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Periodicity of polar and nonpolar amino acids is the major determinant of secondary structure in self-assembling oligomeric peptides

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ABSTRACT **The tendency of a polypeptide chain to form α-helical or β-strand secondary structure depends upon local and nonlocal effects. Local effects reflect the intrinsic propensities of the amino acid residues for particular secondary structures, while nonlocal effects reflect the positioning of the individual residues in the context of the entire amino acid sequence. In particular, the periodicity of polar and nonpolar residues specifies whether a given sequence is consistent with amphiphilic α-helices or β-strands. The importance of intrinsic propensities was compared to that of polar/nonpolar periodicity by a direct competition. Synthetic peptides were designed using residues with intrinsic propensities that favored one or the other type of secondary structure. The polar/nonpolar periodicities of the peptides were designed either to be consistent with the secondary structure favored by the intrinsic propensities of the component residues or in other cases to oppose these intrinsic propensities. Characterization of the synthetic peptides demonstrated that in all cases the observed secondary structure correlates with the periodicity of the peptide sequence—even when this secondary structure differs from that predicted from the intrinsic propensities of the component amino acids. The observed secondary structures are concentration dependent, indicating that oligomerization of the amphiphilic peptides is responsible for the observed secondary structures. Thus, for self-assembling oligomeric peptides, the polar/nonpolar periodicity can overwhelm the intrinsic propensities of the amino acid residues and serves as the major determinant of peptide secondary structure.**

The folded structures of proteins are stabilized by a variety of different features, including hydrogen bonding, van der Waals interactions, electrostatic interactions, the hydrophobic effect, and the intrinsic propensities of amino acid side chains for particular secondary structures (1). In recent years, the importance of each of these types of interactions individually has been probed in model peptide systems and in mutagenically altered proteins (2–15).

The importance of one type of interaction *relative* to another type of interaction has received far less attention. This is not surprising since natural proteins typically are stabilized by the concerted action of numerous interrelated features, and it is not possible to isolate any one of these features from all the others. The study of proteins, however, is no longer limited to natural proteins. It is now possible to construct proteins that are designed entirely *de novo* (16–20). With the ability to design proteins from first principles comes the possibility to design structures by focusing on one type of interaction with the hope that optimizing this type will compensate for the mistakes that may result from an incomplete understanding of other features. Thus, the emerging field of *de novo* protein design highlights the importance of probing the *relative* contributions of different types of interactions.

Among natural proteins, two structural features are common to virtually all globular and water-soluble structures: (*i*) an abundance of hydrogen-bonded secondary structure (α-helices and β-strands) and (*ii*) a hydrophobic interior that excludes aqueous solvent.

While hydrogen-bonded secondary structure is abundant in protein structures, the type (α-helix, β-strand, or turn) and the locations of secondary structure differ substantially from one protein to another. This observation led to early proposals that different local sequences have inherently different propensities to form secondary structures and that these local preferences derive from intrinsic propensities of the individual amino acids (21–23). Models in which these intrinsic propensities play a dominant role in determining global structure are appealing because they suggest that structure is dictated by local interactions and thus might be predicted from a thorough understanding of these intrinsic propensities. This hope motivated a great deal of research aimed at determining the intrinsic propensities of each amino acid for the α-helical and β-sheet structures (2–10). Such studies have shown that different side chains indeed show different preferences for local structures. However, it has not been demonstrated that these local preferences actually dictate the global structures of native proteins. Furthermore, theoretical and experimental work has demonstrated that large collections of *de novo* proteins can be constructed using design principles that ignore intrinsic propensities (18, 24).

The second feature common to all water-soluble proteins is the presence of a compact hydrophobic core. The ubiquitous presence of a hydrophobic core in protein structures coupled with thermodynamic studies on nonpolar molecules in water has led to the view that burial of hydrophobic surface area provides the dominant force driving a polypeptide chain to fold into its compact native structure (1, 24–27).

In this paper we ask: What happens when a sequence is designed in such a way that the intrinsic propensities of the amino acids favor one type of secondary structure, but the periodicity of polar and nonpolar amino acids allows burial of hydrophobic side chains only in the alternative secondary structure? How important are intrinsic propensities *relative* to the burial of hydrophobic surface area? To answer this question we have synthesized and determined the secondary structures of a family of synthetic peptides.

MATERIALS AND METHODS

Peptide Synthesis and Purification. Peptides were synthesized on model 9600 peptide synthesizer (Biosearch, Millipore) using fluorenyl-9-methoxycarbonyl (Fmoc) solid-phase chemistry (28, 29). Fmoc amino acids were purchased from either Peninsula Laboratories or Propeptide (Princeton, NJ). PAL resin

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was purchased from Millipore. This resin yields peptides amidated at their C termini. N-terminal acetylation was performed using acetylimidazole in *N,N'*-dimethyl formamide. Cleavage reactions were carried out in a mixture of 95% trifluoroacetic acid/2.5% anisole/2.5% ethanedithiol. Peptides were purified by reverse-phase HPLC using a Dynamax C_{18} column. The correct molecular weights were verified by electrospray mass spectrometry.

Circular Dichroism (CD) Spectroscopy. Lyophilized peptide powders were dissolved in deionized water at initial concentrations ranging between 0.5 and 1.0 mg/ml. Concentrated buffer was added to give a final buffer concentration of 4 mM sodium phosphate (pH 7). Solutions were stirred for 15 min and insoluble material was removed by centrifugation. Peptide concentrations in CD experiments ranged from 10 μM to 200 μM and were determined from the tyrosine absorbance at 280 nm (30). CD spectra were recorded on an Aviv 62DS spectropolarimeter using an averaging time of 1.0 sec and a step size of 0.2 nm. All measurements were carried out at 20°C, using cuvettes with a 1-mm pathlength.

RESULTS

Peptide Design. To bias the intrinsic propensities of each peptide toward a particular secondary structure—either α-helix or β-strand—we chose two groups of amino acids with distinct conformational preferences. Each group would contain one hydrophobic residue, one negatively charged residue, and one positively charged residue.

Group 1 contains amino acids that have an intrinsic propensity for α-helical secondary structure. The hydrophobic amino acid in group 1 was chosen to be leucine. This was a straightforward choice, since leucine is one of the most frequently found residues in the helices of natural proteins (22). Furthermore, of all the hydrophobic amino acids, leucine has the greatest intrinsic α-helical propensity in the model peptides of Chakrabartty *et al.* (3). The negatively charged residue in group 1 was chosen to be glutamic acid. This was also a straightforward choice, since glutamic acid has a high intrinsic helical propensity while aspartic acid has a much lower helical propensity (3). Glutamic acid also has the largest P_α value (1.51) in the Chou–Fasman statistical compilation of helical preferences in natural proteins (22). The positively charged residue was chosen to be lysine, since it has a high helical propensity in model peptides and in natural proteins (3, 22), and, according to the Chou–Fasman analysis, lysine is found in α-helices significantly more often than is arginine (P_α = 1.16 for lysine, P_α = 0.98 for arginine) (22).

Group 2 is composed of residues that have much lower α-helical propensities and tend to prefer β-structure or reverse turns. The hydrophobic amino acid in group 2 was chosen to be isoleucine. This residue has either the highest or second highest β-sheet-forming propensity in two recent studies (9, 10), and, according to the Chou–Fasman analysis, it is the second most commonly observed residue in the β-structure of natural proteins (P_β = 1.60) (22). The only residue having a higher β-sheet-forming propensity in the model system of Minor and Kim (10) is threonine, which is not hydrophobic. The only residue with a higher P_β value in the Chou–Fasman analysis is valine (P_β = 1.70). We chose isoleucine rather than valine because of its greater hydrophobicity (31). The negatively charged residue chosen for group 2 was aspartic acid. This residue is a poor helix former in model peptides and in natural proteins (3, 22) but is among the most commonly occurring residues in β-turns (P_t = 1.46 for aspartic acid) (22). The positively charged residue chosen for group 2 is arginine. This was not a totally satisfying choice since, although the Chou–Fasman analysis finds arginine to be relatively indifferent to helix formation, more recent studies using model peptides rank this residue as a reasonably good helix former (3).

The sequences of the peptides (Fig. 1) were designed to have a repeating pattern of polar and nonpolar amino acids that would match the structural periodicity either of an α-helix (3.6 residues per turn) or of a β-strand (2 residues per turn). Thus, if a given peptide formed the secondary structure matching its sequence periodicity, then the structure would be amphiphilic (32–34) (see Fig. 2). Such structures could bury nonpolar residues by oligomerization of several amphiphilic units. In essence, removal of hydrophobic surface area from contact with solvent water would depend upon the formation of an amphiphilic secondary structure consistent with the sequence periodicity.

Our strategy was to design four peptides: two peptides would be composed of group 1 amino acids (α-intrinsic propensity), and two peptides would be composed of group 2 amino acids (α-avoiding). Within each pair, one peptide would have a sequence periodicity consistent with amphiphilic α-helices (the "A" peptides), while the other would have a sequence periodicity consistent with amphiphilic β-strands (the "B" pep-

Leu, Glu, Lys *(α-HELICAL INTRINSIC PROPENSITIES)*

PEPTIDE 1A *(α-Helical Periodicity)*

```
Tyr-Leu-Glu-Glu-Leu-Leu-Lys-Lys-Leu-Glu-Glu-Leu-Leu-Lys-Lys-Leu
 •   •   -   -   •   •   +   +   •   -   -   •   •   +   +   •
```

PEPTIDE 1B *(β-Strand Periodicity)*

```
Tyr-Lys-Leu-Glu-Leu-Lys-Leu-Glu-Leu
 •   +   •   -   •   +   •   -   •
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Ile, Asp, Arg *(NON α-HELICAL INTRINSIC PROPENSITIES)*

PEPTIDE 2A *(α-Helical Periodicity)*

```
Tyr-Ile-Asp-Asp-Ile-Ile-Arg-Arg-Ile-Asp-Asp-Ile-Ile-Arg-Arg-Ile
 •   •   -   -   •   •   +   +   •   -   -   •   •   +   +   •
```

PEPTIDE 2B *(β-Strand Periodicity)*

```
Tyr-Arg-Ile-Asp-Ile-Arg-Ile-Asp-Ile
 •   +   •   -   •   +   •   -   •
```

FIG. 1. Sequences of the peptides. Peptides 1A and 1B are composed of residues with α-helical intrinsic propensities, while peptides 2A and 2B are composed of residues with non-α-helical intrinsic propensities. Within each pair, the A peptide has a sequence periodicity of polar (+ and −) and nonpolar (●) residues matching the structural periodicity of an α-helix, whereas the B peptide has a sequence periodicity matching the structural periodicity of a β-strand.

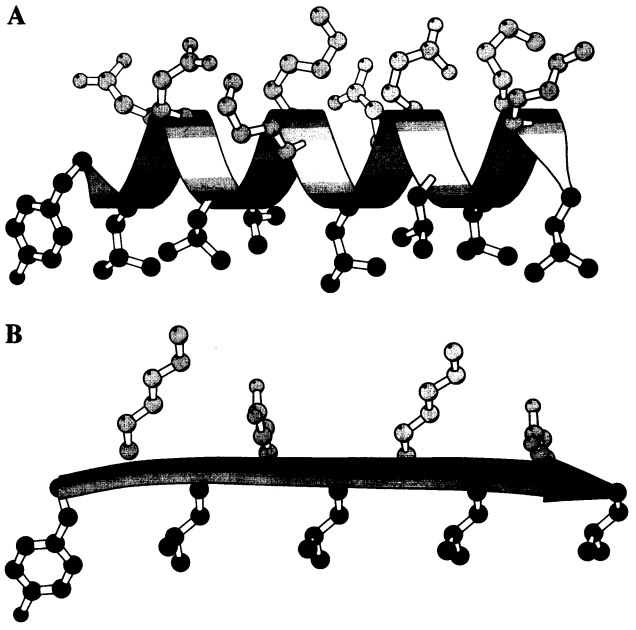

FIG. 2. (*A*) Peptide 1A is shown in α-helical conformation. (*B*) Peptide 1B is shown in β-strand conformation. Polar side chains are shown in gray and nonpolar side chains are shown in black. In each case the sequence periodicity matches the structural periodicity and so the structures are amphiphilic. Diagrams were made using the program MOLSCRIPT (35).

tides). As shown in Fig. 3, the structures formed by each peptide would reflect the relative importance of the intrinsic propensities for a particular secondary structure in competition with the requirement for amphiphilic structure and hydrophobic burial. Peptide "1A" is composed of α-favoring amino acids arranged with the α periodicity, and thus it serves as an α-helical control. Peptide "1B" is also composed of α-favoring amino acids, but they are arranged with the β periodicity; in this peptide the intrinsic propensity and the desire to bury hydrophobic surface area are in direct competition. Peptide "2B" is composed of β-favoring amino acids arranged with the β periodicity and thus serves as a β-strand control. Peptide "2A" is also composed of β-favoring amino acids but arranged with the α periodicity; again, intrinsic propensity competes with hydrophobic burial.

The secondary structures for each peptide as would be predicted either from the intrinsic propensities of the amino

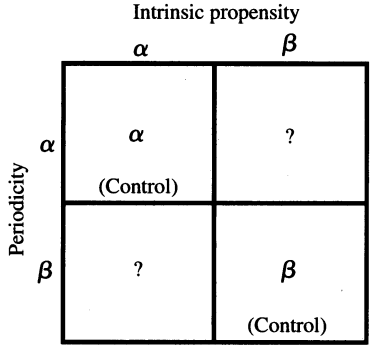

FIG. 3. Schematic diagram showing the competition between intrinsic propensity and polar/nonpolar periodicity. Peptide 1A would be expected to form α-helical structure by both parameters and it serves as the "α control" in the top left. Peptide 2B would be expected to form β structure by both parameters and it serves as the "β control" in the bottom right. For peptides 1B (top right) and 2A (bottom left) there is a competition between the two effects.

acids (22) or from the sequence periodicity are compared in Table 1.

The sequences of the four peptides shown in Fig. 1 are not all the same length but, instead, were designed to have the same number (five) of nonpolar repeats. Ideally, one would also like to compare a set of peptides that were all the same length. However, we found that peptides with alternating polar and nonpolar residues (i.e., the β periodicity) were extremely "sticky;" longer versions of these peptides typically formed gels, suggesting extensive aggregation of β-sheet structure (34, 36, 37) (see *Discussion*).

Each sequence has a tyrosine residue at its N terminus so that peptide concentration could be determined spectrophotometrically (30). Peptides 1A and 2A are acetylated at their N termini and amidated at their C termini. Peptides 1B and 2B are amidated at their C termini. Versions of peptides 1B and 2B that were blocked at both ends were not soluble and so only the singly blocked versions of these peptides were used for further study (see *Discussion*).

Secondary Structures of Peptides. The secondary structures of the designed peptides were probed by CD spectroscopy. This technique is particularly diagnostic for distinguishing between α-helices and β-sheets. The signatures of α-helical structure are two minima at 208 and 222 nm, and a crossover at 200 nm. In contrast, the CD spectrum of β-sheets is dominated by a single minimum at 217 nm and a crossover of ≈205 nm (38, 39). The CD spectra of the four model peptides are shown in Fig. 4. Peptides 1A and 2A are clearly α-helical, while peptides 1B and 2B are clearly β-sheet. Thus, in all cases, the observed secondary structure (Table 1, column 5) correlates with the *periodicity* of the peptide sequence (Table 1, column 4), even when this secondary structure differs from the structure that would have been predicted from the intrinsic propensities of the component amino acids (Table 1, column 3).

Concentration Dependence of Secondary Structure. The results shown in Fig. 4 demonstrate that the periodicity of polar and nonpolar amino acids dominates the decision between α-helices and β-strands. According to the design, these observed secondary structures would be stabilized by the burial of hydrophobic surface area, which accompanies oligomerization of the amphiphilic peptides. Therefore, one would expect the observed secondary structures to be concentration dependent. Fig. 5 shows this is indeed the case. All four peptides show greater secondary structure at high concentrations. Furthermore, the spectra of peptides 1A and 2A show an isosbestic point at 202 nm, indicating a concentration-dependent equilibrium between random coil and α-helix; the spectra of peptides 1B and 2B show an isosbestic point at 205 nm, indicating an equilibrium between random coil and β-sheet.

Peptide 1A shows the least concentration dependence. This is not surprising, since this peptide is composed of strong α-helix formers arranged with an α periodicity, and related peptides are known to form stable four-helix bundles even at relatively low concentrations (40).

DISCUSSION

The results presented above demonstrate that the choice between α-helical and β-sheet secondary structure is controlled by the sequence periodicity of polar and nonpolar amino acids. Thus, although amino acid residues may differ in their intrinsic preferences for one secondary structure versus another (2–10, 21, 22), these preferences can be overwhelmed by the drive to form amphiphilic structures capable of burying hydrophobic surface area. These results corroborate theoretical studies by Dill and coworkers (1, 24, 27), who showed that secondary structures arise from the requirement to bury hydrophobic moieties. Dill's theoretical work and the experiments described here demonstrate that the precise identity of a residue at a particular location in a sequence may be less

Table 1. Secondary structure for each peptide

Peptide	Composition	Intrinsic propensity*	Periodicity	Result
1A	Leu, Glu, Lys	α (P_α = 1.28; P_β = 0.90)	α	α
1B	Leu, Glu, Lys	α (P_α = 1.25; P_β = 0.90)	β	β
2A	Ile, Asp, Arg	β (P_β = 1.14; P_α = 0.97)	α	α
2B	Ile, Asp, Arg	β (P_β = 1.16; P_α = 0.96)	β	β

*Based on the method of Chou and Fasman (22).

important than the simple choice of whether it is polar or nonpolar. This helps to explain why a given fold can be encoded by many different amino acid sequences (41, 42).

While the observed secondary structures of our model peptides correlate with the sequence periodicity of polar and nonpolar amino acids, it must be noted that the structures also correlate with the relative sequence locations of positive and negative charges. Thus, peptides 1A and 2A have charged residues at spacings that would allow formation of multiple salt bridges in α-structure, while peptides 1B and 2B have charged residues at spacings consistent with multiple salt bridges in β-structure. Although formation of salt bridges may contribute to the stabilities of the observed structures, several lines of evidence indicate that salt bridges are not essential:

(*i*) Peptides 1A and 2A are α-helical, not only at pH 7 as described above but also at pH 4.0, where salt bridges would not form (data not shown).

(*ii*) A 13-residue peptide (sequence: Tyr-Arg-Ile-Arg-Ile-Arg-Ile-Arg-Ile-Asp-Ile-Asp-Ile-amide) with the alternating periodicity of polar and nonpolar amino acids forms β-structure, despite having a sequence that is not consistent with the formation of multiple salt bridges (unpublished results).

(*iii*) Previous work with periodic amphiphilic sequences showed that salt bridges are not required for secondary structure formation. For example, early studies demonstrated that poly(Val-Lys) formed β-structure (43). Further studies using either long copolymers (44) or short peptides (33, 45) demonstrated that sequences containing only leucine and lysine residues form β-structures when the leucine and lysine alternate, but form α-helices otherwise. Since those sequences contained no negatively charged side chains, it is apparent that the periodicity of polar and nonpolar residues can dictate secondary structure even in the absence of salt bridges.

While the periodicity of polar and nonpolar residues dominates the decision between α and β secondary structure, it does not ensure that a designed peptide will be water-soluble. Insolubility is a particular problem for sequences with the β periodicity, and amphiphilic β-strands often aggregate into gels or precipitates (34, 36, 37, 46). β-strands are inherently gregarious: The formation of backbone hydrogen bonds and the burial of hydrophobic side chains require a β-strand to interact with neighboring elements of structure. Thus, a given β-strand might form hydrogen bonds to neighboring strands on either side and hydrophobic interactions to a third strand above or below it. (This is quite different from α-helices, which form hydrogen bonds within the same helix.) Such neighborliness can promote nonspecific open-ended aggregation leading to formation of gels or precipitates (37). The tendency toward uncontrolled aggregation can be stemmed (*i*) by maintaining a net charge on the peptide and (*ii*) by choosing relatively short sequences. Thus, peptides 1B and 2B, which are blocked only at their C termini, have a net charge of +1 and form soluble β-structure, but versions of these peptides blocked at both termini are neutral and are not soluble in water. Likewise, while the 9-mers 1B and 2B formed soluble β-structure, longer variants of these peptides typically formed gels or precipitates (unpublished results).

Our studies with synthetic peptides can be considered in terms of models proposed to describe the folding pathways of native proteins (47). Among these models are the framework model and the hydrophobic collapse model. According to the framework model, the secondary structure of a protein forms before the tertiary structure is locked into place. According to the hydrophobic collapse model, nonpolar residues are sequestered away from aqueous solvent early in folding to yield a "molten globule" state; this state then continues on to the native structure by accumulating the precise tertiary interactions of the native fold (47). Our results suggest that these two models should not be considered as competing alternatives but rather as simultaneous and interdependent occurrences. Early events in folding would resemble oligomerization of our amphiphilic peptides. Thus, the secondary structures of the framework model would be stabilized early in folding because of the hydrophobic collapse of nonpolar faces. If protein folding indeed follows a path of hydrophobic collapse with

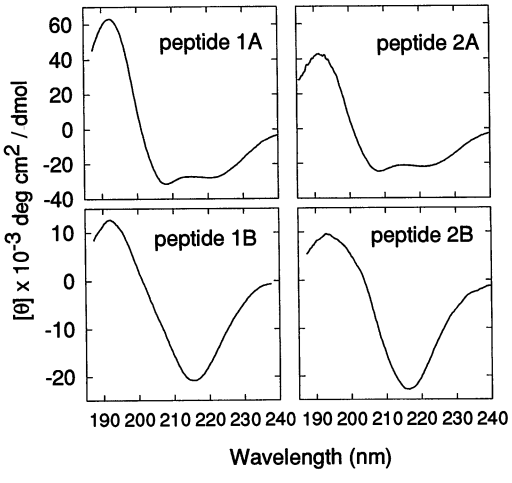

FIG. 4. CD spectra (and concentrations) for peptides 1A (66 μM), 2A (98 μM), 1B (174 μM), and 2B (90 μM). The layout is the same pattern as in Fig. 3.

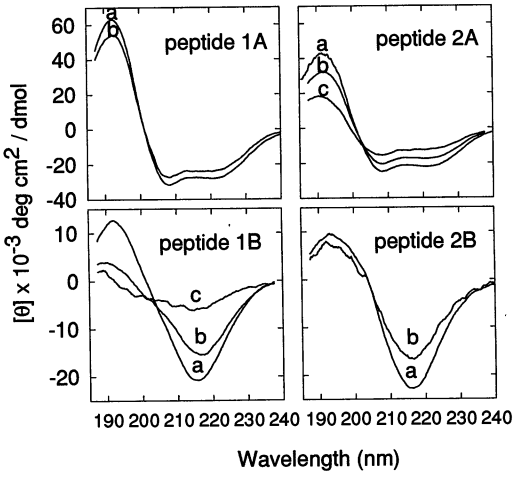

FIG. 5. Concentration-dependent CD spectra for model peptides. Concentrations are as follows: peptide 1A, 13.2 μM and 65.8 μM; peptide 2A, 24 μM, 49 μM, and 98 μM; peptide 1B, 44 μM, 87 μM, and 174 μM; peptide 2B, 31 μM and 90 μM.

concomitant formation of secondary structure, proteins could find their native structures without extensive conformational searching (27). Indeed, the early stages in protein folding may be approximated more closely by these self-assembling peptides than by the monomeric peptides typically used to study intrinsic propensities of local sequences.

Our findings also have implications for *de novo* protein design. The design of novel proteins typically requires a balance between designing *for* the desired structure and *against* all possible alternative structures. To accomplish this task, the would-be designer must consider the various features known to be important in stabilizing proteins. We have shown previously that four-helix bundles can be designed by using a "binary code" with the appropriate sequence periodicity of polar and nonpolar residues (18). Intrinsic propensities were not included as part of that design strategy, and so it was difficult to gauge their importance. Because that collection of sequences was generated by semi-random combinatorial methods, no individual sequence was suitable for explicitly comparing the importance of intrinsic propensities relative to sequence periodicity. The current experiments use defined peptide sequences to demonstrate explicitly that when the periodicity of an amino acid sequence matches the repeat pattern of a secondary structure, the sequence will form that secondary structure—regardless of the intrinsic propensities of the component amino acids. Thus, the periodicity of polar and nonpolar amino acids appears to be a major determinant for *de novo* protein design.

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1. Dill, K. A. (1990) *Biochemistry* **29,** 7133–7155.
2. Wójcik, J., Altman, K. H. & Scheraga, H. A. (1990) *Biopolymers* **30,** 121–134.
3. Chakrabartty, A., Kortemme, T. & Baldwin, R. L. (1994) *Protein Sci.* **3,** 843–852.
4. Lyu, P. C., Liff, M. I., Marky, L. A. & Kallenbach, N. R. (1990) *Science* **250,** 669–673.
5. Merutka, G., Lipton, W., Shalongo, W., Park, S. H. & Stellwagen, E. (1990) *Biochemistry* **29,** 7511–7515.
6. Zhou, N. E., Kay, C. M., Sykes, B. D. & Hodges, R. S. (1993) *Biochemistry* **32,** 6190–6197.
7. O'Neil, K. T. & DeGrado, W. F. (1990) *Science* **250,** 646–651.
8. Kim, C. A. & Berg, J. M. (1993) *Nature (London)* **362,** 267–270.
9. Smith, C. K., Withka, J. M. & Regan, L. (1994) *Biochemistry* **33,** 5510–5517.
10. Minor, D. L. & Kim, P. S. (1994) *Nature (London)* **367,** 660–663.
11. Blaber, M., Zhang, X.-J. & Matthews, B. W. (1993) *Science* **260,** 1637–1640.
12. Horovitz, A., Matthews, J. M. & Fersht, A. R. (1992) *J. Mol. Biol.* **227,** 560–568.
13. Alber, T., Sun, D. P., Wilson, K., Wozniak, J. A., Cook, S. P. & Matthews, B. W. (1987) *Nature (London)* **330,** 41–46.
14. Eriksson, A. E., Baase, W. A., Zhang, X.-J., Heinz, D. W., Blaber, M., Baldwin, E. P. & Matthews, B. W. (1992) *Science* **255,** 178–183.
15. Anderson, D. E., Becktel, W. J. & Dahlquist, F. W. (1990) *Biochemistry* **29,** 2403–2408.
16. Regan, L. & Degrado, W. F. (1989) *Science* **241,** 976–978.
17. Hecht, M. H., Richardson, J. S., Richardson, D. C. & Ogden, R. C. (1990) *Science* **249,** 884–889.
18. Kamtekar, S., Schiffer, J. M., Xiong, H., Babik, J. M. & Hecht, M. H. (1993) *Science* **262,** 1680–1685.
19. Mutter, M. (1988) *Trends Biochem. Sci.* **13,** 260–265.
20. Ghadiri, M. R., Soares, C. & Choi, C. (1992) *J. Am. Chem. Soc.* **114,** 4000–4002.
21. Chou, P. Y. & Fasman, G. D. (1974) *Biochemistry* **13,** 211–222.
22. Chou, P. Y. & Fasman, G. D. (1978) *Annu. Rev. Biochem.* **47,** 251–276.
23. Levitt, M. (1978) *Biochemistry* **17,** 4277–4285.
24. Yue, K. & Dill, K. A. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 4163–4167.
25. Kauzman, W. (1959) *Adv. Protein Chem.* **14,** 1–63.
26. Lau, K. F. & Dill, K. (1989) *Macromolecules* **22,** 3986–3997.
27. Dill, K. A., Fiebig, K. M. & Chan, H. S. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 1942–1946.
28. Atherton, E. & Sheppard, R. C. (1989) *Solid Phase Peptide Synthesis: A Practical Approach* (IRL, Oxford).
29. Fields, G. B. & Noble, R. L. (1990) *Int. J. Pept. Protein Res.* **35,** 161–214.
30. Edelhoch, H. (1967) *Biochemistry* **6,** 1948–1954.
31. Fauchere, J. L. & Pliska, V. (1983) *Eur. J. Med. Chem.* **18,** 369–375.
32. Brack, A. & Spach, G. (1981) *J. Am. Chem. Soc.* **103,** 6319–6323.
33. DeGrado, W. F. & Lear, J. D. (1985) *J. Am. Chem. Soc.* **107,** 7684–7689.
34. Osterman, D. & Kaiser, E. T. (1985) *J. Cell. Biochem.* **29,** 57–72.
35. Kraulis, P. (1991) *J. Appl. Crystallogr.* **24,** 946–950.
36. Zhang, S., Holmes, T., Lockshin, C. & Rich, A. (1993) *Proc. Natl. Acad. Sci. USA* **90,** 3334–3338.
37. Hecht, M. H. (1994) *Proc. Natl. Acad. Sci. USA* **91,** 8729–8730.
38. Greenfield, N. & Fasman, G. D. (1969) *Biochemistry* **8,** 4108–4116.
39. Johnson, W. C. J. (1990) *Proteins Struct. Funct. Genet.* **7,** 205–214.
40. Ho, S. P. & Degrado, W. F. (1987) *J. Am. Chem. Soc.* **109,** 6751–6758.
41. Bajaj, M. & Blundell, T. (1984) *Annu. Rev. Biophys. Bioeng.* **13,** 453–492.
42. Argos, P. (1987) *J. Mol. Biol.* **197,** 331–348.
43. Brack, A. & Orgel, L. E. (1975) *Nature (London)* **256,** 383–387.
44. Brack, A. & Spach, G. (1981) *J. Am. Chem. Soc.* **103,** 6319–6323.
45. Johnsson, K., Allemann, R. K., Widmer, H. & Benner, S. A. (1993) *Nature (London)* **365,** 530–532.
46. Richardson, J. S. & Richardson, D. C. (1987) *Protein Engineering,* eds. Oxander, D. L. & Fox, C. F. (Liss, New York), pp. 149–163.
47. Kim, P. S. & Baldwin, R. L. (1990) *Annu. Rev. Biochem.* **51,** 631–660.