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ARTICLE *in* BIOPOLYMERS · DECEMBER 2006

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The Interplay of Sequence and Stereochemistry in Defining Conformation in Proteins and Polypeptides

Received 5 June 2006; revised 20 July 2006; accepted 31 July 2006
Published online 3 August 2006 in Wiley InterScience (www.interscience.wiley.com). DOI 10.1002/bip.20584

Abstract: *Sequential specification of conformation in proteins and polypeptides is a triangular interplay involving the system of linked peptides, the sequences in side chains, and water as solvent. Stereochemistry in side chain linkages is obviously important in the interaction between all of the players, but no specification of its explicit role, if any, in linking sequence with conformation has been made. Flory and coworkers made a puzzling observation in 1967 that, when mutated from poly-L to alternating-L,D stereochemical structure, polypeptides will suffer a reduction in overall dimension or characteristic ratio by an astonishing factor of 10 and to a value even lower than that predicted for free rotation (Miller, W. G.; Brant, D. A.; Flory, P. J. J Mol Biol 1967, 23, 67–80). Enquiring into this longstanding puzzle, Durani and coworkers found that the stereochemical modification will also abolish conformational sensitivity in polypeptide structure to solvent, because electrostatic interactions in the system of linked peptides are transformed from a condition of mutual conflict to one of harmony (Ramakrishnan, V.; Ranbhor, R.; Kumar, A.; Durani, S. J Phys Chem B 2006, 110, 9314–9323). Thus, poly-L stereochemistry could be the fulcrum linking sequences with ϕ,ψs in protein and polypeptide structures, via dielectric arbitrations in a conflicting type of interpeptide electrostatics, in agreement with the electrostatic screening model of Avbelj and Moult (Avbelj, F.; Moult, J. Biochemistry 1995, 34, 755–764).* © 2006 Wiley Periodicals, Inc. Biopolymers 83: 537–545, 2006

This article was originally published online as an accepted preprint. The "Published Online" date corresponds to the preprint version. You can request a copy of the preprint by emailing the Biopolymers editorial office at biopolymers@wiley.com

Keywords: *protein folding; peptide conformation; peptide dipoles; electrostatic interactions; random coils; chain stereochemistry*

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INTRODUCTION

The ϕ,ψs across interpeptide linkages in folded proteins, according to Anfinsen,[1] are the combination of the lowest free energy for the chain of linked peptides, the sequences in side chains, and water as solvent. Thus, sequences could lend ϕ,ψs from the knowledge of interaction between all of the players in the folding triangle of proteins, but problems arise due to the diversity of sequences, the complexity of the effects of solvent, and the constancy of the system of linked peptides. For enquiries of the protein folding mechanism, sequences are mutated and the conditions in solvent are modified, but not the system of linked peptides—arguably the most critical player at the receiving end in folding triangle—which is left untouched. We draw attention here to experiments, some long standing, which involved a mutation in the system of linked peptides in its stereochemical structure, and with results that implicate interpeptide interaction in a critical role in linking ϕ,ψs with sequences in side chains in both protein and polypeptide structures.

The stereochemistry in side chain linkages is important in protein conformation. Always L-chiral (Figure 1), the accessible ϕ,ψs around the linkages are mainly in the Ramachandran α_R and β basins[2,3] (Figure 2) while correspondingly α-helix and β-sheet (Figure 3) are the principal options in the secondary structure.[4,5] Instrumental in defining electrostatic interaction between linked peptides, poly-L chirality could be also critical in sensitizing ϕ,ψs to sequence effects,[6] as we shall discuss. The implied involvement of electrostatics in specification of protein conformation was detected by Avbelj and Moult[7] in folded proteins and more recently by Avbelj and coworkers[8] in unfolded polypeptides. Thus, the conclusion seems incontrovertible that homochirality in interpeptide linkages is critical to the sequence-dependent character of conformation in both proteins and polypeptides, with the following overall reasoning.

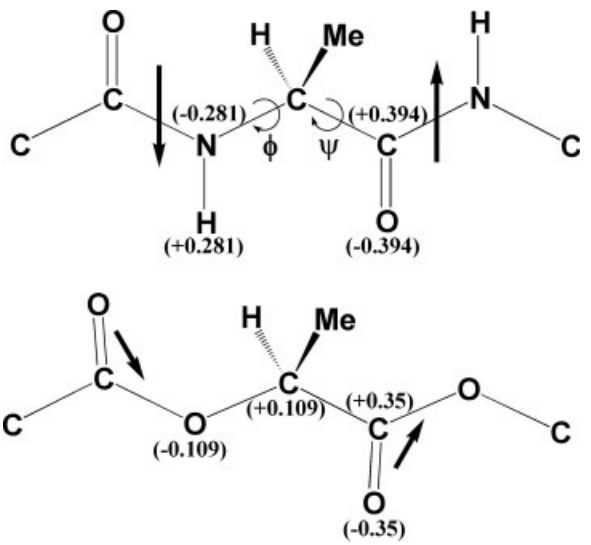


FIGURE 1 Schematic structure of poly-L-alanine (top) and poly-L-lactate (bottom) repeat units, showing the torsion angles ϕ,ψ, the atomic partial charges (in parenthesis), and the resultant dipoles vectors (arrows), used by Flory[15] in calculating the statistical distribution of ϕ,ψs in alanine dipeptide and the characteristic ratios in poly-L-peptides and poly-L-lactates.

ϕ,ψ DISTRIBUTIONS IN UNORDERED PEPTIDES AND UNFOLDED PROTEINS

There are two interrelated questions about the ϕ,ψs in proteins and polypeptides: the effects that restrain them in folded proteins and the freedom gained when the restraints are either abolished or absent. The former question has been in the mainstay of protein folding

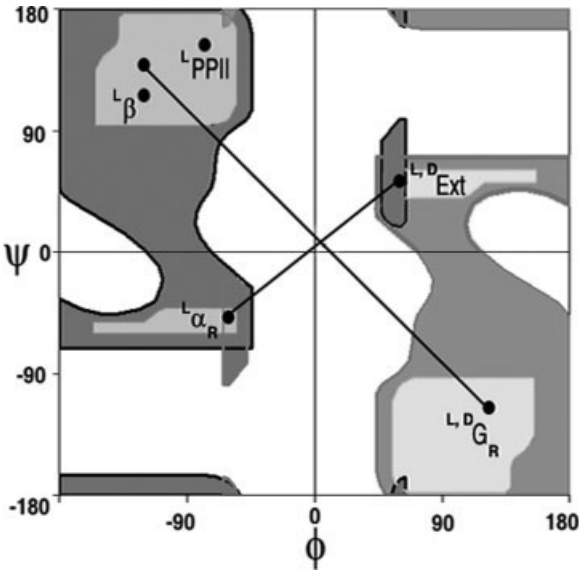


FIGURE 2 Ramachandran diagram with schematic representation of the sterically allowed ϕ,ψ basins for L (darker shades) and D (lighter shades) residues, showing the positions corresponding to ideal "PPII" ($^{L}\phi = -75$, $^{L}\psi = 145$), and "poly-L-helix" ($^{L}\phi = -57$, $^{L}\psi = -47$), "alternating-L,D-helical" ($^{L}\phi = -120$, $^{L}\psi = 140$, $^{D}\phi = 120$, $^{D}\psi = -110$), "poly-L-extended" ($^{L}\phi = -120$, $^{L}\psi = 120$), and "alternating-L,D-extended" ($^{L}\phi = -57$, $^{L}\psi = -47$, $^{D}\phi = 57$, $^{D}\psi = 47$) structures, as modeled in Figure 3. The positions for the "alternating-L,D-helical" structure are approximately those in gramicidin β-helix, as in PDB 1GRM.pdb.[49] $^{L}\beta$, poly-L-extended; $^{L}\alpha_R$, right-handed α-helix; ^{L,D}Ext, alternating-L,D-extended; $^{L,D}G_R$, alternating-L,D-helix.

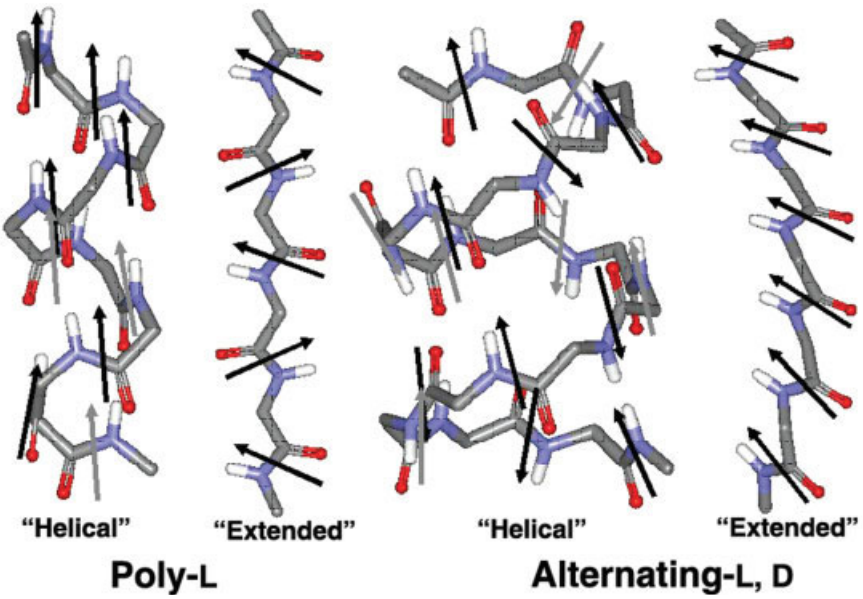


FIGURE 3 The canonical "helical" and "extended" conformations for poly-L and alternating-L,D alanines, showing the peptide dipole vectors (arrows). The modeled structures correspond to the standard ϕ,ψs as defined in the legend to Figure 2.

research while the latter, although equally vital to specificity and stability of folded proteins, came into focus only recently. The classical view that, when denatured, proteins will assume a random distribution in ϕ,ψs is attributed to Tanford and coworkers,[9,10] who came to this influential conclusion upon finding that denatured proteins were similar in dimensions to Flory's random coils.[11,12] The specific experiments of Brant and Flory in direct relevance for denatured proteins,[11,12] however, did not explicitly support the conclusion. These experiments were performed with poly-L-glutamic acid and poly-L-lysine in water at neutral pH, the pH of maximal ionization unfolding the polymers from "helix" to what was then regarded as "random coil" conformation.[13] Finding these "random coils" to be unusually large in the average end-to-end distance, with the characteristic ratio 9.0 $\pm$ 0.5 against the calculated value 2.97,[11,12] and finding this discrepancy smaller in poly-L-lactates—the experimental value is 2.1 $\pm$ 0.3 in this case against the calculated value 1.24—Flory suspected electrostatic interaction between peptide dipoles, unfavorable for α-type ϕ,ψs, as a possible reason. Indeed, upon inclusion of a term for the electrostatics, the calculated characteristic ratio increased to 9.27 in poly-L-peptides and to 2.13 in poly-L-lactates, in close agreement of the above quoted experimental values. The building blocks of polypeptide and polyester structures and the atomic partial charges used in Flory's calculations,[14–16] corresponding to the dipole strength 3.7 D for the peptide unit and 1.8 D for the ester unit, are shown in Figure 1, along with the approximate location cum direction of the dipole vectors. Flory found that, upon inclusion of the electrostatic effect between peptide dipoles, the statistical sampling of ϕ,ψs in the alanine dipeptide structure was 5.6% in α_R, 1.0% in α_L, and 93.4% in β basins. Clearly, the pH unfolded poly-L-glutamic acid and poly-L-lysine, and by implication denatured proteins, could not be random coils, but in a biased sampling of the generalized β Ramachandran basin and possibly specifically its poly-proline II (PPII) subbasin (Figure 2), being the minimum of energy in the calculation of Flory with the effect of interpeptide electrostatics included. Examining the pH denatured poly-L-glutamic acid and poly-L-lysine with circular dichroism, Tiffany and Krimm[17–19] provided direct proof for the existence of PPII-type bias in these systems. Follow up studies have suggested that some degree of bias for PPII may be universal in both unfolded polypeptides[20] and denatured proteins.[21] There is a sampling of PPII type ϕ,ψs in folded proteins as well, but to about 10% in the elements outside of the α and β secondary structure.[22–24] The observations of Han et al.[25] and Poon et al.[26] that alanine dipeptide is predominantly in PPII conformation suggested that the preference for PPII could be intrinsic to the system of linked peptides independent of side chain effects and thus appearing in polypeptides and proteins when either lacking in or relieved of the effects of sequence.

In an hepta-L-alanine peptide model with pairs of positively charged end residues added as solublizers, Kallenbach and coworkers[20,27] found evidence to

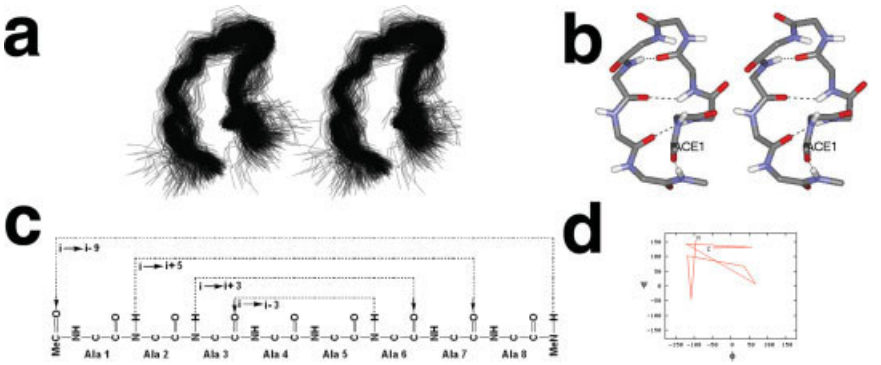


FIGURE 4 The "native" 2:2 type I′ β-hairpin (stereo-image in b) of blocked octa-L-alanine (c), being the most populous conformational cluster (stereo-image in a) in its equilibrium ensemble in water, showing its ϕ,ψs (d) and interpeptide hydrogen bonds (c).

suggest an almost uninterrupted PPII helix to >90% at 2°C in water and in equilibrium with extended β-conformation, the latter increasing with temperature to almost complete exclusion of the α-type ϕ,ψs. Performing a molecular dynamics (MD) study with an octa-alanine peptide, some of us[28] questioned the conclusion, finding that even modest sampling of the α-type ϕ, ψs, not detectable with either NMR or CD, could be compatible with "folds"—specifically β-turns and β-hairpins—even when the distribution in ϕ,ψs was overtly of the PPII/β type. Detecting a broad distribution in ϕ,ψs, Durani and coworkers[28] identified 2:2 type I′ β-hairpin (Figure 4)—the hairpin of greatest natural abundance among the hairpins found in native protein structures[29,30]—as a possible "native fold" for blocked octa-alanine, being its most populous microstate lowest in free energy, comprising 18% of the equilibrium ensemble of the peptide in water. Examining the Kallenbach and coworker's hepta-alanine peptide with small angle X-ray scattering, Pande and coworkers[31] found a radius of gyration significantly smaller than the expectation for a PPII helix. Revisiting the peptide with the combined use of CD, NMR and MD, Scheraga and coworkers[32] confirmed the existence of fluctuating "folds," mainly in the middle of the peptide, along with a wide spread in the distribution of ϕ,ψs.

While there is preference in alanine dipeptide for β/PPII type ϕ ψs, either there is no consensus about possible physical reasons or there is a convergence of effects in its favor. Finding the solvation of C=O and N–H dipoles in alanine dipeptide to be the most favored in the PPII, then β-strand, and finally α-type ϕ,ψs, Han et al.[25] implicated solvation of peptide C=O and N–H groups as a possible major factor in the preference for PPII-type ϕ,ψs. Modeling steric interactions in a Monte Carlo study, Pappu and Rose[33] and Pappu and coworkers[34] found that the excluded volume effect was sufficient grounds to explain the preference for PPII, in both alanine dipeptide and oligo-L-alanines. However, according to the studies of first Brant and Flory[11,12] and later Avbelj and Moult,[7] the β/PPII type preference in alanine dipeptide could be an indirect effect of electrostatic aversion for the α-type ϕ,ψs. Whatever the origins of the conformational preference in alanine dipeptide, the detection of "turns" in longer alanine chains,[28,32] mandating occupancy in α_R and α_L basins, implies that intrachain interactions could be responsible for the shift in ϕ,ψ distribution away from the intrinsic β/PPII type preference.

CHAIN STEREOCHEMISTRY, INTERPEPTIDE ELECTROSTATICS, AND DISTRIBUTION IN ϕ,ψS

What are the competitive "forces" that cause alanine dipeptide to "unfold" to β/PPII conformation,[25,26] shorter poly-L-alanines to "fold" in β-turns and β-hairpins,[28,32,35] and longer poly-alanines ($\geq$20 residues) to fold in α-helices?[36] Further, what are the reasons for the lack of substantive disorder in "unfolded" poly-L-alanines, at least not to the extent implied in random-coil hypothesis? Thus, what exactly are the antecedents of cooperativity in the α-helical fold if not entropy and enthalpy as the "forces" in mutual opposition, as implied in the random-coil hypothesis?

To probe the opposing "forces" responsible for "folding" and "unfolding" in poly-L-alanines, the systems could be mutated in the stereochemical structure. A relevant mutation experiment performed by Flory and coworkers[16] in 1967, modifying poly-L peptides to an alternating-L,D structure, produced a 10-fold reduction of the unusual large characteristic ratio ~9.0 to the astonishing small value ~0.9, as predicted

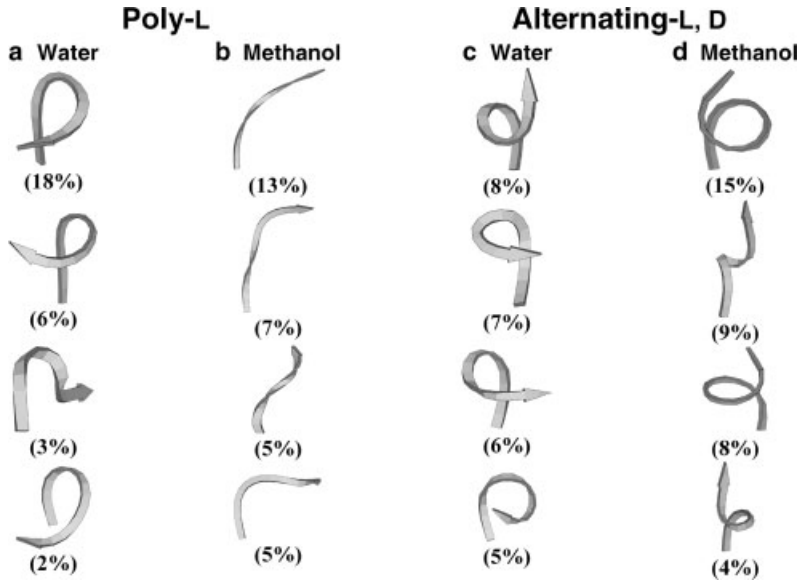


FIGURE 5 The four most populous microstates in equilibrium ensembles of isomeric octa-alanines in water and methanol, shown in ribbon representations of the central members along with the percent population in parenthesis.

theoretically and verified experimentally.[14,16] The change is astonishing not just in the magnitude but also in the absolute value in alternating-L,D peptides, being even lower than the theoretical minimal characteristic ratio 1.0 for free rotation. Further, in alternating-L,D peptides, the characteristic ratio diminishes with length, in contrast of the commonsense expectation of increase with length, as is borne out in every other random-coil system, including the poly-L peptide random coils, as described by Flory[15] in his 1969 book.

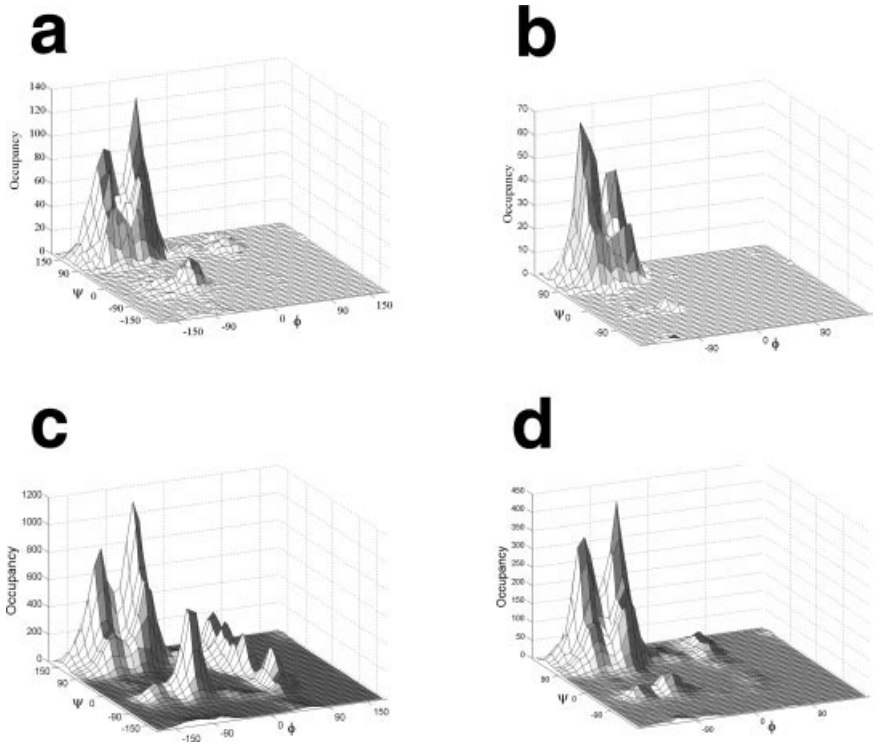


FIGURE 6 Occupancies of ϕ,ψ basins in equilibrium ensembles of alanine-dipeptide in water (a) and methanol (b), and of blocked octa-L-alanine in water (c) and methanol (d).

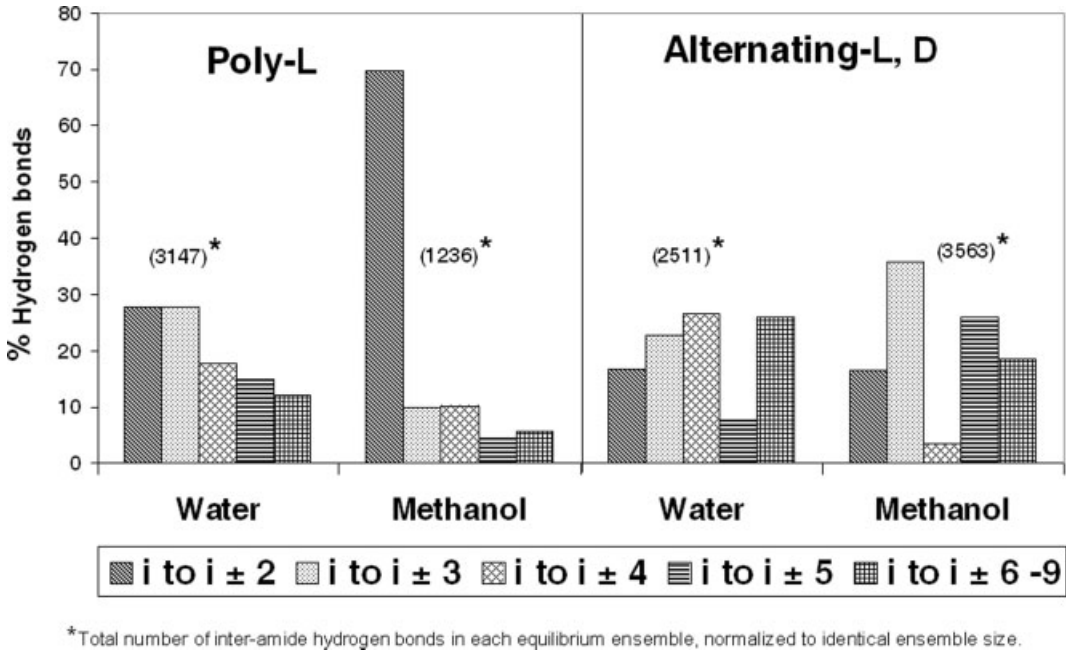
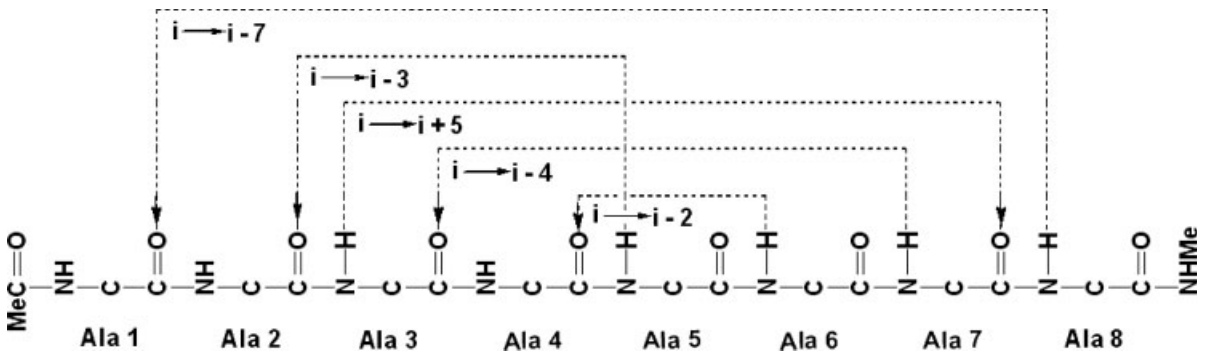


FIGURE 7 Inventory of different types of interpeptide hydrogen bonds (shown in Scheme 1) in the equilibrium ensembles of isomeric blocked octa-alanines in water and methanol.

Using MD and examining octa-L-alanine for effect of mutation to alternating-L,D structure, Ramakrishnan and coworkers[6] found an almost complete loss of sensitivity of the peptide to solvent. The most populous conformational clusters of octa-alanine found in its equilibrium ensemble prepared by Ramakrishnan et al.[6] with MD are shown in Figure 5. Octa-L-alanine populates mainly in β-turns and β-hairpins in water (Figure 5a) and largely in PPII-helices in methanol (Figure 5b), while alternating-L,D octa-alanine populates exclusively in similar looking turns in a helical type twist in both water and methanol (Figure 5c and d). Analysis of the effect of solvent on distribution in ϕ,ψs (Figure 6) and inventory of hydrogen bonds (Figure 7) provided clues about the possible underlying reasons. For alanine dipeptide in water, the ϕ,ψs were predominantly in the β/PPII basin and in a bimodal distribution with PPII as the preferred subbasin (Figure 6a). The α_L basin, scarcely populated in this system, diminished even further in population in methanol (Figure 6b), in conformity with the role of electrostatics in the control of this preference. In octa-L-alanine, there was an appreciable increase in the occupancy of α_L and α_R basins in water (Figure 6c) relative to the occupancy of these basins in both octa-L-alanine in methanol (Figure 6d) and alanine dipeptide in water (Figure 6a). Quite clearly intrachain "interaction," in active "assistance" of



SCHEME 1 Specific intrachain hydrogen bonds in blocked octa-alanine and corresponding secondary structures: γ-turn (i to $i-2$), β-turn (i to $i-3$), α-turn (i to $i-4$), and the gramicidin turns (i to $i+5$ and i to $i-7$).

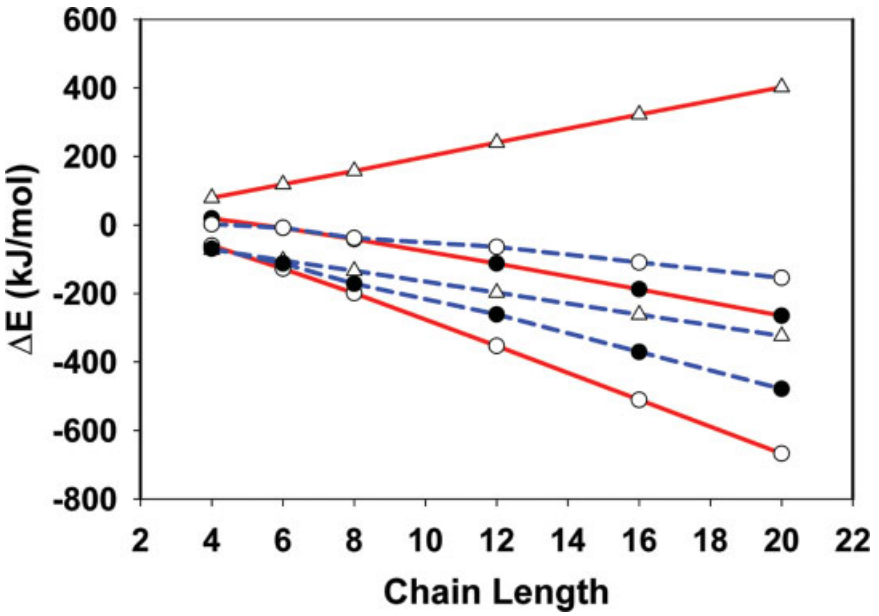
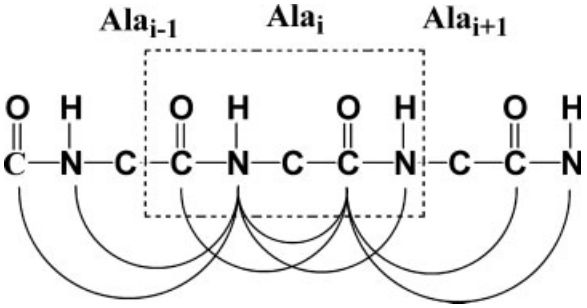


FIGURE 8 Length-dependent change in Coulomb energy (ΔE in kJ/mol at $\varepsilon = 1$) in isomeric poly-alanines (— Poly-L (solid red line), - - - Alternating-L,D (dashed blue line); △ ΔE_{Local}, ○ $\Delta E_{Non\text{-}local}$, ● ΔE_{Total}) when folded from canonical "extended" to canonical "helical" conformations, as defined in the legend to Figure 2.

water as the solvent, is the reason for the increase in α-type ϕ,ψs in octa-L-alanine. Hydrogen bonds between linked peptides are an obligatory accompaniment of folding in polypeptides and the possible donor–acceptor combinations corresponding to different secondary structures, as illustrated in Scheme 1, are dependent on stereochemistry. The mix of secondary structure reflected in the inventory of hydrogen bonds noted in Figure 7 implies that poly-L octa-alanine is "folded" in water but "unfolded" in methanol, while alternating-L,D octa-alanine is "folded" to a comparable extent in both water and methanol. Clearly, there is active involvement of solvent in the folding of octa-L-alanine but not of the alternating-L,D isomer.

The poly-L and alternating-L,D peptides are contrasted in the geometrical relationship between the



SCHEME 2 The N–H and C=O groups of a residue and its near sequence neighbors that define E_{Local}.

C=O and N–H dipoles in the canonical "helical" and "extended" conformations, as noted in Figure 3. Thus, the interactions between the dipoles may induce a polypeptide to "fold" or "unfold" depending upon the chain stereochemistry. Analyzing peptide dipolar interactions for a possible role in protein conformation, Avbelj and Moult[7] made a distinction between the interactions that are "local" (E_{Local}) and "nonlocal" ($E_{Nonlocal}$) in sequence. E_{Local} in a polypeptide defines the interactions in its tripeptide segments as shown in Scheme 2 and summed over the entire polypeptide, while $E_{Nonlocal}$ defines all of the remaining dipolar interactions in the polypeptide, including those definable as hydrogen bonds. E_{Local} and $E_{Nonlocal}$ are related in $E_{Local} + E_{Nonlocal} = E_{Total}$. Calculating the length dependent change in E_{Local} and $E_{Nonlocal}$ in poly-alanines on folding from canonical "extended" to "helical" structures, shown in Figure 3 and defined in Figure 2, Ramakrishnan et al.[6] found that ΔE_{Local} and $\Delta E_{Nonlocal}$ were in mutual "harmony" in alternating-L,D peptides, but in "antagonism" in poly-L peptides, as noted in Figure 8 and Table I. The result is that ΔE_{Total}, a linear function of chain length, is much less favorable for folding in poly-L peptides, as the slope is -17.9 kJ/mol, than in alternating-L,D peptides, as the slope is -25.6 kJ/mol. The effect of mutation on the mutual relationship between ΔE_{Local} and $\Delta E_{Nonlocal}$ explains Flory's puzzle[14,16]; both ΔE_{Local}, with a slope of -15.8 kJ/mol, and $\Delta E_{Nonlocal}$, with a slope of -9.8 kJ/mol, are favor-

Table I Length-Dependent Change in Coulomb Energy (ΔE in kJ/mol) in poly-Alanine Structures in the Folding from Canonical "Extended" to "Helical" Conformations[a]

Chain Length	Poly-L			Alternating-L,D		
	ΔE_{Local}	$\Delta E_{Nonlocal}$	ΔE_{Total}[b]	ΔE_{Local}	$\Delta E_{Nonlocal}$	ΔE_{Total}[b]
4	79	−60	19	−72	2	−70
6	119	−128	−9	−104	−8	−112
8	158	−199	−41	−134	−38	−172
12	240	−353	−113	−197	−64	−261
16	322	−510	−188	−262	−109	−371
20	402	−667	−265	−324	−154	−479

[a] As defined in the Figure 2 legend. The calculations were performed under dielectric constant $\varepsilon = 1$ with the atomic partial charge assignments ±0.38 in C=O and ±0.28 in N—H groups.

[b] $\Delta E_{Total} = \Delta E_{Local} + \Delta E_{Nonlocal}$.

able for folding in even the shortest possible alternating-L,D peptides, and even more so on increase in chain length, leading to the diminished characteristic ratios with chain length. The "harmony" between ΔE_{Local} and $\Delta E_{Nonlocal}$, evidenced in Flory's puzzle and corroborated in the solvent-insensitive nature of alternating-L,D octa-alanine,[6] could be why the alternating-L,D chiral gramicidin-A is "folded" to β-helical conformations irrespective of the solvent, whether methanol or detergents, or the internal lipid phase in membranes.[37–40] The effect of antagonism between ΔE_{Local}, with a slope of +20.3 kJ/mol, and $\Delta E_{Nonlocal}$, with a slope of −38.2 kJ/mol, in poly-L structure is manifest in the conformational sensitivity of polypeptides to effects of chain length, solvent, denaturants, and even sequences in side chains. Indeed, the length-dependent variation in ΔE_{Total} in poly-L peptides can explain why the longer oligo-L-alanines are stable "folds," the shorter oligo-L-alanines are quasi-stable "folds," and alanine dipeptide is "unfolded." Furthermore, the conflict between ΔE_{Local} and $\Delta E_{Nonlocal}$ can even explain why α-helix is a cooperative fold, strong in sensitivity to solvents, to denaturants, and even to side chain effects, which could all manifest conformational effects via chemical arbitrations in the conflicting electrostatics, with dielectric modulation of E_{Local} as a possible fundamental mechanism.

Indeed, in their electrostatic screening model, Avbelj and Moult[7] implicate unfavorable ΔE_{Local} of local folding as a fundamental basis for side chain effects in ϕ,ψs. Blocking access of C=O and N—H groups in the main chain to either the solvent or the rest of protein structure, side chains could influence the screening of E_{Local} and thus determine whether the local preference in ϕ,ψs is for α or β basin. The phenomenon, having been confirmed in proteins,[7,41–47] in unfolded polypeptides,[8,48] and more recently even in individual blocked amino acids,[8] lends credence to the notion that interpeptide electrostatics, riding on the fulcrum of poly-L stereochemistry, is indeed the primary link of conformation with sequence in both protein and polypeptide structures.

This work was supported by grants from the Board of Research in Nuclear Sciences (BRNS) and Council of Scientific and Industrial Research (CSIR), Government of India. RR is the recipient of a fellowship from the Lady Tata Memorial Trust, India.

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Reviewing Editor: David Wemmer