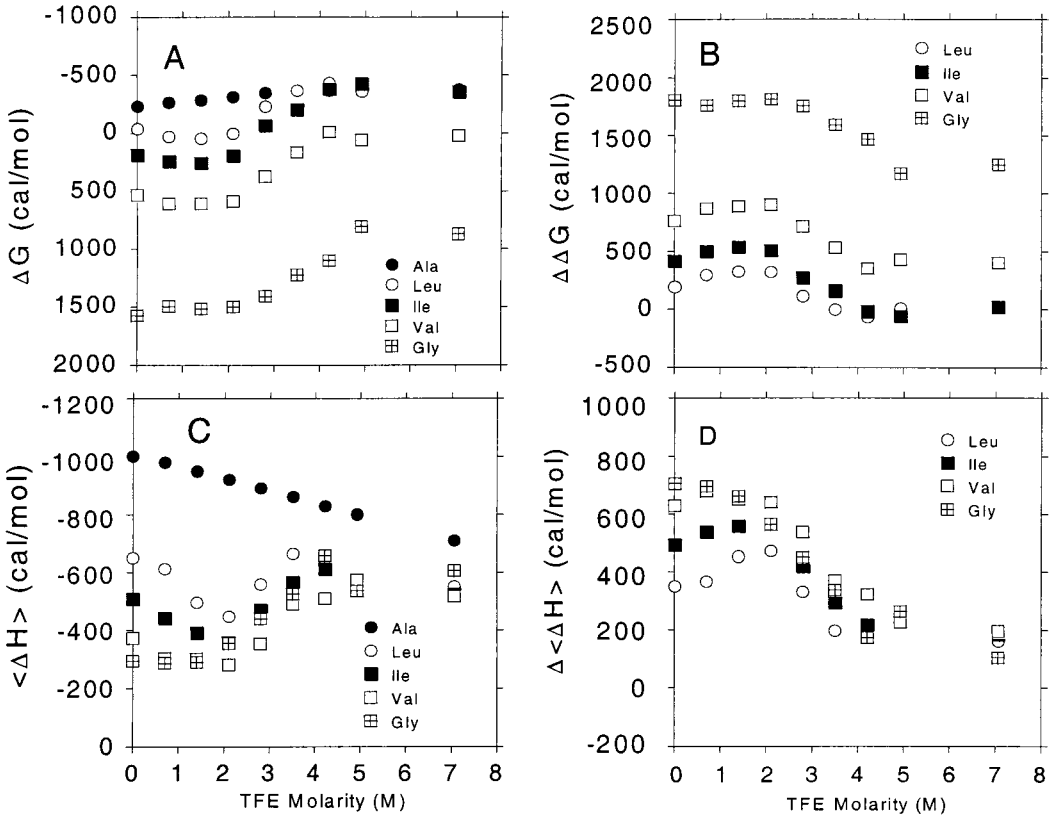

FIG. 2. Free energy changes corresponding to helix propensity values at 0°C and enthalpy changes for formation of the entire peptide helix but expressed per residue as a function of TFE molarity measured from 0 to 50 volume% TFE. (*A*) Free energy change of $\Delta G = -RT\ln[w_X/(1+v)]$ (see text) as a function of TFE molarity, where w_X is the helix propensity of residue X = Ala, Leu, Ile, Val, and Gly. (*B*) The helix propensity ratio relative to alanine (expressed as $\Delta\Delta G_X = \Delta G_X - \Delta G_A$) versus [TFE]. (*C*) The average enthalpy change per residue, $<\Delta H>$, for formation of the entire helix versus [TFE], and $\Delta<\Delta H_X> = <\Delta H_X> - <\Delta H_A>$ is shown in *D* for comparison.

becomes stronger in the TFE/water mixtures, presumably because TFE affects the competition between water and the peptide H-bond for peptide CO and NH groups. Unlike the results for Ala, however, the dependence of ΔG and ΔH for Leu, Ile, and Val (Fig. 2) on [TFE] is complex. The complexity probably reflects a fine balance between the effects of TFE and of the side chains that shield the interaction between water and polar groups. At low [TFE] (<2 M), the values of $<\Delta H>$ change in a side-chain-dependent manner (Fig. 2*C*), whereas there is little change in ΔG with [TFE] (Fig. 2*A*). At high [TFE] (>3 M), the effects of TFE are dominant, and Leu, Ile, and Val show similar changes in both ΔG and $<\Delta H>$. (*iv*) Gly presents a special and puzzling case: probably the increased access of water to the helix backbone (see below) is a main factor affecting the behavior of glycine. Nonetheless, the change in its helix propensity above 2 M [TFE] (Fig. 2*A*) correlates with the change in helix enthalpy (Fig. 2*B*).

Fitting the Helix Unfolding Curves with Varying Values of ΔCp Rather Than $<\Delta H>$. The thermal unfolding curves (Fig. 1) show that the helix propensities of Leu, Ile, Val, and Gly all increase relative to Ala as the temperature increases. At first sight, a possible explanation is that this effect is caused by greater burial of the nonpolar surface upon helix formation by Leu, Ile, and Val compared with Ala, because the negative free energy change for burial of nonpolar surface area increases with temperature (14). Note, however, that the values of helix content shown in Fig. 1 depend on the helix propensities as equilibrium constants, not as standard free energies (see the discussion by Schellman, ref. 15). If hydrophobic burial is responsible for the change in rank order with temperature, then it can be characterized by assigning different values of ΔCp to the five peptides, where ΔCp is the change in heat capacity upon helix formation. The main result of fixing ΔH at the value of the Ala peptide and letting ΔCp vary for the other four peptides is that ΔCp has the opposite sign to that expected for burial of nonpolar surface area (data not shown). If the helix propensities of Leu and Ile become larger at high temperatures than that of Ala because of greater burial of nonpolar surface, then their ΔCp values for helix formation should be negative like the free energy of the folding reaction, but instead they are positive (see predicted ΔCp values of Leu and Ile in table 5 of ref. 6). Moreover, TFE also is known to reduce the hydrophobic effect in water, and the difference between the high temperature values of Leu and Ile relative to that of Ala becomes even larger at high [TFE]. Consequently, burial of nonpolar surface area upon helix formation is not a plausible explanation for the change in rank order of the helix propensities. Richards and Richmond (16) computed the amount of nonpolar surface area burial on helix formation in an alanine background and found only small differences among the amino acids studied here.

The values of $-<\Delta H>$ shown by the Leu, Ile, Val, and Gly peptides, compared with that of the Ala peptide, explain why their helix propensities increase relative to Ala with increasing temperature. If ΔH is zero, the helix propensity (like any equilibrium constant) is independent of temperature. If two amino acids have different values of $-\Delta H$ for helix formation, the helix propensity of the one with the smaller value of $-\Delta H$ gains on the other with increasing temperature.

Model Building Results. Nonpolar side chains readily block the access of water to CO groups in an alanine-based helix. This effect is easily demonstrated by model building, using a standard helical conformation with preferred side-chain rotamers of Leu, Ile, and Val at a central position of an alanine-based helix. The changes in water-accessible surface area of the peptide backbone are computed by using a standard program and are shown in Fig. 3. Side-chain shielding of the interaction between water and the peptide backbone is specific: it occurs at i and/or i−4 positions, depending on the specific rotamer and side-chain types. Summarized in simple terms, as shown schematically in Fig. 3, Ile and Val each can desolvate two helical peptide CO groups at the i and i−4 positions; Leu desolvates one, either at i−4 in the g+ rotamer or at i in the t rotamer, whereas none is desolvated by Ala or Gly, and Gly markedly increases the access of water to peptide groups at i and i−4 and, to a lesser extent, at i−3 and i+1. Side-chain shielding of helix polar groups has been observed earlier, both in modeling the helices of myoglobin (16) and experimentally in analyzing structures of T4 lysozyme mutants (17).

The Polar Interaction Between Water and the Peptide Group Is Enthalpic. To obtain the polar interaction between water and the peptide group, we analyze model compound data by a procedure similar to the one used by Honig and coworkers (18). Their aim was to represent the polar interactions of numerous compounds by using a suitable set of partial charges. Our aim is to find out whether different amides, used as models for the peptide group, give essentially the same value for the polar interaction of the amide group with water, and then to find out whether the polar interaction is enthalpic by comparison with calorimetric data.

Transfer data from aqueous solution to the gas phase are analyzed. The observed values of transfer free energy (ΔG_{obs}) for nonpolar compounds (hydrocarbons) are taken to be the sum of two terms, one for formation of a cavity in the solvent (ΔG_{cav}) and one for van der Waals interactions between the solute and the solvent (ΔG_{vdw}), and both terms are assumed to scale linearly with water-accessible surface area (ASA). For polar compounds, (ΔG_{obs}) is assumed to be a sum of three terms, $\Delta G_{cav} + \Delta G_{vdw} + \Delta G_{pol}$, where ΔG_{pol} is the polar interaction between solute and water. The van der Waals interaction term, for a given value of ASA, is assumed to be approximately the same for the polar and nonpolar compounds. The cavity term describes the work of making an empty cavity, and it is not affected by the type of solute placed in the cavity. The transfer enthalpies are analyzed by the same procedure, using calorimetric data from the literature.

We will present data for various amides elsewhere and illustrate the procedure with data for just one amide in Table 1. The other amides studied give equivalent results. The first main point in Table 1 is that, although ΔH_{obs} is nearly twice as large as ΔG_{obs} for acetamide, ΔG_{pol} is the same as $\Delta H_{pol.}$ Thus, the polar interaction between water and the CO and NH_2 groups of acetamide is entirely enthapic. The second main point is that the value of ΔH_{pol}, −11.6 kcal/mol, is large compared with the enthalpy change for forming the helix backbone in an alanine-based helix, −1.0 kcal/mol (19, 20). If we assume that the enthalpy of helix formation represents only changes in the polar interactions made by the peptide CO and NH groups, then we may write it as $\Delta H_{hc} = \Delta H_1 + \Delta H_2 - \Delta H_3$, where ΔH_1 is the enthalpy of the peptide H-bond (for example, say −10 kcal/mol), ΔH_3 is the polar interaction (with water) of the free peptide CO and NH groups (−11.6 kcal/mol) and, if ΔH_{hc} (the enthalpy of helix formation) is −1.0 kcal/mol, then in this example ΔH_2 is −2.6 kcal/mol, where ΔH_2 is the enthalpy of interaction between water and the peptide CO and NH groups in the helix. In the structure of ordinary ice, every oxygen makes two equivalent H-bonds. Thus, ΔH_2 also may be described as an H-bond enthalpy, but the relative sizes of ΔH_1 and ΔH_2 are presently unknown (see ref. 21).

The Context Dependence of Helix Propensities Arises from an Enthalpic Interaction. We present evidence that the rank order of helix propensities changes with temperature and that the temperature dependence is affected by TFE concentration. These observations indicate that an enthalpic interaction is a major determinant of helix propensities. The model we propose, namely that nonpolar side chains tend to exclude water from helix polar groups whereas water interacts enthalpically with helix polar groups, may not be the only model able to explain our results, but we do not have a second model.

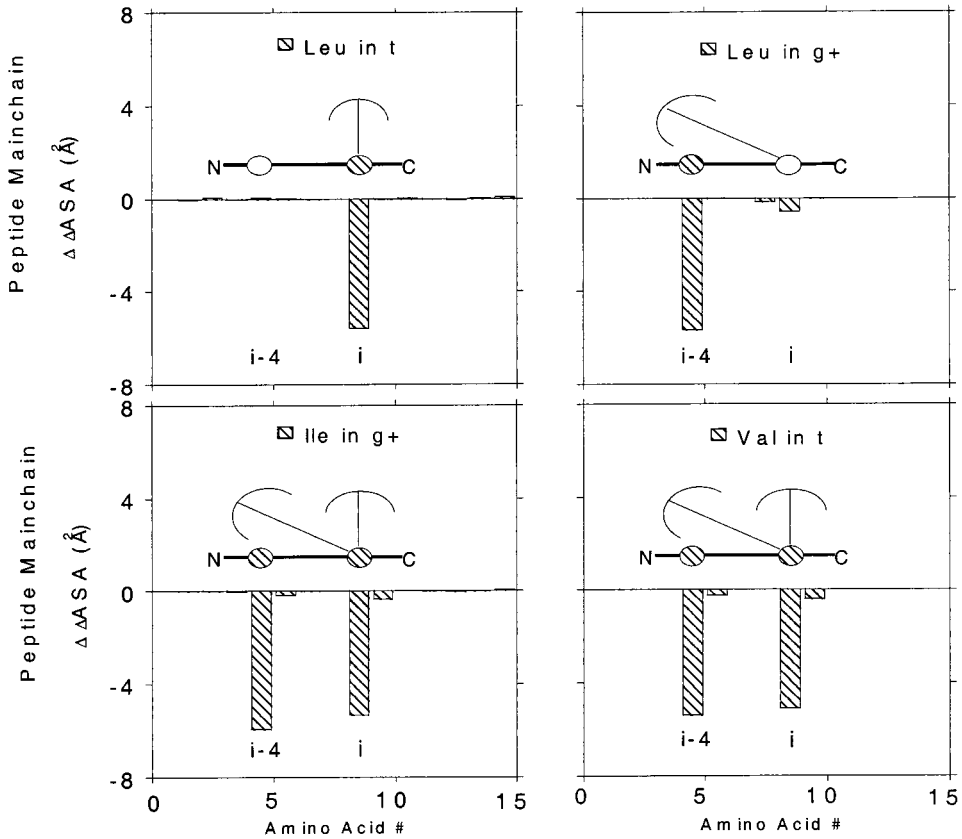

FIG. 3. Difference in solvent-accessible surface area of peptide main-chain atoms, ΔΔASA versus amino acid position in the sequence. The difference is taken from $\Delta\Delta ASA_X = \Delta ASA_X - \Delta ASA_A$, where ΔASA_X is the change in solvent-accessible surface area of peptide main-chain atoms, for a peptide with X = Leu, Ile, Val, or Gly in the middle of a polyalanine α-helix, on helix formation; ΔASA_A is the reference with X = Ala. Relative to an all-alanine helix, one peptide group in a leucine-containing helix is desolvated by a leucine side chain at either i or i−4 in the trans (or t) or gauche plus (or g^+) conformation, whereas two peptide groups at both i and i−4 are desolvated by Ile and Val with β-branched side chains. These results also are shown schematically: the side chain is represented by an umbrella that points upward in t and tilts toward the N terminus in g^+. The desolvated peptide group is shaded because a nearby side chain (umbrella) blocks its access to water, whereas the solvated group is unshaded.

Our model can explain both observations that motivated this study. First, as pointed out earlier (1), the loss of side-chain conformational entropy on helix formation accounts for only one-third of the free energy differences arising from the helix propensities. There must be another major factor. Second, as also pointed out earlier (3, 22), the ratios of helix propensity relative to glycine are much larger in alanine-based peptides than in natural sequence peptides. Our explanation is that the helix-stabilizing effect of substituting one amino acid for another depends on the desolvation of polar groups in the helix backbone caused by the substitution. Neighboring side chains desolvate not only their own peptide CO groups but also those of other residues four away. Thus, neighboring side chains affect the desolvation that accompanies a given amino acid substitution. The largest change in desolvation for a given substitution always will occur in an alanine-based peptide. This explains why the helix propensity ratios are larger in alanine-based peptides than in natural sequence peptides.

Table 1. Calculation of the polar interaction between water and acetamide

	ΔG, kcal/mol	ΔH, kcal/mol
Observed*	−9.70	−16.87
vdw + cavity†	1.95	−5.22
Polar‡	−11.65	−11.65

*ΔG from Wolfenden and coworkers (28, 29) at 25°C, and ΔH from (30).

†The sum of the contributions from van der Waals interactions and cavity formation by a nonpolar compound with the same ASA, found from vapor-water transfer data for nonpolar compounds. The polar and nonpolar surface areas of acetamide are 95 and 97 (Å^2), respectively.

‡The polar hydration energies found by subtracting $\Delta G_{vdw+cav}$ from ΔG_{obs} and similarly for ΔH.

Side-Chain Shielding of Helix Polar Groups Is an Important Factor in the Energetics of Protein Folding. Both Ben-Naim (21) and Honig and coworkers (23) pointed out that desolvation of peptide CO and NH groups in peptide H-bonds will occur when a unit of secondary structure is buried during folding, and the desolvation will be energetically unfavorable for folding. Murphy and coworkers (24) discussed this issue in connection with experiments on the thermodynamics of solution of cyclic peptides. We point out that the presence of side chains larger than alanine also causes partial desolvation of a helix.

The magnitude of the effect on protein folding can be illustrated by the following example. The enthalpy of unfolding sperm whale myoglobin is almost zero at 25°C (25). Myoglobin has approximately 116 helical residues. If the enthalpy of unfolding an alanine helix, 1.0 kcal/mol per residue, is applicable, then the enthalpy of unfolding myoglobin should be 116 kcal/mol plus contributions from other sources. The other known main factor is the hydrophobic interaction. According to the liquid hydrocarbon model (14), the contribution from exposing buried nonpolar surface area on unfolding should be very small at 25°C. If we try to construct an enthalpy balance by comparing the observed unfolding enthalpy with expected contributions from various sources, there is an extremely large

imbalance, far outside experimental error. Honig and coworkers (23), using a related argument, already have pointed this out. They attribute the deficit to the same reason given here (and also postulated by Ben-Naim in ref. 21), namely that there is an enthalpy change accompanying desolvation of peptide CO and NH groups when units of secondary structure are buried during folding. Note that this effect favors a hierarchic mechanism of folding, because it preferentially stabilizes units of secondary structure before they are buried during folding.

The enthalpy changes on helix formation that we determine here are referred to as apparent values because they are determined by using helix-coil theory, not by direct calorimetric measurement. In an earlier study (19), direct calorimetric measurement gave the same value for the unfolding enthalpy of a 50-residue alanine-based helix as found by fitting helix-coil theory to the thermal unfolding curves of a series of repeating-sequence peptides (19, 20). Consequently, we expect the apparent enthalpies of helix formation given here to be reliable, but they should be checked in future research by calorimetry. This should be possible by studying repeating-sequence peptides with repeating units similar to the sequences studied here.

We report the apparent enthalpy of helix formation for the entire peptide, rather than computing the ΔH for the helix propensity of the test amino acid, because $<\Delta H>$ for the entire peptide can be determined with much better precision. More reliable values of the ΔH for the helix propensity of the test amino acid could be obtained by studying peptides in which the proportion of the test amino acid is increased considerably.

CONCLUDING COMMENTS

From the perspective of side-chain entropy, it is easy to understand why alanine is the only amino acid with a favorable helix propensity, because helix formation is driven by the enthalpy change of forming the helix backbone (-1.0 kcal/mol, refs. 19 and 20), and alanine is the only amino acid forming a standard peptide backbone that does not suffer a loss of side-chain conformational entropy on helix formation. From the perspective of side-chain desolvation of the helix backbone, it is equally easy to understand why alanine has a uniquely favorable helix propensity. It is the only amino acid forming a standard peptide backbone whose side chain does not desolvate polar groups in the helix backbone. As expected for the side-chain desolvation mechanism, the enthalpy values for helix formation by the five peptides (Fig. 1*C*) follow the same rank order as the helix propensities at 0°C.

The contribution of side-chain conformational entropy to helix propensity can be quantitated, and different methods of estimating the entropy change on helix formation (4–6) agree reasonably well. Can the contribution arising from side-chain desolvation of polar groups in the helix backbone be quantitated? At high TFE concentrations (above 4 M), the apparent enthalpy differences among the five peptides approach zero (Fig. 2*D*). If this behavior means that the side-chain desolvation effect also approaches zero at high TFE concentrations, then the helix propensity values should depend on side-chain entropies alone. As pointed out earlier (1), this prediction is approximately correct in 40% TFE.

The helix propensity ratios measured in the RNase T1 peptide system are, however, still substantially smaller (26) than the values measured in alanine-based peptides (1) in 40% TFE. TFE can alter the interaction between nonpolar side chains by clustering around nonpolar side chains (27), as well as by affecting H-bonds formed between polar side chains (8). Mechanisms of this sort may affect helix propensities measured in natural sequence peptides.

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