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Helix propensities of the amino acids measured in alanine-based peptides without helix-stabilizing side-chain interactions

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Abstract

Helix propensities of the amino acids have been measured in alanine-based peptides in the absence of helix-stabilizing side-chain interactions. Fifty-eight peptides have been studied. A modified form of the Lifson–Roig theory for the helix–coil transition, which includes helix capping (Doig AJ, Chakrabartty A, Klingler TM, Baldwin RL, 1994, *Biochemistry 33*:3396–3403), was used to analyze the results. Substitutions were made at various positions of homologous helical peptides. Helix-capping interactions were found to contribute to helix stability, even when the substitution site was not at the end of the peptide. Analysis of our data with the original Lifson–Roig theory, which neglects capping effects, does not produce as good a fit to the experimental data as does analysis with the modified Lifson–Roig theory. At 0 °C, Ala is a strong helix former, Leu and Arg are helix-indifferent, and all other amino acids are helix breakers of varying severity. Because Ala has a small side chain that cannot interact significantly with other side chains, helix formation by Ala is stabilized predominantly by the backbone ("peptide H-bonds"). The implication for protein folding is that formation of peptide H-bonds can largely offset the unfavorable entropy change caused by fixing the peptide backbone. The helix propensities of most amino acids oppose folding; consequently, the majority of isolated helices derived from proteins are unstable, unless specific side-chain interactions stabilize them.

Keywords: alanine-based peptides; helix capping; helix propensities; helix stability; modified Lifson–Roig theory; peptide helices; protein folding

Several de novo designed peptides form stable α-helices in water (reviewed by Scholtz & Baldwin, 1992). Most isolated helical segments of proteins, on the other hand, are unstable in water. There are, however, a growing number of examples of protein helices that exhibit partial helix formation in water (Brown & Klee, 1971; Hughson et al., 1991; Sancho et al., 1992; Jimenez et al., 1993; Waltho et al., 1993). The results indicate that helix stability is determined both by the helix propensities, or intrinsic helix-forming tendencies, of the amino acids and by interactions between side chains. The relative contributions of these 2 types of interactions toward helix stability in peptides and proteins are still not established. One reason why this issue is unresolved is the disagreement between helix propensities measured in different peptide (Lyu et al., 1990; Merutka et al., 1990; O'Neil & DeGrado, 1990; Padmanabhan et al., 1990; Chakrabartty et al., 1991; Gans et al., 1991; Kemp et al., 1991; Scholtz et al., 1991b; Rohl et al., 1992; Stellwagen et al., 1992) and protein (Bell et al., 1992; Serrano et al., 1992a, 1992b) systems. The reasons for the disagreement are not yet well understood.

We pointed out earlier that, in peptides containing specific helix-stabilizing side-chain interactions, the free energies assigned to these interactions can affect the values obtained for the helix propensities (Chakrabartty & Baldwin, 1993). For this reason, it is important to measure helix propensities in the absence of specific side-chain interactions. Here we use an alanine-based peptide without ion-pair or other specific interactions as the reference peptide for measurement of helix propensities. The choice of alanine as the major constituent is dictated by the high helix propensity of alanine: at present, only alanine is able to form a stable peptide helix in water in the absence of helix-stabilizing interactions between side chains. The choice of alanine is, moreover, fortunate because it has a methyl side chain that makes only minimal interactions with other side chains.

Fifty-eight homologous peptides, with different amino acids substituted in various positions of alanine-based sequences, were examined. The data have been interpreted by a modified Lifson–Roig theory that includes helix-capping interactions (Doig et al.,

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1994); including the capping interactions improves the fit between predicted and observed helix contents for substitutions made at various positions in the helix. The helix propensity measurements reported here, along with previously reported measurements on charged amino acids (Armstrong & Baldwin, 1993; Huyghues-Despointes et al., 1993; Scholtz et al., 1993), complete the measurement of the helix propensities of all 20 naturally occurring amino acids obtained in the absence of helix-stabilizing interactions.

Results and discussion

Peptide design and helix contents

Our aim was to measure the helix propensities of the amino acids in alanine-based peptides without helix-stabilizing interactions. Because the ends of peptide helices are partly frayed (Rohl et al., 1992), residues in the interior of the peptide have finite probabilities of being either the N-cap or C-cap residue (Chakrabartty et al., 1993a). Consequently, evaluation of helix propensity by substitution experiments in peptide helices requires the simultaneous evaluation of the N- and C-cap propensities. Although N-cap interactions can stabilize peptide helices (Bruch et al., 1991; Forood et al., 1993; Lyu et al., 1993), and the N-cap propensities of the amino acids vary widely (Chakrabartty et al., 1993a), C-cap propensities, on the other hand, show little variation (Chakrabartty et al., 1993a; A.J. Doig & R.L. Baldwin, unpubl. data). Doig et al. (1994) modified the Lifson–Roig theory to include capping; the modification involves assigning different weightings (the n- and c-values for N- and C-cap propensities, respectively) to the amino acid immediately preceding or following a stretch of helical residues. The modified theory does not alter, however, the properties or definition of the helix propensity parameter (the w-value) of the original Lifson–Roig theory. All the parameters of the modified theory describe intrinsic properties of the amino acids, and, by definition, they are not affected by position in the sequence or by neighboring residues. We use the modified theory here to determine simultaneously helix propensities (w-values) and N-cap propensities (n-values).

The peptides used in this study are designed to avoid complications arising from side-chain–side-chain interactions and side-chain–helix-macrodipole interactions. They are based on the sequence $K(AAAAK)_3$, they undergo a monomeric helix to coil transition in water, and they are similar to Ala-Lys peptides previously used by our laboratory (Marqusee et al., 1989; Padmanabhan et al., 1990; Chakrabartty et al., 1991; Rohl et al., 1992). Each peptide contains a terminal Tyr residue that is separated from the Ala-Lys block of residues by 1 or 2 Gly residues. The Tyr residue enables accurate determination of peptide concentration by Tyr absorbance, and the Gly residues provide a flexible linker that reduces induced CD bands arising from interactions between Tyr side chain and the helix; the induced CD introduces error in measurements of helix content (Chakrabartty et al., 1993b). The Lys residues, which ensure water solubility, are distributed symmetrically throughout the helical block of the peptide and are spaced 5 residues apart. The symmetrical distribution of the Lys residues causes the attractive and repulsive interactions of Lys^+ with the helix macrodipole to cancel approximately. The $(i, i + 5)$ spacing causes the Lys side chains to spiral around the helix and be well separated in space, thus minimizing long-range repulsive interactions between Lys^+ side chains. The repulsive interactions are further reduced by making measurements in 1.0 M NaCl, where such repulsive interactions are screened. Side-chain interactions between guest and host residues were minimized by choosing substitution sites that are flanked by Ala residues and have $(i, i \pm 4)$ neighbors that are either Ala or Gly. If more than 1 residue is substituted, then the sites are spaced 5 residues apart to avoid interactions between substituted residues.

The peptides used in this study are designed to maximize the sensitivity of the system to measurement of helix propensities and N-cap propensities. The optimum position and number of substitution sites are determined from simulated substitution experiments using the modified Lifson–Roig theory. That exercise indicates that the unblocked N-terminus is most sensitive to N-cap propensity. Sites with the greatest sensitivity to the helix propensity of the substituted residue depend on the value of the helix propensity. Multiple sites, dispersed symmetrically, are best when a helix-forming or helix-indifferent residue is substituted, whereas a single site in the middle of the sequence is sufficient (and often is optimal) for a helix-breaking residue. We also wanted to measure the N-cap propensity of the acetyl group, and consequently several peptides with and without an N-terminal acetyl group were synthesized. The names, sequences, and helix contents of the peptides used in this study are listed in Table 1.

Application of the modified Lifson–Roig theory

All the data in Table 1—with the exception of peptides YGG-1P, YGG-1C, YGG-1F, NP- GY, W16, and Y16—are simultaneously fitted to the modified Lifson–Roig theory to obtain helix propensities (w-values) and N-cap propensities (n-values). The nucleation parameter for all residues is fixed at $v = 0.048$ (Scholtz et al., 1991b; Rohl et al., 1992) and the n-value of Ala was set at $n(Ala) = 1$; the reasons why these parameters have been fixed are outlined in the Materials and methods section. The parameters that are allowed to float include the w-value of Ala, the n-value of the acetyl group, and the w- and n-values of Asn, Gln, Gly, Ile, Leu, Lys^+, Met, Ser, Thr, and Val. The w-values for Cys, Phe, Pro, Trp, and Tyr and the n-value for Pro are obtained separately by analyzing helix contents of peptides YGG-1P, YGG-1C, YGG-1F, NP-GY, W16, and Y16; the w- and n-values of Ala, Lys, and Gly are fixed to the best-fit values determined above. These peptides are analyzed separately to prevent propagation of errors caused by side-chain contributions to CD, as well as to avoid difficulty in fitting the 0% helix content of YGG-1P.

The helix propensity of Lys^+ is determined without consideration of interactions of the charged side chain with the helix macrodipole. The propensity measurement is therefore an apparent value. Because the Lys^+ residues in each peptide are distributed symmetrically throughout the helical block, the helix-dipole interactions cancel approximately.

Determination of the helix propensity values of Trp, Tyr, and Phe residues is complicated by the error in measurement of helix contents caused by aromatic contributions to CD at 222 nm (Chakrabartty et al., 1993b). In our analysis of the helix propensities of Tyr and Trp, the helix contents of peptides W16 and Y16 were corrected for aromatic contributions (see Materials and methods). The method of correction was based on the assumption that the magnitude of the induced CD band varies linearly with the helix content. We also analyzed the data without

Table 1. *Sequences and helix contents of peptides*

Name	Sequence	f_{helix}[a]
YG[b]	Ac-YGKAAAAKAAAAKAAAAK-$CONH_2$	0.737
YGG[b]	Ac-YGGKAAAAKAAAAKAAAAK-$CONH_2$	0.677
YGGG[b]	Ac-YGGGKAAAAKAAAAKAAAAK-$CONH_2$	0.616
YGG-G18[b]	Ac-YGGKAAAAKAAAAKAAGAK-$CONH_2$	0.465
YGG-G8[b]	Ac-YGGKAAGAKAAAAKAAAAK-$CONH_2$	0.373
YGG-G13[b]	Ac-YGGKAAAAKAAGAKAAAAK-$CONH_2$	0.256
NN-GY[c]	NH_2-NAKAAAAKAAAAKAAGY-$CONH_2$	0.595
NG-GY[c]	NH_2-GAKAAAAKAAAAKAAGY-$CONH_2$	0.525
NS-GY[c]	NH_2-SAKAAAAKAAAAKAAGY-$CONH_2$	0.525
NT-GY[c]	NH_2-TAKAAAAKAAAAKAAGY-$CONH_2$	0.457
NL-GY[c]	NH_2-LAKAAAAKAAAAKAAGY-$CONH_2$	0.449
NI-GY[c]	NH_2-IAKAAAAKAAAAKAAGY-$CONH_2$	0.419
NM-GY[c]	NH_2-MAKAAAAKAAAAKAAGY-$CONH_2$	0.402
NP-GY[c]	NH_2-PAKAAAAKAAAAKAAGY-$CONH_2$	0.404
NA-GY[c]	NH_2-AAKAAAAKAAAAKAAGY-$CONH_2$	0.378
NV-GY[c]	NH_2-VAKAAAAKAAAAKAAGY-$CONH_2$	0.375
NQ-GY[c]	NH_2-QAKAAAAKAAAAKAAGY-$CONH_2$	0.293
YG-AC[c]	Ac-YGAAKAAAAKAAAAKAA-COOH	0.490
YG-GC[c]	Ac-YGAAKAAAAKAAAAKAG-COOH	0.481
YGG-1L	Ac-YGGKAAAAKALAAKAAAAK-$CONH_2$	0.546
YGG-1M	Ac-YGGKAAAAKAMAAKAAAAK-$CONH_2$	0.507
YGG-1Q	Ac-YGGKAAAAKAQAAKAAAAK-$CONH_2$	0.507
YGG-1I	Ac-YGGKAAAAKAIAAKAAAAK-$CONH_2$	0.460
YGG-1C	Ac-YGGKAAAAKACAAKAAAAK-$CONH_2$	0.452
YGG-1S	Ac-YGGKAAAAKASAAKAAAAK-$CONH_2$	0.426
YGG-1F	Ac-YGGKAAAAKAFAAKAAAAK-$CONH_2$	0.426
YGG-1N	Ac-YGGKAAAAKANAAKAAAAK-$CONH_2$	0.397
YGG-1T	Ac-YGGKAAAAKATAAKAAAAK-$CONH_2$	0.295
YGG-1P	Ac-YGGKAAAAKAPAAKAAAAK-$CONH_2$	0.000
YGG-3L	Ac-YGGKALAAKALAAKALAAK-$CONH_2$	0.492
YGG-3M	Ac-YGGKAMAAKAMAAKAMAAK-$CONH_2$	0.364
YGG-3Q	Ac-YGGKAQAAKAQAAKAQAAK-$CONH_2$	0.312
YGG-3S	Ac-YGGKASAAKASAAKASAAK-$CONH_2$	0.223
YGG-3I	Ac-YGGKAIAAKAIAAKAIAAK-$CONH_2$	0.216
YGG-3N	Ac-YGGKANAAKANAAKANAAK-$CONH_2$	0.163
YGG-3V	Ac-YGGKAVAAKAVAAKAVAAK-$CONH_2$	0.093
YGG-3T	Ac-YGGKATAAKATAAKATAAK-$CONH_2$	0.079
2L-GGY	Ac-KALAAKALAAKAAAAKGGY-$CONH_2$	0.487
2M-GGY	Ac-KAMAAKAMAAKAAAAKGGY-$CONH_2$	0.403
2Q-GGY	Ac-KAQAAKAQAAKAAAAKGGY-$CONH_2$	0.395
I-GGY	Ac-KAAAAKAIAAKAAAAKGGY-$CONH_2$	0.484
S-GGY	Ac-KAAAAKASAAKAAAAKGGY-$CONH_2$	0.448
V-GGY	Ac-KAAAAKAVAAKAAAAKGGY-$CONH_2$	0.433
N-GGY	Ac-KAAAAKANAAKAAAAKGGY-$CONH_2$	0.428
T-GGY	Ac-KAAAAKATAAKAAAAKGGY-$CONH_2$	0.402
N2L-GGY	NH_2-KALAAKALAAKAAAAKGGY-$CONH_2$	0.291
N2M-GGY	NH_2-KAMAAKAMAAKAAAAKGGY-$CONH_2$	0.244
N2Q-GGY	NH_2-KAQAAKAQAAKAAAAKGGY-$CONH_2$	0.220
NI-GGY	NH_2-KAAAAKAIAAKAAAAKGGY-$CONH_2$	0.288
NS-GGY	NH_2-KAAAAKASAAKAAAAKGGY-$CONH_2$	0.213
NN-GGY	NH_2-KAAAAKANAAKAAAAKGGY-$CONH_2$	0.211
NV-GGY	NH_2-KAAAAKAVAAKAAAAKGGY-$CONH_2$	0.196
NT-GGY	NH_2-KAAAAKATAAKAAAAKGGY-$CONH_2$	0.182
Qref	Ac-YGGQAAAAQAAAAQAAAAQ-$CONH_2$	0.518
Q12	Ac-YGGQAAAAQAQAAQAAAAQ-$CONH_2$	0.378
Q7,12,17	Ac-YGGQAQAAQAQAAQAQAAQ-$CONH_2$	0.266
W16	Ac-KAAAAKAWAAKAAAAK-$CONH_2$	0.504 (0.462)[d]
Y16	Ac-KAAAAKAYAAKAAAAK-$CONH_2$	0.507 (0.556)[d]

[a] $f_{helix} = [\theta]_{222}^{obs}/(-40{,}000[1 - 2.5/\text{chain length}])$. Conditions: 0 °C, 1.0 M NaCl, 1 mM each of sodium borate, sodium citrate, and sodium phosphate; pH 7.0 for acetylated peptides and pH 9.55 for unacetylated peptides.

[b] Data taken from Chakrabartty et al. (1993b).

[c] Data taken from Chakrabartty et al. (1993a).

[d] f_{helix} values in parentheses have been corrected for aromatic contributions to $[\theta]_{222}^{obs}$.

this correction and report the helix propensities of Tyr and Trp as a range of values. Because our previous study indicated that the aromatic contribution of Phe was smaller than that of Tyr or Trp, we did not employ any aromatic correction for the peptide YGG-1F.

The helix propensities of the amino acids are listed in Table 2 and the N-cap propensities are listed in Table 3. A complete list of N-cap propensities will be published shortly (A.J. Doig & R.L. Baldwin, unpubl. data). The goodness of fit of the experimental data to the modified Lifson-Roig theory is indicated in Figure 1B. In that figure the experimental helix content of each peptide in Table 1 is plotted against its calculated helix content based on the best-fit helix propensity and N-cap propensity values (Tables 2, 3). The RMS deviation between experimental and calculated helix contents is 3.1%; this deviation is very close to the experimental error of the measurement of helix content, which is ±2.5%; thus, the fit of the data to the modified Lifson-Roig model is excellent. On the other hand, the goodness of fit of the experimental data to the original Lifson-Roig theory, which is shown in Figure 1A, is not as good; the RMS deviation for that plot is 8.0%. The helix propensities obtained by applying the original Lifson-Roig theory are on average 10% higher than those reported in Table 2 (data not shown). The modified Lifson-Roig theory therefore provides a better description of the helix-coil transition in peptides than the original model, and helix-capping interactions need to be considered when substitution experiments are being evaluated, even when the substitution sites are in interior positions.

Table 2. *Helix propensity values measured at 273 K*

Residue	s-Value[a]	w-Value[a]	$-RT\ln(w)$ (kcal/mol)	$\Delta\Delta G^0$ (kcal/mol)
Ala	1.54	1.61	−0.258	−1.88
Arg^+	1.1[f]	1.2[f]	−0.047	−1.67
Leu	0.92	0.96	0.022	−1.60
Lys^+	0.78	0.82	0.108	−1.52
Glu°	0.63[b]	0.66[b]	0.225	−1.40
Met	0.60	0.63	0.251	−1.37
Gln	0.53	0.56	0.314	−1.31
Glu^-	0.43[b]	0.45[b]	0.433	−1.20
Ile	0.42	0.44	0.445	−1.18
Tyr	0.37–0.50[c]	0.39–0.53[c]	0.344[c]–0.511	−1.28[c] to −1.11
His^0	0.36[d]	0.38[d]	0.525	−1.10
Ser	0.36	0.38	0.525	−1.10
Cys	0.33	0.35	0.570	−1.06
Asn	0.29	0.31	0.635	−1.00
Asp^-	0.29[e]	0.31[e]	0.635	−1.00
Asp^0	0.29[e]	0.31[e]	0.635	−1.00
Trp	0.29[c]–0.36	0.30[c]–0.38	0.525–0.653[c]	−1.10 to −0.97[c]
Phe	0.28	0.29	0.672	−0.95
Val	0.22	0.23	0.797	−0.83
Thr	0.13	0.14	1.07	−0.56
His^+	0.06[d]	0.06[d]	1.53	−0.10
Gly	0.05	0.05	1.62	0
Pro	≈0.001	≈0.001	≈4	>5

[a] Values obtained by applying Lifson-Roig theory modified to include either N-capping or charged group-helix macrodipole interactions. Conditions: 273 K, 1.0 M NaCl for uncharged residues and Lys, and 273 K, 10 mM NaCl for Arg, Asp, Glu, and His.
[b] Values from Scholtz et al. (1993).
[c] Values corrected for error in fraction helix measurement caused by aromatic contribution.
[d] Values from Armstrong and Baldwin (1993).
[e] Values from Huyghues-Despointes et al. (1993).
[f] Values from Huyghues-Despointes and Baldwin (unpubl.).

Table 3. *Helix N-cap propensity values measured at 273 K*

Residue or group	n-Value	$\Delta\Delta G^0$ (kcal/mol)
Asn	13.3	−1.41
Ser	7.9	−1.12
Gly	7.3	−1.08
Leu	3.7	−0.71
Thr	3.3	−0.64
Ile	2.9	−0.58
Met	2.1	−0.39
Pro	1.6	−0.33
Val	1.2	−0.10
Ala	1	0
Lys^+	0.4	0.50
Gln	0.2	0.93
Acetyl group	9.7	−1.23

Helix propensities and N-cap propensities

In Table 2, the helix propensities or w-values of the amino acids have been listed. In addition, the w-values are converted to the helix propagation parameter s of Zimm-Bragg theory (Zimm & Bragg, 1959) by using the transformations given by Qian and Schellman (1992); the w-values are also converted to standard free energies (ΔG^0) and the difference in ΔG^0 values of an amino acid and Gly ($\Delta\Delta G^0$) is given (Table 1). The rank order of helix propensities has been rationalized by others (Creamer & Rose, 1992; Hermans et al., 1992; Horovitz et al., 1992; Blaber et al., 1993), and their explanations appear to be applicable to our data. Loss of side-chain entropy upon helix formation, hydrophobic interactions between apolar side-chain atoms and the peptide backbone, and formation of side-chain to backbone H-bonds in the unfolded state appear to be the critical determinants of the rank order of helix propensities.

The helix propensity data indicate that, out of the 20 naturally occurring amino acids, only Ala possesses a helix propensity significantly greater than 1, the propensities of Arg and Leu are close to 1, and for all the other amino acids they are less than 1. Thus, under these conditions (0 °C, 1 M NaCl), Ala is the only strong helix former, Arg and Leu are helix-indifferent, and the rest of the amino acids are helix breakers of varying severity. This result implies that short peptides that do not contain significant amounts of Ala will not form stable helices in water at 0 °C unless they are otherwise stabilized by side-chain–side-chain interactions or association. The current published data on peptide helix formation appear to support this statement (see Dyson & Wright, 1991; Scholtz & Baldwin, 1992, for reviews).

The N-cap propensities or n-values are listed in Table 3. As discussed by Doig et al. (1994), application of the modified theory to substitution experiments gives relative N-cap propen-

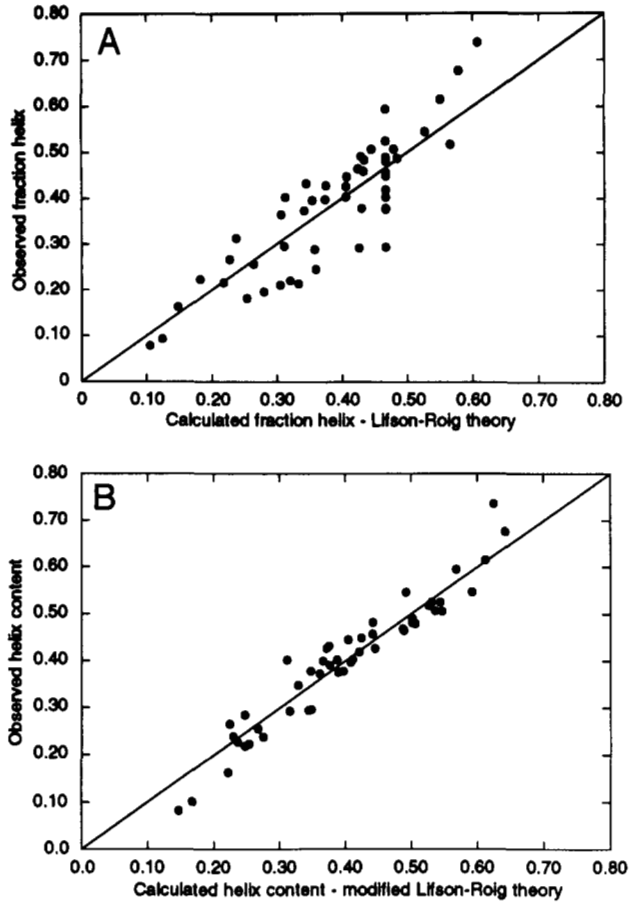

Fig. 1. A: Goodness of fit of experimental data to the Lifson-Roig theory. The observed helix content of each peptide in Table 1 is plotted against its calculated helix content, based on the best-fit helix propensity obtained from application of the Lifson-Roig theory. **B:** Goodness of fit of experimental data to the modified Lifson-Roig theory. The observed helix content of each peptide in Table 1 is plotted against its calculated helix content, based on the best-fit helix propensity and N-cap propensity obtained from application of the modified Lifson-Roig theory.

sities and not absolute values. Consequently, the n-value of Ala is set to 1, and the n-values of the other residues are thus measured relative to Ala. The rank order of the N-cap propensities has been discussed previously (Chakrabartty et al., 1993a; Doig et al., 1994). Residues with small polar side chains (Asn, Ser, Thr) possess high to moderate N-cap propensities because they can form side-chain to peptide backbone H-bonds that stabilize the helix (Presta & Rose, 1988). Gly possesses a high N-cap propensity presumably because, unlike residues with side chains, it does not hinder the solvation of non-H-bonded NH groups of the first turn of the helix (Serrano et al., 1992). The nonpolar residues have moderate to low N-cap propensities, and their rank order correlates with the size of the side chain, suggesting that burial of hydrophobic surface contributes to N-cap propensity (Chakrabartty et al., 1993a). The extremely low N-cap propensity of Gln has been attributed to formation of side-chain to peptide backbone H-bonds in the unfolded state (Chakrabartty et al., 1993a). The relative free energy changes ($\Delta\Delta G^0$) associated with N-capping (Table 3) are in the same range, approximately 2 kcal/mol, as the relative free energy changes upon helix formation (Table 2).

Comparison of results with other systems

Scheraga and coworkers were the first to measure helix propensities using the host-guest technique (Sueki et al., 1984). Host-guest experiments indicate that all 20 naturally occurring amino acids, rather than comprising a diverse group of helix formers and breakers, apparently possess similar intrinsic helix-forming tendencies, except for proline, that neither strongly favor nor disfavor isolated helix formation in aqueous solution (Altmann et al., 1990). The helix propensities measured here do not correlate with the host-guest results.

The helix propensities of the nonaromatic uncharged amino acids have been determined by Kallenbach and coworkers (Lyu et al., 1990; Gans et al., 1991) in a host peptide composed of 8 Glu and 8 Lys residues appropriately placed so that 8 possible (i, $i \pm 4$) salt bridges may form (generic sequence: succinyl-$E_4K_4XXXE_4K_4$, where X is a guest residue). They analyzed their results using the 1-sequence approximation of the Zimm-Bragg helix-coil theory (Zimm & Bragg, 1959), which was further modified to include an interaction parameter for (i, $i \pm 4$) salt bridges (Gans et al., 1991). Although the numerical values are different, the helix propensity values of Gans et al. (1991) show a good linear correlation with our results (Fig. 2A). The correlation coefficient is 0.98 and the slope is close to 1. The correlation curve is, however, offset from the origin by 0.6 units, indicating that there is a systematic anomaly. This offset has been observed before, and we believe it is caused by side-chain interactions that are not accounted for in their analysis (Chakrabartty & Baldwin, 1993). The configurations of the Lys and Glu residues in their host peptide are compatible with the formation of 6 additional (i, $i \pm 3$) salt bridges, and significant interaction between charged side chains and the helix macrodipole should also be present. Scholtz et al. (1993) have recently shown that the energetics of (i, $i \pm 3$) salt bridges ($\Delta G^0 = -0.38$ kcal/mol) and (i, $i \pm 4$) salt bridges ($\Delta G^0 = -0.47$ kcal/mol) for Glu and Lys are similar, and that charged side-chain to helix-macrodipole interactions for these residues are sizable. Therefore, neglect of (i, $i \pm 3$) salt bridges and the helix-macrodipole interaction may have introduced a systematic error in the analysis that accounts for the 0.6-unit offset.

Park et al. (1993a, 1993b) measured helix propensities in a host peptide containing 3 possible Glu-Lys salt bridges (generic sequence: acetyl-YEAAAKEAXAKEAAAK-amide, where X is a guest residue), using the standard Lifson-Roig theory. There is a moderate correlation between their and our results (Fig. 2B). Their rank order of propensities is, however, significantly different, especially with regard to the charged residues. For example, in their propensity scale, Arg^+ possesses the highest s-value (s = 1.83), and the s-values of Lys^+ (s = 1.25) and Glu^- (s = 0.88) are also very high relative to the propensity measurements reported here (Table 2). The design of their peptide sequence, as well as their method of analysis, may explain this disparity. The substitution site in their peptide (position 9) is spaced (i, $i - 3$) from Lys 6 and spaced (i, $i + 3$) from Glu 12. As mentioned above, Scholtz et al. (1993) demonstrated that significant salt-bridge interactions occur between acidic and basic residues in (i, $i \pm 3$) orientations. In the host peptide of Park et al. (1993a, 1993b), helix fraying will tend to destabilize the

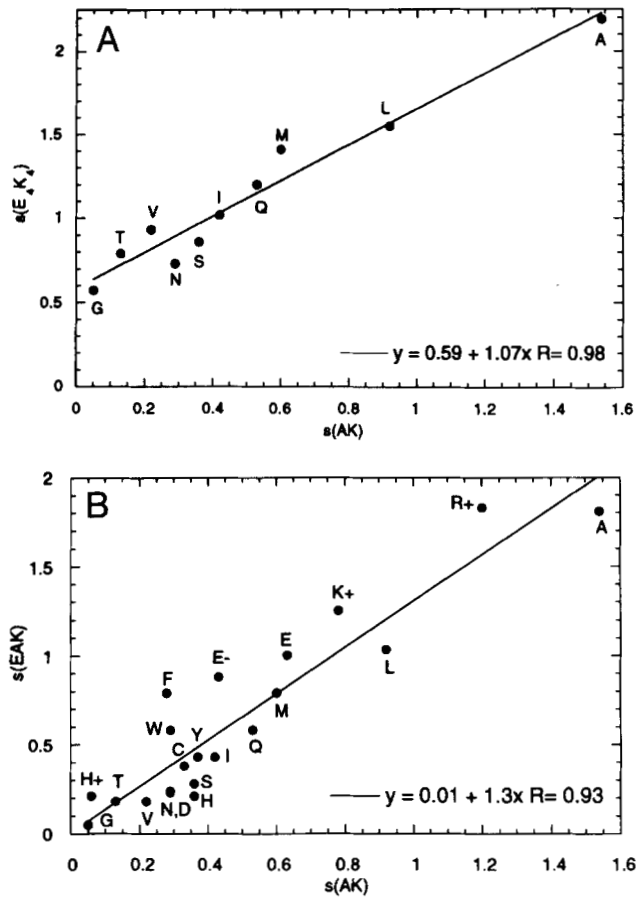

Fig. 2. Correlation of helix propensities (s-values) measured in Ala-Lys peptide system and listed in Table 2, s(AK), with those measured in **(A)** the multiple salt-bridge system of Gans et al. (1991), $s(E_4K_4)$, and **(B)** the triple salt-bridge system of Park et al. (1993a, 1993b), s(EAK).

Glu 2–Lys 6 and Glu 12–Lys 16 salt bridges, and this will leave Lys 6 or Glu 12 free to interact with the guest residue in position 9. Because side-chain interactions are not accounted for in the Lifson–Roig model used by Park et al. (1993a, 1993b), any side-chain interaction will be subsumed in the helix propensity measurement. Park et al. (1993b) suggested that, by making linear extrapolations from measurements in high salt solutions, they removed all ionic effects. Scholtz et al. (1993) showed, however, that Glu^-–Lys^+ salt-bridge interactions (i.e., H-bonded ion pairs) in helices are screened only partially by high salt. Because all the peptides examined by Park et al. (1993a, 1993b) contain salt bridges, it is likely that omission of salt-bridge interactions affected their measurements of helix propensities, the ionizing amino acids being affected the most.

Neighbor-dependent interactions besides the specific side-chain–side-chain interactions studied thus far may also affect the measured values of helix propensities. The same substitution made either on the hydrophilic or hydrophobic face of an amphipathic helix produces different changes in helix content (Zhou et al., 1993).

Helix propensities have been measured by O'Neil and DeGrado (1990) in a coiled-coil peptide system. All 20 amino acids were substituted at a single solvent-exposed site opposite the helix packing interface of a peptide designed to form a homodimeric coiled-coil. An apparent association constant of the coiled-coil was measured by applying the 2-state equation, and the association constant was related to the helix propensity of the substituted amino acids. Their rank order of helix propensities is in good agreement with the values in Table 2. Their measured $\Delta\Delta G^0$ values for helix formation are, however, significantly different from the values in Table 2 (data not shown). Recent crystallographic data indicate that their designed peptides actually form triple-stranded coiled-coils, and ultracentrifugation analysis suggests that the peptide is in equilibrium between monomeric, dimeric, and trimeric states (Lovejoy et al., 1993). Consequently, the 2-state model may not be applicable to their system, and a more complicated multistate analysis may be required.

Helix propensities have also been measured in 2 proteins at solvent-exposed sites in 2 helices of barnase (Horovitz et al., 1992) and at solvent-exposed sites in 2 helices of T4 lysozyme (Blaber et al., 1993). The protein systems, unlike the peptide systems, give only ratios of helix propensities and not absolute values. Consequently, the protein data can be used to compare the helix propensities of 2 residues, but the data cannot be used to determine whether a residue is a helix former or breaker. The relative helix propensities ($\Delta\Delta G^0$) measured in the protein systems are moderately correlated with our results (Fig. 3A,B). The scatter in the plot is likely to result from the dependence of the $\Delta\Delta G^0$ measurements on the surrounding context, provided by the remainder of the protein, in the protein systems. Attempts to correct for this context dependence have been slightly successful (Lin et al., 1993; Pinker et al., 1993). What is remarkable, however, is that the peptide systems appear to be more sensitive to changes in helix propensity than the protein systems. For example, an Ala → Gly replacement in our peptide system results in a change in free energy close to 2 kcal/mol, whereas in barnase or T4 lysozyme the free energy change is close to only 1 kcal/mol (Fig. 3). The free energy measurements were made under different conditions of temperature, ionic strength, and denaturants present; consequently, complete agreement between values is not expected.

Helix propensities may be compared with the frequency of occurrence of residues in helices of proteins. In Figure 4A, the helix propensities of the amino acids are plotted against the frequency of occurrence of those residues in middle helix positions, and in Figure 4B the N-cap propensities are plotted against the frequency at N-cap positions in helices of proteins (Richardson & Richardson, 1988). There is only a moderate correlation between the helix propensity values and the middle helix preferences in proteins (Fig. 4A). Helix propensities measured in other systems also correlate only moderately with the middle helix preferences (data not shown). Strong helix breakers such as Pro or Gly appear in middle positions of protein helices only about 4 times less often than Ala (after correcting for the relative abundance of each amino acid). These findings indicate that helix propensities can account for only part of the conformational preferences of the amino acids, and other factors such as tertiary interactions account for the remainder. The same is not true, however, for the N-cap propensity, which correlates well with the N-cap preference of amino acids (Fig. 4B). The better correlation may be a result of the ends of helices in proteins being more exposed to solvent than the interiors. Consequently, ends of helices are less likely to participate in tertiary interac-

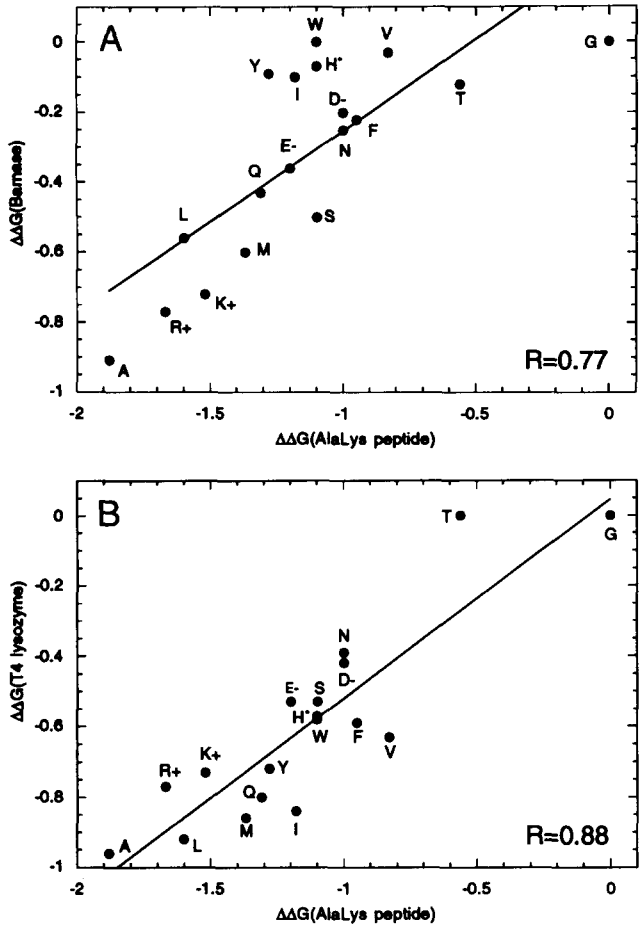

Fig. 3. Correlation of free energy changes for helix formation (relative to glycine) measured in Ala-Lys peptide system and listed in Table 2, $\Delta\Delta G$(AK), with those measured in (**A**) barnase (Horovitz et al., 1992) and (**B**) T4 lysozyme (Blaber et al., 1993).

tions. Prediction of helices in proteins may therefore be more accurate if helix boundary parameters are weighted more strongly than helix propensities.

Implications for protein folding

The calculated free energy change for helix formation by Ala is −0.26 kcal/mol (Table 2) at 0 °C. The alanine helix propensity is determined predominantly by the enthalpy of the helical H-bond and the entropy change for freezing the backbone in a helical conformation, and by a small contribution caused by interactions between the methyl side chain and the peptide backbone (Richards & Richmond, 1978; Blaber et al., 1993); there is no change in side-chain entropy for alanine helix formation (Creamer & Rose, 1992). The small negative free energy for alanine helix formation therefore indicates that, on a per-residue basis, the favorable enthalpic contribution from a helical H-bond (approximately −1 kcal/mol·residue; Scholtz et al., 1991a) is roughly equivalent to the unfavorable entropy change caused by conformational restriction of the peptide backbone. The implication for protein folding is that formation of peptide H-bonds can largely offset the unfavorable entropy change of fixing the conformation of the peptide backbone. Solvation of the peptide CO groups by H_2O may be different in the α-helix and in β-strand conformations (cf. Ben-Naim, 1991), and the enthalpy of a peptide H-bond may be different in α-helical and β-strand conformations.

How do energetic contributions from secondary-structure propensities compare with those of tertiary interactions during protein folding? Thomas and Dill (1993) have considered this question theoretically. Using a 2-dimensional lattice model and representing the protein molecule as a polymer composed of 2 monomer types, they explored the consequences of varying the relative energetic contributions of helix propensities versus side-chain-side-chain interactions. The helix propensities and side-chain-side-chain interactions were both assumed to favor folding ($\Delta G^0 < 0$). The behavior of their system resembled globular proteins most closely when side-chain-side-chain interactions dominate over helix propensities. Based on this result, they concluded that helix propensities are probably not a dominant force in driving protein folding.

The results of this study indicate that the helix propensities do have a major influence on structure formation during folding, for 2 reasons. First, because there are large differences between the helix propensities of the amino acids, the amino acid composition of a sequence has a major influence on the probability that the sequence will form an α-helix. Second, because the free energy change for helix formation for most residues is positive (the average $\Delta G^0 = 0.54$ kcal/mol; Table 2) and the helix propensities of all amino acids except Ala, Leu, and Arg oppose folding, these unfavorable interactions must be overcome either by side-chain-side-chain interactions within the helix or by tertiary interactions, in order for helix formation to occur. The individual free energy changes that accompany helix formation for single amino acids (−0.26 kcal/mol for Ala to 1.6 kcal/mol for Gly) are similar in magnitude to intrahelical side-chain-side-chain interactions such as Glu^-–Lys^+ salt bridges (−0.3 to −0.5 kcal/mol; Scholtz et al., 1993), or burial of a methylene group (−1.8 kcal/mol; Lee, 1993) in a tertiary interaction. Thus, side-chain-side-chain interactions and helix propensities can make roughly equal and opposite contributions to protein folding.

The implication for structure prediction is that accurate prediction of helices from protein sequence will not be possible using methods that employ only helix propensities. Incorporation of short-range side-chain-side-chain interactions (e.g., salt bridges) may, however, improve the accuracy of helix prediction.

Concluding remarks

This study completes the measurement of the helix propensities of all 20 naturally occurring amino acids in the absence of helix-stabilizing side-chain interactions. The helix propensities and N-cap propensities reported here can be used to show that the distribution of helicity in an apparently symmetrical sequence such as Ac-(AAKAA)$_3$-amide is in fact skewed (Fig. 5). This effect is caused by the high N-cap propensity of the acetyl group. The propensity data obtained in this study will be useful in understanding early events in protein folding: they are needed to identify regions in protein sequences that can form helices in isolation, and such regions may represent initiation sites for protein folding.

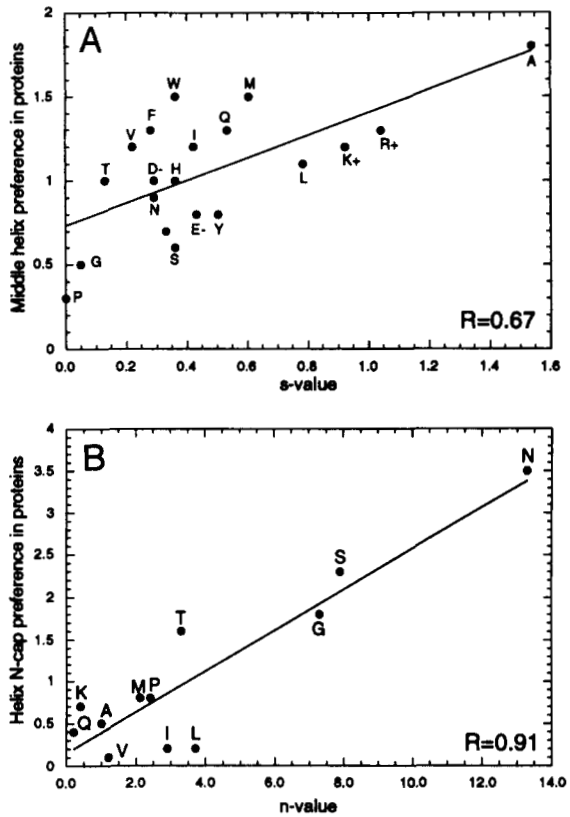

Fig. 4. **A:** Correlation of helix propensities (s-values) of the amino acids listed in Table 2 with their frequency of occurrence in middle positions of helices in proteins (Richardson & Richardson, 1988). **B:** Correlation of N-cap propensities (n-values) of the amino acids listed in Table 3 with their frequency of occurrence in N-cap positions of helices in proteins (Richardson & Richardson, 1988).

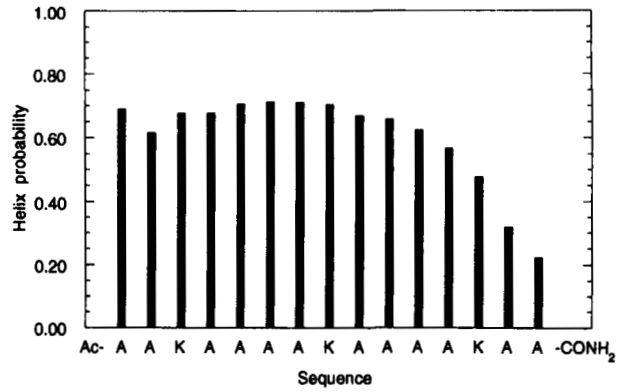

Fig. 5. Predicted distribution of helicity in peptide Ac-(AAKAA)$_3$-amide. The helix probability distribution was determined using the modified Lifson-Roig theory. The input parameters were: w(Ala) = 1.61; w(Lys) = 0.82; n(Ala) = 1; n(Lys) = 0.4; n(Acetyl) = 9.7; v = 0.048; N = 17; and the helix probability is the probability that a residue will receive a statistical weight of w or v.

Materials and methods

Peptide synthesis

Peptides were synthesized by the solid-phase method, as either peptide-amides or peptide-acids using Rink-resin (Advanced Chemtech) or Pepsyn KA resin (Milligen), respectively. An active ester coupling procedure, employing pentafluorophenyl esters of 9-fluorenylmethoxycarbonyl amino acids, was used for all cases except for Ser and Thr, where 3,4-dihydro-4-oxo-1,2,3-benzotriazine esters were used instead. The N-termini were either acetylated with acetic anhydride or left unblocked. The peptides were cleaved from the resin with 95:5 trifluoroacetic acid:anisole mixture; ethanedithiol and thioanisole were added if Cys was present; indole was added if Trp was present. The peptides were purified by C_{18} reverse-phase chromatography and peptide identity was confirmed by FAB mass spectrometry. Peptide purity was assessed by analytical C_{18} reverse-phase chromatography using the Pharmacia FPLC system.

Circular dichroism measurements

CD measurements were made using an Aviv 60DS spectropolarimeter in a 1.0-cm quartz cell. Measurements at 0 °C, pH 7.0 or pH 9.55, were made on peptides dissolved in 1.0 M NaCl and 1 mM each of sodium borate, sodium citrate, and sodium phosphate; 0.1 mM dithiothreitol was present when Cys-containing peptides were examined. pH was adjusted with 0.5 M HCl or 0.5 M NaOH. Peptide concentration was determined by measuring tyrosine absorbance of aliquots of stock solutions diluted in 6.0 M guanidine hydrochloride solutions using ϵ_{275nm} = 1,450 M^{-1} cm^{-1} (Brandts & Kaplan, 1973). CD measurements are reported as mean residue ellipticity in units of deg cm^2/dmol.

Helix content was calculated from $[\theta]_{222}$, the ellipticity measurement at 222 nm, using $-40{,}000(1 - 2.5/n)$ and 0 deg cm^2/dmol as the values for $[\theta]_{222}^{helix}$ and $[\theta]_{222}^{coil}$, respectively; n is the number of amino acid residues (Chakrabartty et al., 1991).

Measurements of $[\theta]_{222}$ for peptides YGG-3L, YGG-3N, YGG-3S, and N2L-GGY were independent of peptide concentration over the range 20–100 μM. The concentration independence of the $[\theta]_{222}$ values of the other peptides was established by performing measurements at 2 separate concentrations that differed approximately by a factor of 2. Ultracentrifugation analysis of similar alanine-based peptides indicates that helix formation is monomeric (Padmanabhan et al., 1990).

When helix contents of peptides containing Tyr or Trp in an internal position (peptides W16 and Y16) were measured, a correction for aromatic CD contributions to $[\theta]_{222}$ was made. Previously we showed that in highly helical alanine-based peptides, interactions between side chains of Tyr or Trp with the helix can induce side-chain CD bands that contribute to $[\theta]_{222}$ ($[\theta]_{222}^{aromatic}$) by +4,400 ± 1,000 and −2,300 ± 600 deg cm^2/dmol, respectively; separation of the aromatic group from the helix by Gly insertions reduces the induced CD to insignificant levels (Chakrabartty et al., 1993). To subtract the aromatic CD contribution from $[\theta]_{222}$ of peptides W16 and Y16, a linear relationship between the magnitude of the induced aromatic CD band and the helix content of the peptide was assumed, and the fraction helix was calculated by: $[\theta]_{222}^{observed}/([\theta]_{222}^{helix} + [\theta]_{222}^{aromatic})$.

Application of modified Lifson–Roig theory

CD measurements on peptides were analyzed using 2 computer programs simultaneously. The first computer program imple-

ments the Lifson–Roig theory modified to include helix-capping parameters. A detailed description of the modified theory has been published (Doig et al., 1994). Briefly, the modified theory describes the fractional helix content of a peptide in terms of 5 parameters: w, propagation; v, nucleation; n, N-terminal capping; c, C-terminal capping; N, chain length. The parameters w, v, n, and c are measures of the helix propensity, nucleation propensity, N-cap propensity, and C-cap propensity, respectively, and by definition these statistical weights are independent of position in sequence and neighboring residues. The nucleation propensity is a property of the peptide backbone, and various experimental data suggest that it is not influenced significantly by the side chain (Ptitsyn, 1972; Scholtz et al., 1991b; Rohl et al., 1992). For our analysis, we assumed that the nucleation parameter v for all residues is equal to the value of 0.048 determined by Rohl et al. (1992). The greater flexibility of the Gly backbone and the greater rigidity of the Pro backbone may cause the nucleation parameters of these 2 residues to differ from the rest. The analysis employed here is, however, relatively insensitive to moderate changes in the nucleation parameter. Thus, any error associated with the assumption that the nucleation parameter is constant is likely to be small. Previous experiments have shown that the C-cap propensities of the amino acids do not vary significantly (Chakrabartty et al., 1993a). Consequently, we set the C-cap propensity of each amino acid equal to 1; this procedure essentially eliminates the C-cap parameter from the analysis. The computer program was written by J.A. Schellman and was modified by H. Qian, C.A. Rohl, and T. Kortemme.

The second computer program used to analyze the experimental CD data implements a binary search algorithm. It was used to determine the propensity parameters that produced the best fit to the experimental CD data. The following are the inputs to the fitting program: the amino acid sequences and experimental helix content measurements of various peptides, and a library file containing values of the propensity parameters w, v, n, and c of the constituent amino acids. The fitting program calls on the modified Lifson–Roig theory program to calculate the helix contents of the input sequences using the values in the propensity parameter library. The program varies the values in the propensity library until the best-fit parameters are obtained. The program uses the following test to evaluate each fit. Each parameter is, in turn, increased and decreased by a factor of 10^{-6} to obtain upper and lower values. The sum of the residuals squared between the experimental and calculated helix contents is calculated using each upper and lower value. If the ratio of the change in the sum of the residuals squared to the change in the absolute value of that parameter is less than 0.0001, convergence is assumed. This program was written by S.L. Mayo and modified by T. Kortemme.

Acknowledgments

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