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Conformational Energy Estimates for Helical Polypeptide Molecules[1]

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ABSTRACT: Conformational energy functions previously used successfully to account for the mean square unperturbed dimensions and dipole moments of randomly coiling polypeptides are used, after modification to account for the possibility of intramolecular hydrogen bonding, to evaluate the conformational energies of polypeptide helices. Contributions to the potential energy from van der Waals, dipole, and hydrogen-bonded interactions are evaluated separately to assess the importance of each in determining the stability of regular polypeptide helical structures. An empirical potential function for hydrogen bonding is described which reproduces in semiquantitative fashion known features of CO···NH hydrogen bonds and which merges continuously with the potential function for ordinary van der Waals and dipolar interactions at the assumed geometric limits of hydrogen bonding. Double counting of dipolar contributions to the hydrogen bond is avoided. Energy accounting is accomplished in a way which permits evaluation of the contribution from the intramolecular conformational energy to the nucleation parameter of polypeptide helix–coil transition theory. It is concluded that van der Waals energies are predominantly responsible for dictating the helical residue conformations of least energy. The relative stability of these conformations is greatly enhanced by the possibility for formation in the corresponding helical structures of intramolecular hydrogen bonds and to a lesser extent by favorable dipole interactions which occur in addition to the hydrogen-bonded interaction. Calculation of α-helical peptide unit contributions to the interfacial free energy of a helix–coil junction shows the interface to have a practical depth of 5–7 helical units and that terminal helical units are unstable relative to interior units with respect to conformational energy. An intramolecular enthalpy contribution to the Zimm–Bragg parameter σ of 3.46 kcal/mol of helical sequences is calculated; it is argued that intermolecular contributions are small. The unfavorable enthalpy change for creation of a helix–coil interface with no change in the number of hydrogen bonds is shown to be a consequence of disruption of long-range interaction of peptide dipoles in α-helical array. Experimental results bearing on these conclusions are discussed.

The power of approximate conformational energy calculations to predict correctly the stable helical conformation[2] characteristic of crystalline synthetic polymers has been amply demonstrated.[3] Application of similar methods to helical polypeptides has been no less successful despite the necessity to account for the increased variety and range of interactions reflecting the polar character of these chains.[4-7] The implicit assumption that factors which determine the chain conformation of the crystalline polymer are largely *intramolecular* would seem therefore to be substantiated.

It is a primary aim of the present work to conduct a systematic analysis of the separate contributions from the several terms comprising the conformational potential energy functions employed. Functions accounting for van der Waals repulsions, London attractions, and dipole interactions,[8,9] which have been used to predict successfully the mean square unperturbed dimensions and mean square dipole moments of random polypeptide molecules,[10-14] are modified to include intramolecular peptide hydrogen bonding which may make an important contribution to the conformational energy

(1) Presented in part at the 155th National Meeting of the American Chemical Society, San Francisco, Calif., April 1968.

(2) The term *conformation* is used to denote the rotational isomeric state of a residue or peptide unit and also with reference to the spatial arrangement of a polymeric sequence of residues or peptide units.

(3) A. M. Liquori, *J. Polym. Sci., Part C*, **12**, 209 (1966).

(4) P. De Santis, E. Giglio, A. M. Liquori, and A. Ripamonti, *Nature*, **206**, 456 (1965).

(5) G. N. Ramachandran, C. M. Venkatachalam, and S. Krimm, *Biophys. J.*, **6**, 849 (1966).

(6 R. A. Scott and H. A. Scheraga, *J. Chem. Phys.*, **45**, 2091 (1966).

(7) T. Ooi, R. A. Scott, G. Vanderkooi, and H. A. Scheraga, *ibid.*, **46**, 4410 (1967).

(8) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2791 (1965).

(9) D. A. Brant, W. G. Miller, and P. J. Flory, *J. Mol. Biol.*, **23**, 47 (1967).

(10) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 2788 (1965).

(11) W. G. Miller, D. A. Brant, and P. J. Flory, *J. Mol. Biol.*, **23**, 67 (1967).

(12) P. R. Schimmel and P. J. Flory, *Proc. Nat. Acad. Sci. U.S.*, **58**, 52 (1967).

(13) P. J. Flory and P. R. Schimmel, *J. Amer. Chem. Soc.*, **89**, 6087 (1967).

(14) P. R. Schimmel and P. J. Flory, submitted for publication.

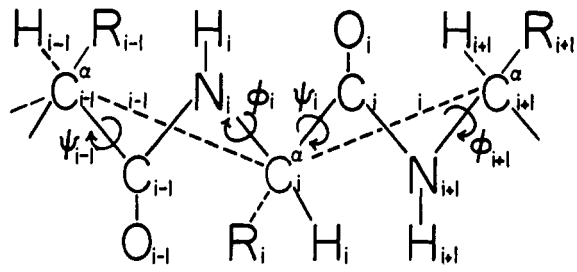

Figure 1. Segment of an α-L-polypeptide chain. Virtual bonds are shown as dashed lines.

for some helical structures. Interactions of long range in the chain sequence are taken into account, and the role of each contribution to the conformational energy in determining the least energetic helical conformation is evaluated. Energy bookkeeping is accomplished in a way which permits analysis of the origin of the nucleation parameter of helix–coil transition theory.[15-17] From the dependence upon helix length of the conformational energy of a helical peptide unit intramolecular energy contributions to the nucleation parameter[18] are determined as is the depth of the helix–coil interface. The influence on the cooperativeness of the polypeptide helix–coil transition of the various intramolecular energy contributions is investigated, and all results are discussed in light of relevant experimental data.[19,20]

Calculations

Polypeptide Chain Conformations. A segment of a polypeptide chain is depicted in its completely extended conformation in Figure 1. Geometric descriptions of the chain contained herein are in accordance with conventions recently proposed.[21] Thus, serial subscripts denote the sequence of *amino acid residues* –NHCHR-CO–, and positive values of the rotation angles result for right-handed rotations from the reference conformations shown in Figure 1. Bond angles and bond lengths published earlier[8] are adhered to in the present work. As before, these parameters are treated as fixed; only torsional angles are varied in the generation of chain conformations with consideration being restricted to chain elements not beyond the first atom or group in the side chain R. For all that follows, this group is taken to be a methylene group, and the absolute configuration at the α-carbon is chosen to be L.

Each *peptide unit*[21] –CHRCONH– is spanned by a virtual bond of constant length 3.80 Å in consequence of the rigid planar, *trans* character assumed for all amide groups. Each virtual bond and its associated peptide unit is indexed to the participating amino acid residue of lower serial index. Accordingly, the orientation of peptide unit i relative to peptide unit $i-1$ is controlled by the pair of rotation angles ϕ_i, ψ_i. A given chain conformation is determined uniquely by specifying all such pairs of angles in the chain. In particular, a regular helical chain conformation may be generated by requiring that a set of values adopted by the pair of angles ϕ_i, ψ_i be repeated in all such angle pairs throughout the chain. Under some conditions the chain may comprise sequences of peptide units in both random and helical conformations. A given peptide unit i is said to have a helical conformation *if*, over a period long compared with the period of bond torsion in a di- or tripeptide, the torsional angles ϕ_i, ψ_i retain values characteristic of some particular helical conformation. A consecutive array of such peptide units, having identical values ϕ_i, ψ_i, is said to comprise a *helical sequence.* Peptide units whose conformations are not so restricted are called coil units, and these in consecutive array comprise a *coil sequence.*

Conformational Energies. Conformational energies of peptide units are estimated here using approximate potential functions[9] which account for bond torsional strain and for attractive and repulsive interactions between nonbonded elements of the chain. This potential function is reproduced as eq 1. The former

$$V(\phi_i,\psi_i) = V_\phi(\phi_i) + V_\psi(\psi_i) + \sum_{j,k}[V_{r,jk}(\phi_i,\psi_i) + V_{l,jk}(\phi_i,\psi_i) + V_{c,jk}(\phi_i,\psi_i)] \quad (1)$$

terms, V_ϕ and V_ψ, depend explicitly on the torsional angles ϕ_i, ψ_i, whereas the latter, $V_{r,jk}$, $V_{l,jk}$, and $V_{c,jk}$, representing, respectively, repulsive, London, and coulombic interactions of atoms j and k, are explicit functions of internuclear separation which depend implicitly only on the torsional angles in consequence of the assumed constancy of all bond angles and lengths. Numerical parameters required for the various terms are identical with those chosen earlier[9] to be consistent with experimentally determined random polypeptide solution properties,[10-14] except where modifications described below to account for the possibility of hydrogen bonding are introduced. No attempt is made to account for polymer–solvent interactions.

It is convenient to consider at this point the energy accounting procedure appropriate for the purposes at hand. For a peptide unit contained in an *unperturbed* coil sequence it is legitimately argued that the average conformational energy may be correctly evaluated if interactions only with first neighbor peptide units are taken into account.[8,9,22] Contributions from interactions with other near neighbor units in the chain sequence are small owing to the spatial separation effected by the planar, *trans* amide groups, and the resultant contribution of longer range interactions is nullified by confining consideration to ideal solvent media. For the moderately good solvents[10] in which the polypeptide helix–coil transition is observed one may without serious approximation restrict attention for peptide units in *any* coil sequence to interactions of first

(15) B. H. Zimm and J. K. Bragg, *J. Chem. Phys.*, **31**, 526 (1959).
(16) S. Lifson and A. Roig, *ibid.*, **34**, 1963 (1961).
(17) P. J. Flory and W. G. Miller, *J. Mol. Biol.*, **15**, 284 (1966).
(18) D. A. Brant and P. J. Flory, *J. Amer. Chem. Soc.*, **87**, 663 (1965).
(19) R. L. Snipp, W. G. Miller, and R. E. Nylund, *ibid.*, **87**, 3547 (1965).
(20) F. E. Karasz and J. M. O'Reilly, *Biopolymers*, **4**, 1015 (1966); **5**, 27 (1967).
(21) J. T. Edsall, P. J. Flory, J. C. Kendrew, A. M. Liquori, G. Nemethy, G. N. Ramachandran, and H. A. Scheraga, *J. Biol. Chem.*, **241**, 1004 (1966).
(22) P. J. Flory in "Conformation of Biopolymers," G. N. Ramachandran, Ed., Vol. I, Academic Press Inc., New York, N. Y., 1967, p 399.

neighbors[23] and adopt the convention that the mutual energy of units $i - 1$ and i be assigned to unit i.

The conformational energy of a helical unit should, however, reflect the interactions with many other units within the same helical sequence. A correct accounting of the total energy of the polypeptide chain is achieved if to helical unit i is assigned the mutual energy of interaction of unit i with all helical units of lower index within the sequence *and* with the first coil unit of lower index in the adjoining coil sequence. Consideration of the energy to be assigned to the unit of lowest index in a helical sequence makes clear the requirement for the latter addition. To obtain the total conformational energy of unit i the energy thus accumulated must be augmented by the self-energy which arises from torsional rotations ϕ_i and ψ_i plus the energy of interaction of nonbonded atoms within unit i.

In the calculations reported below, the conformational energy of helical peptide unit i, as just defined, has been evaluated as a function of the number m of units of lower serial index. Here m counts all helical units plus the first coil unit of lower serial index and may take values $m = 1, 2, 3, \cdots m^*$, where m^* represents the practical limit for convergence of the energy. For $m^* \simeq 20$ convergence of the energy has been achieved for any polypeptide helix of interest. The first adjoining coil unit is rigidly aligned in the helical array, and the conformational energy obtained may therefore be interpreted as the energy per peptide unit at the center of a regular helical array of $2m + 1$ units.[24] This is the quantity desired to assess the helical conformation of lowest energy. In an alternative interpretation, the conformational energy for a given value of m corresponds to the total energy for adding a helical unit to the end of a helical sequence $m - 1$ units in length to generate a sequence of m units. When reduced by the average energy of a random coil unit,[9] this quantity may be taken to represent, within the approximation involved in the present potential functions, the contribution from intrapolymeric factors to the standard enthalpy change for converting from the coil conformation to the helical conformation a coil unit adjacent to a helical sequence of $m - 1$ units. In this interpretation the conformational energy of helical unit i, and particularly its dependence upon m, has significance for the theory of the polypeptide helix–coil transition.

Parameters of Helix–Coil Transition Theory. If the peptide unit conformation space, with coordinates ϕ_i, ψ_i, is partitioned into just two regions[16] to be designated "coil" and "helix," then, following Zimm and Bragg,[15] for $m > m^*$ we denote as $s = \exp(-\Delta G^\circ_s/RT)$ the equilibrium constant[25] for the above process converting a peptide unit from coil into helix. For smaller m the equilibrium constant, here designated $s(m) = \omega(m)s = \exp(-\Delta G^\circ_s(m)/RT)$, can be expected to vary with m because of the diminished range of interactions within a short helical sequence.[15,17] The quantity $\Delta G^\circ_\omega(m) = -RT \ln \omega(m)$ is thus recognized as the contribution from a peptide unit near the end of a helical sequence to the total standard interfacial free energy

$$\Delta G^\circ_\omega = \sum_{m=1}^{m^*} \Delta G^\circ_\omega(m) = -RT \ln \omega$$

associated with a junction between long helix and coil sequences. In further compliance with the treatment of Zimm and Bragg the quantity

$$\omega = \prod_{m=1}^{m^*} \omega(m)$$

is a lumped nucleation parameter which enters as a factor in the statistical weight for a given chain conformation once for each helical sequence at least m^* units in length. Here $m^* + 1$ is that value of m for which $\omega(m)$ becomes unity. A factor $\omega(m)$ enters the statistical weight once for each helical sequence $m \leqslant m^*$ units in length.

In accordance with elementary nucleation theory[26] the coil to helix transition is expected to occur abruptly in a narrow range of s for $s \simeq 1$ if the thermodynamic potential for the process, $\Delta G^\circ_s(m)$, changes for small m from positive to negative values as m increases with helix growth. That is, the observed character of the transition in polypeptides results from the occurrence of an initial maximum in the free energy function

$$\Delta G^\circ = \sum_m \Delta G^\circ_s(m)$$

The size of the critical nucleus for helix initiation is defined by the position of this maximum.

The thermodynamic potential and its associated equilibrium constant $s(m)$ are not subject to evaluation by methods employed here. Restriction to consideration of *regular* helical structures, *i.e.*, points in conformation space, precludes estimation of the entropy contribution, and indeed, the entropy of the random coil is evaluated only to within an arbitrary additive constant so long as the rotational isomeric state model is employed.[9] Finally, the overriding influence of polymer–solvent interactions erases all hope of obtaining estimates by present methods of $s(m)$ or the related parameters $\omega(m)$ and ω. On the other hand, consideration of the nucleation parameter σ of Zimm and Bragg, as it is usually interpreted, largely avoids the difficulties which confront a calculation of ω. Contributions to σ from intermolecular interactions should be small, since σ is taken to represent the equilibrium constant for creation of one additional (long) helical sequence by a process maintaining constant the number of hydrogen-bonded carbonyl groups.[25] Thus, to an excellent approximation, the free energy change associated with changes in strong solvation does not enter $\Delta G^\circ_\sigma = -RT \ln \sigma$. Intramolecular entropy contributions to ΔG°_σ are widely recognized;[15,25] intramolecular energy factors may be estimated from the dependence of the conformational energy of a helical peptide unit upon helix length, as will be demonstrated below.

Hydrogen Bond Potential Function. From an empirical standpoint the noncovalent interaction referred

(23) N. Go, M. Go, and H. A. Scheraga, submitted for publication.

(24) The helical array in question here is to be distinguished from the helical *sequence* previously defined, since it contains peptide units formally designated "coil."

(25) J. Applequist, *J. Chem. Phys.*, **38**, 934 (1963).

(26) L. Mandelkern, "Crystallization of Polymers," McGraw Hill Book Co., Inc., New York, N. Y., 1964, Chapter 8.

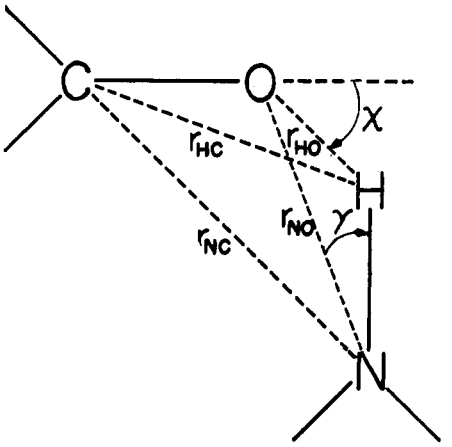

Figure 2. Definitions of geometric parameters employed in discussion of the hydrogen bond potential function.

to as the "hydrogen bond" differs from the more prevalent van der Waals interactions in that energies are greater by approximately an order of magnitude, equilibrium internuclear distances are considerably smaller, and the strength of the interaction is more highly orientation dependent than is the case for the latter class of interactions. These features can be treated semiquantitatively within the framework of eq 1 without the need for introducing any additional terms into the potential function. Moreover, the potential function for hydrogen-bonded interactions can be made to merge continuously with the appropriate van der Waals function when the mutual orientation of hydrogen-bonding groups becomes unacceptable for hydrogen bond formation.

Klotz and Franzen[27] have shown that the standard enthalpy of amide hydrogen bond formation varies from about −4.2 kcal mol^{-1} in the nonhydrogen-bonding solvent CCl_4 to virtually zero in aqueous solution. A survey of amide and peptide crystal structure parameters by Pimentel and McClellan[28] indicates that H bonds of type N–H···O display N···O distances as low as 2.67 Å. Remembering that an H atom of radius 1.2 Å is interposed between N and O in a hydrogen-bonded interaction, this distance is to be compared with the sum of the conventional van der Waals radii[29] for N and O of 3.05 Å. This tabulation also gives evidence regarding the requirements for mutual orientation of N–H and C–O. Consider the representation of these groups shown in Figure 2. If cylindrical symmetry is assumed about the two bonds, then their mutual orientation can be described in terms of the parameters r_{HO}, χ, and γ. For nonlinear hydrogen bonds, *i.e.*, $\gamma \neq 0°$, the strength of the bond is believed to diminish rapidly for $\gamma > 30°$, while the angle χ is found to vary from 15 to 85° in known hydrogen-bonded crystalline compounds.[28] Although involvement in hydrogen bonding of the unshared oxygen electrons, which presumably lie at $\chi = \pm 60°$ when coplanar with the bonds depicted in Figure 2, is an attractive postulate,

(27) I. M. Klotz and J. S. Franzen, *J. Amer. Chem. Soc.*, **84**, 3461 (1962).
(28) G. C. Pimentel and A. L. McClellan, "The Hydrogen Bond," W. H. Freeman and Co., San Francisco, Calif., 1960, Chapter 10.
(29) A. Bondi, *J. Phys. Chem.*, **68**, 441 (1964).

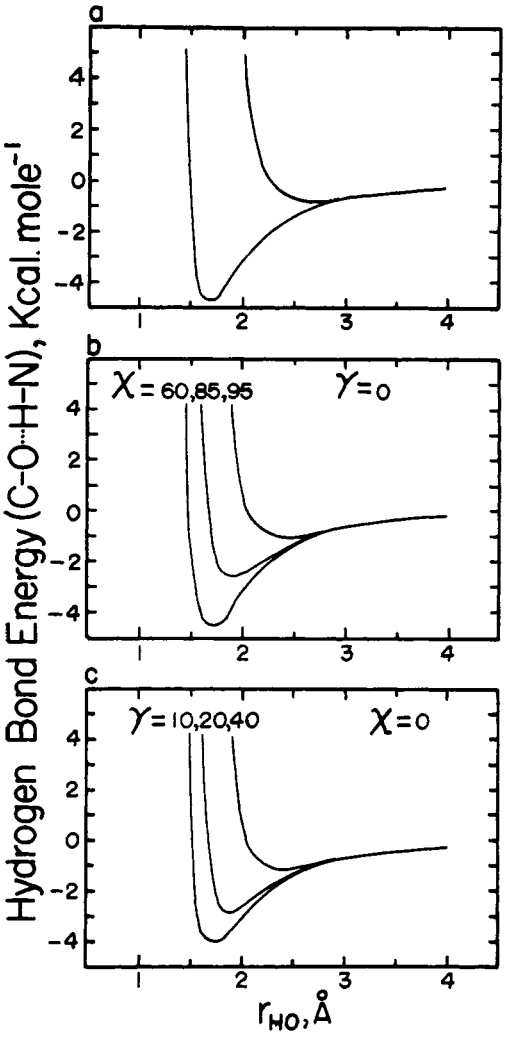

Figure 3. Potential energy of atoms depicted in Figure 2 *vs.* the distance r_{HO}. (a) Lower curve, energy for "perfect hydrogen bond" with $\gamma = \chi = 0$; upper curve, energy in absence of hydrogen bond, *i.e.*, van der Waals plus coulombic energies, with $\gamma = \chi = 0$. (b) Hydrogen bond energy as a function of χ for $\gamma = 0$. (c) Hydrogen bond energy as a function of γ for $\chi = 0$.

the evidence is not sufficiently strong to warrant a model which makes $\chi = \pm 60°$ preferred to other similar values. Neither is there sufficient evidence to require an assumption of other than cylindrical symmetry about the C=O bond.

As shown previously,[9] a suitable potential function for van der Waals interaction of the four atoms depicted in Figure 2 can be obtained if the function $V_{r,jk} + V_{1,jk}$ for each pair of noncovalently bonded atoms be required to have a minimum at a distance 0.20 Å greater than the sum of their van der Waals radii. If a "perfect hydrogen bond" is defined as one in which χ and γ of Figure 2 are zero while $r_{NO} = 2.70$ Å, then if all covalent bond lengths and angles adopt the values given previously,[8] the other interatomic distances are $r_{HO} = 1.70$ Å, $r_{HC} = 2.72$ Å, and $r_{NC} = 3.68$ Å. Each of these distances save r_{NC} is less than the sum of the normal van der Waals radii. When $V_{r,jk} + V_{1,jk}$ for each atom pair H···O, N···O and H···C is minimized at the distance characteristic of the "perfect hydrogen bond" and for the pair N···C at a distance 0.2 Å greater than

the sum of the van der Waals radii for these atoms, the resultant potential function for nonbonded interaction of the four atoms displays a minimum when plotted *vs.* r_{HO} near 1.70 Å with an energy −4.5 kcal mol^{-1} for the mutual orientation characterized by $\chi = \gamma = 0$. The lower curve of Figure 3a corresponds to this result. When this minimization is carried out following the procedure of Brant, *et al.*,[9] the same plot yields a minimum near 2.70 Å with an energy of −0.5 kcal mol^{-1}, as expected for this mutual orientation in the absence of hydrogen bonding. A curve illustrating this result is plotted as the upper line in Figure 3a.

As $|\gamma|$ deviates from zero and as $|\chi|$ becomes greater than 90°, one expects the hydrogen bond energy to decrease and the position of the minimum along the r_{HO} coordinate to increase; for sufficiently large $|\gamma|$ and/or $|\chi|$ the hydrogen bond potential should become identical with that for the nonhydrogen-bonding set of atoms. Since, as seen in Figure 3a, both the energy of the four atoms and the position of minimum energy are strongly dependent upon the distances at which the functions $V_{r,jk} + V_{l,jk}$ for noncovalently bonded atom pairs are minimized, it is convenient to let these distances vary with χ and γ in order to achieve the desired functional behavior. Let us therefore define r°_{jk} as the interatomic distance at which $V_{r,jk} + V_{l,jk}$ for a given atom pair is to be minimized to obtain a satisfactory potential function in the absence of hydrogen bonding[9] and Δ_{jk} as the difference between r°_{jk} and the equilibrium contact distance for this atom pair in the "perfect hydrogen bond." Then for various values of χ and γ we can minimize the functions $V_{r,jk} + V_{l,jk}$ at distances r^*_{jk} given by

$$\begin{aligned} r^*_{HO} &= r^\circ_{HO} - Q(\chi,\gamma)\Delta_{HO} \\ r^*_{NO} &= r^\circ_{NO} - Q(\chi,\gamma)\Delta_{NO} \\ r^*_{HC} &= r^\circ_{HC} - Q(\chi,\gamma)\Delta_{HC} \\ r^*_{NC} &= r^\circ_{NC} \end{aligned} \qquad (2)$$

where $r^\circ_{HO} = 2.90$ Å, $r^\circ_{NO} = 3.25$ Å, $r^\circ_{HC} = 3.10$ Å, $r^\circ_{NC} = 3.45$ Å, $\Delta_{HO} = 1.20$ Å, $\Delta_{NO} = 0.55$ Å, and $\Delta_{HC} = 0.38$ Å. Here $Q(\chi,\gamma)$ is chosen equal to unity for $\chi = \gamma = 0$ and such that Q is diminished as some power of cos γ.[30] We also desire that Q should be virtually independent of χ in the range $|\chi| < 90°$ but that it should approach zero rapidly for $|\chi| > 90°$. The empirical expression given in eq 3 for $Q(\chi,\gamma)$ has been found satisfactory. This function vanishes for

$$Q(\chi,\gamma) = (\cos\gamma)^4\{1 - \exp[0.15(\chi - 100)]\} \qquad (3)$$

$\gamma = \pm 90°$ and for $\chi = \pm 100°$; for $|\chi| > 90°$ and $|\chi| > 100°$ it is assumed that there is no hydrogen-bonding contribution to the conformational energy, and the potential functions of Brant, *et al.*,[9] with which the above functions merge at these limits, are used. Plots of the above hydrogen bond potential function are shown in Figures 3b and 3c as a function of r_{HO} for several values of χ and γ.[31]

(30) K. D. Gibson and H. A. Scheraga, *Proc. Nat. Acad. Sci. U.S.*, **58**, 420 (1967).

(31) The hydrogen bond potential function used in the calculations reported below differed slightly from that presented in eq 2 and 3. The differences are inconsequential, and the two functions yield curves of the sort plotted in Figure 3 which are virtually indistinguishable.

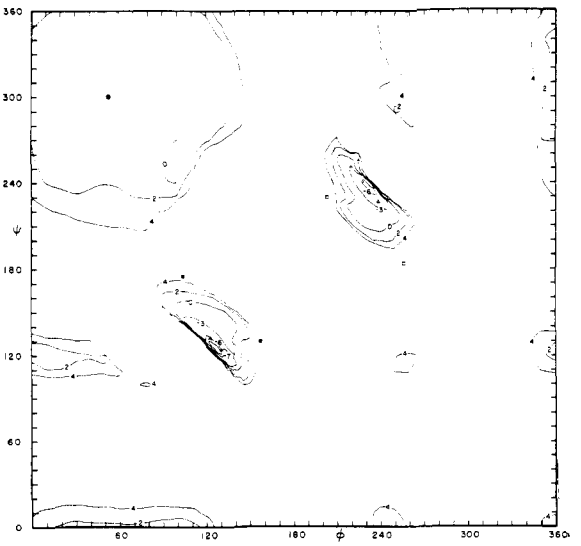

Figure 4. Contour diagram for poly-α-L-alanine of the conformational energy of the central peptide unit in a regular helical array of 25 units *vs.* ϕ_i and ψ_i as calculated from eq 1, including contributions from hydrogen bonding. Absolute energies[44] are shown in kilocalories per mole: ▲, α_R; △, α_L; ■, right-handed 3_{10} helices; □, left-handed 3_{10} helices; ●, pleated sheet.

As pointed out by Donohue,[32] two classes of hydrogen bonds are possible in polypeptide helices, namely, $H_i\cdots O_{i+k}$ where $k = 1, 2, 3, 4$ and $H_i\cdots O_{i-k}$ where $k = 2, 3, 4, 5$. The hydrogen bond potential function just presented was employed for hydrogen-bonded interactions of both types.

Results and Discussion

Analysis of Contributions to the Conformational Energy. The conformational energy, calculated on the basis of eq 1, of the central peptide unit in a helical array[24] of 25 units is plotted in Figure 4 as a contour diagram of the energy *vs.* the rotation angles ϕ_i and ψ_i. Contributions from the hydrogen bond potential function described above are included. Convergence of the energy to within 10% of the asymptotic limit is achieved for a residue thus situated for any helix of interest; only for helices of very low pitch, which are necessarily unstable by virtue of strong steric repulsions, will the results be significantly nonasymptotic for helical arrays of this length. Two prominent minima in steep-walled wells are found at positions (ϕ_i, ψ_i) 126°, 126° and 232°, 236°. These minima have been located to within ±2°, and they agree well in position with the two lowest minima reported by Scheraga and coworkers,[6,7] who used potential functions accounting for hydrogen bonding by a method based on a modified Lippincott–Schroeder function.[33] The former of these helices is at the energy minimum, −7.80 kcal mol^{-1}; the energy of the latter is −6.87 kcal mol^{-1}.

The positions of the standard left- and right-handed Pauling–Corey[34] α-helices (axial translation per residue,

(32) J. Donohue, *Proc. Nat. Acad. Sci. U.S.*, **39**, 470 (1953).

(33) W. G. Moulton and R. A. Kromhout, *J. Chem. Phys.*, **25**, 34 (1956).

(34) L. Pauling, R. B. Corey, and H. R. Branson, *Proc. Nat. Acad. Sci. U.S.*, **37**, 205 (1951).

h = 1.50 Å, axial rotation per residue, t = 100°), as determined by the method of Nagai and Kobayashi[35] and independently confirmed following Shimanouchi and Mizushima,[36] are plotted at 122°, 133° (α_R) and 238°, 227° (α_L). Topologically equivalent helices of the α-helix class occupy elliptical areas around the standard α-helix positions. The domains of these structures coincide approximately with the −3 kcal mol^{-1} contour line.[5,37] Locations of the standard 3_{10} helices[32] are also indicated in Figure 4. In general one obtains up to four solutions for ϕ_i, ψ_i for a given polypeptide helix if described only in terms of h and t.[37] Solutions for the standard 3_{10} helix (h = 2.0 Å, t = 120°) are, for right-handed helices, at 157°, 130° and 104°, 176°; the left-handed counterpart of each is given by $-\phi_i$, $-\psi_i$. The calculated energy difference between the right-handed variants is too small, *i.e.*, 1 kcal mol^{-1}, to permit a reliable choice between them, and in fact the former helix is chosen as the 3_{10} by Leach, *et al.*,[37] whereas Schellman and Schellman[38] choose the latter. Both corresponding left-handed 3_{10} helices have energies higher than either right-handed helix. The domain of helices topologically equivalent to the standard 3_{10} helix does not overlap regions of the diagram having energies −3 kcal mol^{-1} or below.[5,37] Correlation of Figure 4 with the conformations of the helical residues of crystalline egg-white lysozyme[39] has been discussed in an earlier communication,[40] where the predominance of conformations resembling the right-handed α-helix was noted.

Some noteworthy differences exist between the present results and those of Scheraga and coworkers.[6,7] These are reflected primarily in the lower energies observed in the present calculations for the regions of the two principle minima relative to other low energy regions of the helical conformation space. Very similar methods have been used to account for van der Waals and electrostatic interactions, and although the different methods used for including the hydrogen bond energy might be suspect, this appears not to be the source of the differences cited. Thus, it is reported[7] that the hydrogen bond potential function used by Scheraga and coworkers yields an energy of −3.25 kcal mol^{-1} for the best hydrogen bond, such bonds being realized for conformations within the domains of the principle minima of the contour diagram. When it is recognized that this contribution has been included in the calculations of Scheraga and coworkers *in addition* to the electrostatic interaction of the participating N–H and C–O groups, which is *ca.* −2 kcal mol^{-1} for the mutual orientation characterizing these conformations (see below), appreciable numerical differences from this source cannot be anticipated. Rather, the discrepancy appears to arise from the different representations of the conformational energy. Thus, Scott and Scheraga[6] report in their diagrams one-tenth the total energy of a nine residue helix, which yields the average energy per residue for residues in a variety of environments. The present representation of the conformational energy of a helical unit is essentially devoid of contributions from units near the end of a helical sequence, and the unique stability of helices of the α-helix class is made readily apparent. It is of interest therefore to investigate the influence from the several terms of the conformational potential energy functions on the stability and conformation of polypeptide helices.

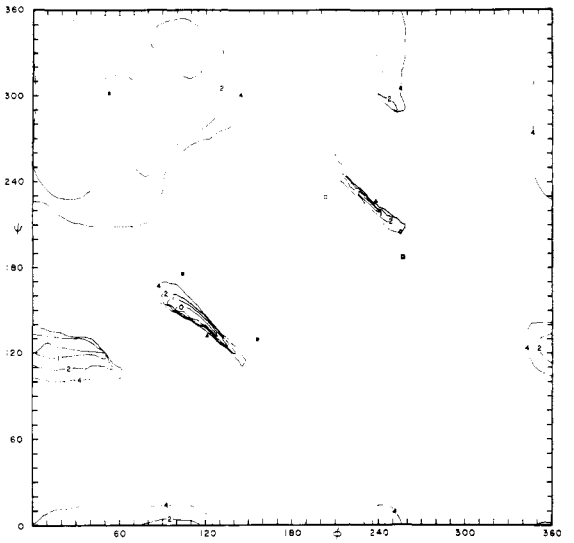

Figure 5. Contour diagram as in Figure 4 but with only torsional and van der Waals contributions included in the potential function.

When the conformational energy of a helical residue at the center of a 25-unit helical array is calculated by including only torsional and van der Waals contributions to the potential function, *i.e.*, ignoring coulombic and hydrogen-bonding contributions, the energy diagram shown in Figure 5 is obtained. It is clear that the principle features of the diagram in Figure 4 are preserved in Figure 5, although both deep wells are narrowed, and the relief in the interesting regions of the map is reduced by a factor of almost 2. The locations of the principal minima are shifted slightly to 120°, 140° and 240°, 220° and accord less well with α_R and α_L than do the minima in Figure 4. Nevertheless, the fundamental role claimed for the van der Waals interactions by de Santis, *et al.*,[4] in dictating the observed structure of polypeptide helices cannot be questioned. Direct comparison of the present results with those of de Santis, *et al.*,[4] or with other potentially comparable calculations due to Ramachandran and coworkers,[5,41] is difficult in the absence of any indication regarding the scheme of energy accounting employed by those workers. The more narrow and steep-sided character of the principle minima observed in Figure 5 in comparison with their results may well reflect the absence of end effects in the present calculations.

Displacement of the minima in Figure 5 from the posi-

(35) K. Nagai and M. Kobayashi, *J. Chem. Phys.*, **36**, 1268 (1962).
(36) T. Shimanouchi and S. Mizushima, *ibid.*, **23**, 707 (1955).
(37) S. J. Leach, G. Nemethy, and H. A. Scheraga, *Biopolymers*, **4**, 887 (1966).
(38) J. A. Schellman and C. Schellman in "The Proteins," Vol. II, 2nd ed, H. Neurath, Ed., Academic Press Inc., NewYork, N. Y., 1964, Chapter 7.
(39) D. C. Phillips, *Proc. Nat. Acad. Sci. U.S.*, **57**, 484 (1967).
(40) D. A. Brant and P. R. Schimmel, *ibid.*, **58**, 428 (1967).
(41) C. M. Venkatachalam and G. N. Ramachandran in "Conformation of Biopolymers," Vol. I, G. N. Ramachandran, Ed., Academic Press Inc., New York, N. Y., 1967, p 83.

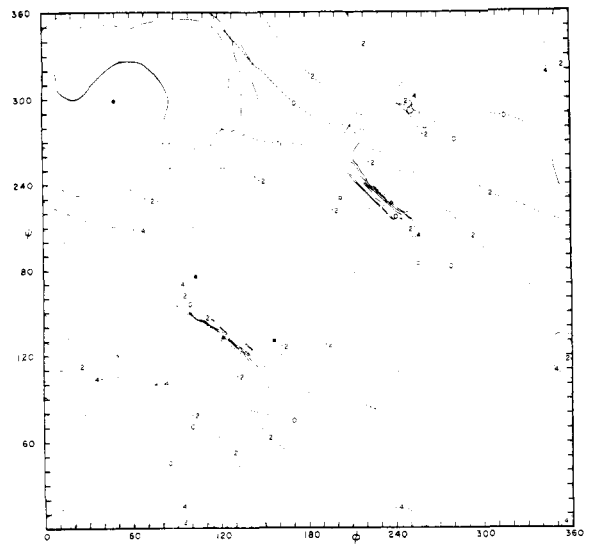

Figure 6. Contour diagram as in Figure 5 but including coulombic contributions, calculated in the point monopole approximation, in additon to torsional and van der Waals energies. Dashed contours show separately the coulombic term.[44]

tions of α_L and α_R is to be expected. In the absence of adjustments in the potential functions to allow for the reduced internuclear contact distances permissible for hydrogen-bonded groups, the conformational energy of the helices α_R and α_L is expected to be large owing to an $N_i \cdots O_{i-4}$ approach of 2.91 Å in these structures. This is less by 0.14 Å than the normal van der Waals contact distance for these atoms, and the interposed hydrogen lies close to the line joining O and N. The resultant van der Waals energy of the constellation N_i–$H_i \cdots O_{i-4}$–C_{i-4} approaches 9 kcal mol^{-1} for α_R and α_L. Consequently, coincidence of the simple van der Waals energy minima with the standard α-helical conformations is not anticipated.

It has been noted previously[18,42,43] that in the α-helix the peptide group dipole moments are arranged in almost parallel array. For peptide group neighbor pairs beyond the first the dipole–dipole interaction works to reduce the conformational energy of the α-helix, and the comparatively long range of these interactions enhances their potential importance as contributions to the conformational energy of all polypeptide helices. In hydrogen-bonded structures the dipole–dipole interaction of one of the peptide group neighbor pairs, *e.g.*, the pair i, $i - 3$ in the α-helix, undoubtedly makes a major contribution to the energy of the hydrogen bond, but calculations presented below demonstrate that important contributions to the energy accrue from other peptide group pairs as well. It is useful to recall that the hydrogen bond potential function introduced above is not superimposed upon the van der Waals and coulombic energies, but rather incorporates these contributions.

The conformational energy map calculated from torsional, van der Waals, and dipole–dipole interactions is presented in Figure 6. Dipole–dipole, or coulombic, interactions are calculated here in the point monopole approximation[9] with dielectric constant 3.5, and the dashed contours in Figure 6 show separately this increment V_c to the total energy.[44] The principle minima of the diagram in Figure 6 occur at 120°, 140° and 240°, 220°, that is, superimposed upon those in Figure 5 which were calculated from van der Waals energies only. Introduction of the coulombic terms into the potential functions is not in itself sufficient to render α_R and α_L low energy conformations, but it should be noted that the lowest contours in both Figures 5 and 6 enclose helices topologically equivalent to α_R and α_L.

It is instructive to observe the relative effect of the coulombic term in various regions of the map. Thus, the effect is neutral along the zero dashed contours of Figure 6, and these lines embrace the large shallow minimum in the upper left of Figures 4–6 wherein are located the more extended helices characterized by large axial translation per residue.[35] One such structure, taken as representative of the class, is the "pleated sheet"[45] with h = 3.34 Å and t = 180°; it is plotted in Figures 4–6 with coordinates 53°, 302°. Detailed analysis of this helix reveals that while first neighbor peptide group dipoles interact with an energy of *ca.* −0.9 kcal mol^{-1}, the remaining more distant neighbors make a total additional contribution of only +0.1 kcal mol^{-1} to the coulombic energy owing to separations greater than 6.5 Å. Helical residues within or near the domains of the principle minima experience an average decrease in energy of about −2 kcal mol^{-1} from the coulombic interactions. Taking the standard α-helices as exemplary of this class, we find V_c = −1.9 kcal mol^{-1}. Of this, the coulombic interaction of hydrogen-bonded peptide groups i and $i - 3$ contributes −1.5 kcal mol^{-1}. The respective quantities for the helix of minimum energy in Figure 4 (126°, 126°) are −2.3 and −1.9 kcal mol^{-1}.

Comparison of the contribution of coulombic interactions to the energy of a helical residue with its effect on the energy of a random coil residue reveals an interesting contrast. As shown in Figure 6 of Brant, Miller, and Flory,[9] a random coil residue in conformations resembling the conformation of the pleated sheet structure experiences a reduction in energy of about −0.75 kcal mol^{-1} from nearest neighbor coulombic interactions, whereas conformations resembling that of the α-helix suffer a comparable increase in energy from the same source. Thus, coulombic interactions favor the more extended random chain conformations, characterized by the extended residue conformations, relative to conformations characterized by the α-helical residue conformation. This circumstance arises because the peptide dipoles of *nearest neighbor* residues are approximately antiparallel, and thereby energetically favored,

(42) A. Wada in "Polyamino Acids, Polypeptides, and Proteins," M. A. Stahmann, Ed., University of Wisconsin Press, Madison, Wis., 1962, p 131.

(43) R. G. C. Arridge and G. C. Cannon, *Proc. Roy. Soc.* (London), **A278**, 91 (1964).

(44) The absolute energy of each dashed contour line in Figure 6 is less by 0.15 kcal mol^{-1} than the energy indicated on on the diagram, due to an error in calculating V_c which was detected after the diagrams were drawn. Thus, the absolute energy of the −1 kcal contour is −1.15, etc. The same constant error exists in every contour line reflecting contributions from V_c, *i.e.*, all solid contours in Figures 4 and 6. The error is not present in energies quoted elsewhere in the text or tables.

(45) L. Pauling and R. B. Corey, *Proc. Nat. Acad. Sci. U.S.*, **37**, 729 (1951).

TABLE I

CONFORMATIONAL ENERGY OF AN α-HELICAL PEPTIDE UNIT[a]

n,m	$V_{c,n}$	$V_{d,n}$	$V_\phi + V_\psi + V_{r,n} + V_{l,n}$	$V_\phi + V_\psi + (V_{r,n} + V_{l,n})^H$	$V_m{}^H$	$V_{c,m}$	$V_{d,m}$	$\Delta H'_\sigma$	Dipole contribution to $\Delta H'_\sigma$
0	0.00	0.00	0.34	0.34	0.34	0.00	0.00	...	...
1	0.52	1.22	0.82	0.82	1.68	0.52	1.22	...	...
2	−0.08	−0.50	−1.54	−1.62	−0.03	0.44	0.72	...	..
3	−1.50	−1.00	7.81	−3.17	−4.69	−1.06	−0.27	−0.79	2.38
4	−0.32	−0.38	−0.67	−0.67	−5.68	−1.38	−0.65	0.20	2.70
5	−0.14	−0.16	−0.07	−0.07	−5.89	−1.52	−0.81	0.62	2.98
6	−0.13	−0.13	−0.03	−0.03	−6.06	−1.66	−0.94	1.13	3.40
7	−0.09	−0.09	−0.02	−0.02	−6.16	−1.75	−1.03	1.53	3.76
8	−0.05	−0.05	−0.01	−0.01	−6.22	−1.80	−1.08	1.83	4.01
9	−0.04	−0.04	−0.00	−0.00	−6.26	−1.84	−1.12	2.07	4.25
10	−0.03	−0.03	−0.00	−0.00	−6.29	−1.87	−1.15	2.28	4.46
11	−0.02	−0.02	−0.00	−0.00	−6.32	−1.89	−1.17	2.52	4.62
12	−0.02	−0.02	−0.00	−0.00	−6.33	−1.91	−1.18	2.61	4.80
13	−0.01	−0.01	−0.00	−0.00	−6.35	−1.92	−1.20	2.81	4.90
14	−0.01	−0.01	−0.00	−0.00	−6.36	−1.93	−1.21	2.92	5.01
15	−0.01	−0.01	−0.00	−0.00	−6.37	−1.94	−1.22	3.04	5.13
20	−0.00	−0.00	−0.00	−0.00	−6.40	−1.97	−1.25	3.46	5.55

[a] Units, kilocalories per mole.

for the extended conformations but nearly parallel for conformations resembling that of the α-helix. For helical residues the longer range attractive interactions possible for α-helical structures cause the total coulombic energy V_c to favor these compared to the more extended structures for which convergence of the coulombic energy with separation in the chain sequence is rapid. The characteristic dependence of coulombic energies upon separation in the chain sequence has consequences for helix–coil transition theory which are explored in the following section.

Results presented here support previous evidence[4] for the predominant influence of the simple van der Waals interactions in determining the least energetic class of residue conformations in poly-L-alanine and presumably in other similar peptide homopolymers. The stability of these favored conformations relative to others is materially enhanced by intramolecular hydrogen bonding which may occur in the corresponding helical structures. Although the introduction of features into the potential functions to account for hydrogen bonding has an effect upon the coordinates of the least energetic conformations, this effect is minor, and helices of least energy closely similar to those predicted to be most stable solely from consideration of van der Waals interactions are observed. Coulombic or dipolar interactions not included in the hydrogen bonding term also contribute to the relative stability of the most stable conformations. It is noted that the relative effect of coulombic interactions upon the stability of residue conformations characterizing the pleated sheet and α-helix is opposite for helical and random coil residues.

Dependence of Conformational Energy on Helix Length. Contributions from the several terms of eq 1 to the conformational energy of a helical polypeptide unit have been calculated as a function of helix length for the standard Pauling–Corey right-handed α-helix, which is chosen as representative of conformations predicted to be most stable for a variety of helical peptide homopolymers.[7] Let the mutual repulsive energy of peptide units i and $i - n$ be designated

$$V_{r,n} = \sum_{\{i,i-n\}} V_{r,jk}$$

where the summation is carried only over atoms within these units. Similar definitions apply to the London energy $V_{l,n}$, the coulomb energy in the point monopole approximation $V_{c,n}$, and the peptide dipole energy $V_{d,n}$ of units i and $i - n$.[9] These quantities, alone and combined with V_ϕ and V_ψ, are shown in columns 2–5 of Table I; the superscript H designates use of potential functions modified as described above to account for hydrogen bonding. Entries for $n = 0$ represent the self-energy of a peptide unit.

The α-helix is characterized by favorable alignment for hydrogen bonding of group N_i–H_i with C_{i-4}–O_{i-4}. The convention adopted for enumerating peptide units places the group N_i–H_i within peptide unit $i - 1$, and consequently interactions reflecting juxtaposition of groups N_i–H_i and C_{i-4}–O_{i-4} occur for $n = 3$ as seen in Table I. The evident instability of the standard α-helix in the absence of hydrogen bonding results from approach of atom pairs N_i, O_{i-4} and H_i, O_{i-4} to distances appreciably smaller than the sums of their conventional van der Waals radii as noted above. Comparison of columns 4 and 5 in Table I shows that hydrogen bonding contributes to the energy appreciably only for interaction of peptide units i and $i - 3$. Although the present functions predict a minor contribution of hydrogen bonding to interaction of units i and $i - 2$, discrimination between alignment conducive to hydrogen bonding and nonhydrogen-bonding alignments is excellent. The range and importance of dipole interactions is also apparent from Table I. The two approximations to this term are similar for small n and become identical as n increases. A dielectric constant of 3.5 was used.[9]

Let V_m be the conformational energy of helical peptide unit i at the end of a helical sequence of length

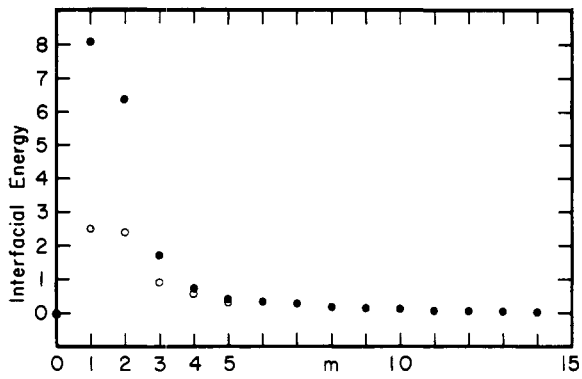

Figure 7. Interfacial energy, $V_m{}^H - V_m{}^{*H}$ (●), and corresponding dipolar contribution, $V_{e,m} - V_{e,m}{}^*$ (○), *vs.* helix length m. The latter quantity is shown only when significantly different from the former.

m. This is given in accordance with its previous definition and eq 1 by

$$V_m = V_\phi + V_\psi + \sum_{n=0}^{m}(V_{r,n} + V_{1,n} + V_{e,n})$$

The contribution from coulombic interactions to V_m may be defined by

$$V_{e,m} = \sum_{n=0}^{m} V_{e,n}$$

and a similar definition made for $V_{d,m}$. Columns 6–8 of Table I give $V_m{}^H$, $V_{e,m}$ and $V_{d,m}$ as functions of helix length m. The quantity $V_m{}^H$ is the rate of change of total helix energy with helix length and as such represents the contribution from intrahelical factors to the thermodynamic potential $\Delta G^\circ{}_s(m)$.

Addition of the third unit to a growing helix is accompanied by a marked decrease in total conformational energy in consequence of its strong hydrogen-bonded interaction with the first adjacent coil unit. Successive units are added with even greater decrease in energy, and the conformational energy has converged for $m = 20$. The remarkable stability of the α-helix in a variety of solvents[46–48] is a manifestation of the large negative conformational energy of an interior helical peptide unit represented by $V_{20}{}^H$. Contributions to helix stability from entropy factors[49] must not be overlooked, but these are not subject to estimate by methods employed here.[9]

The incipient helix for which addition of one more unit decreases the *free energy* of the system represents the critical helical nucleus.[26] This cannot be ascertained from the sign of $V_m{}^H$. For systems in which the helix–coil transition can occur, *i.e.*, $s \simeq 1$, the large negative contribution to $V_m{}^H$ from hydrogen bonding will necessarily be nullified by solvation energy and conformational entropy effects, and the critical nuclear size will probably be greater than that suggested by the present calculations. This supposition is consistent with

(46) W. B. Gratzer and P. Doty, *J. Amer. Chem. Soc.*, **85**, 1193 (1963).
(47) H. E. Auer and P. Doty, *Biochemistry*, **5**, 1708 (1966).
(48) J. S. Franzen, J. B. Harry, and C. Bobik, *Biopolymers*, **5**, 193 (1967).
(49) G. Nemethy, S. J. Leach, and H. A. Scheraga, *J. Phys. Chem.*, **70**, 998 (1966).

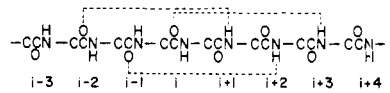

Figure 8. Segment of polypeptide chain showing hydrogen-bonded interaction (dashed lines) of peptide units in the α-helix.

the results of Goodman and coworkers.[50] Dipolar contributions $V_{e,m}$ and $V_{d,m}$ to the conformational energy both become negative for $m = 3$. Nearest neighbor peptide dipoles are approximately parallel and repulsive in the standard α-helix; second neighbors become moderately attractive for this conformation, but the resultant energy of first and second neighbor interactions is positive. Third neighboring dipoles are, however, strongly attractive by virtue of head-to-tail alignment, and succeeding neighbor pairs are all in similar attractive juxtaposition, since the dipoles are nearly parallel to the helical axis.[18,42,43]

The difference $V_m{}^H - V_m{}^{*H}$ represents the contribution from intramolecular factors to the interfacial free energy per peptide unit $\Delta G^\circ{}_\omega(m)$. Figure 7 shows $V_m{}^H - V_m{}^{*H}$ and the contribution to this quantity from $V_{e,m} - V_{e,m}{}^*$ as functions of m. A maximum occurs for each at $m = 1$, and convergence to zero is complete for $m = 20$ (see columns 2 and 5 of Table I). That both functions are positive for all m reflects the instability with respect to conformational energy and dipolar energy of interfacial peptide units relative to interior units. The interface is seen to extend, for practical purposes, about 5–7 residues into the helical sequence; $V_m{}^H$ has reached 90% of its asymptotic value for $m = 5$ for the standard α-helix. Convergence is in general more or less rapid, respectively, in helices of smaller or larger axial translation per residue. Consideration of column 8 of Table I reveals that $V_{d,m} - V_{d,m}{}^*$ follows closely the function $V_{e,m} - V_{e,m}{}^*$ appearing in Figure 7.

The contribution from factors considered here to the total interfacial free energy $\Delta G^\circ{}_\omega$ is given by

$$\sum_{m=1}^{m^*}(V_m{}^H - V_m{}^{*H})$$

where we take $m^* = 20$. Let us denote the latter quantity by $\Delta H'_\omega$, recognizing explicity with the prime the neglect of contributions to $\Delta H^\circ{}_\omega$ from polymer–solvent interactions and from interactions of amino acid side chains other than those involving the β-methylene groups which have been taken into account. We find then $\Delta H'_\omega = 18.70$ kcal/mol of helical sequences. Dipolar interactions contribute 8.07 kcal to this total. The present scheme for identifying helical peptide units recognizes in a sequence of α-helix of length m, m units having rotations ϕ_i, ψ_i restricted to values characterizing this helix and linked by $m - 2$ hydrogen bonds. Thus, in Figure 8 the five residues $i - 1$ to $i + 3$ are accounted as helical, and these are spanned by three hydrogen bonds. Unit $i - 2$, the first adjoining coil unit, participates in the hydrogen bonding, but the angles ϕ_{i-2}, ψ_{i-2} suffer no restriction of torsional free-

(50) M. Goodman, I. Listowsky, and E. Schmitt, *J. Amer. Chem. Soc.*, **84**, 1296 (1962); M. Goodman, F. Boardman, and I. Listowsky, *ibid.*, **85**, 2491 (1963).

dom as a result of this hydrogen bridge. The large positive value recorded for $\Delta H'_\omega$ is therefore a reflection of the deficit of two hydrogen bonds *not formed* at the ends of a helical sequence.

The predominant influence of solvent in determining the energy attributable to the hydrogen bond in polypeptide solutions precludes meaningful interpretation of $\Delta H'_\omega$, which was calculated restricting attention to intramolecular factors. An accounting scheme proposed by Zimm and Bragg[15] recognizes as helical a unit in which the carbonyl oxygen participates in a peptide hydrogen bond. As just demonstrated, there are two fewer such units in any α-helical sequence than there are helical units in the present scheme. When the requirement is imposed that the statistical weight assigned to a long, *i.e.*, $m > m^*$, helical sequence be the same using both bookkeeping schemes, it is found that $\sigma = s^2\omega$. Interpretation of the parameter σ has been discussed in a previous section. Only energetic contributions to ΔG°_σ are considered here, and the relationship of interest is $\Delta H'_\sigma = 2\Delta H'_s + \Delta H'_\omega$. The prime calls attention to neglect of side chain and solvent interactions; the latter have hopefully been rendered negligible by restricting consideration to σ to which contributions from changes in hydrogen-bonded solvation do not arise.

Intramolecular energy contributions to ΔG°_s are obtained readily from $\Delta H'_s = V_{m*}{}^H - \langle V\rangle_{coil}$, where $\langle V\rangle_{coil} = 1.22$ kcal mol^{-1} is the average energy of a coil unit previously evaluated.[9] The contributing dipole energy $\langle V_c\rangle_{coil}$ is -0.71 kcal mol^{-1}. One finds therefore $\Delta H'_\sigma = 3.46$ kcal/mol of helical sequences of which a contribution of 5.55 kcal arises from the dipole term. Evidently dipole interactions greatly disfavor the creation of helix–coil interfaces because of the unfavorable energy change which accompanies destruction of the long-range interaction of peptide dipoles in α-helical array. Dipolar contributions to hydrogen bonding make no contribution to this effect, however, owing to the interpretation afforded σ.

Although ΔG°_ω is free of intramolecular entropy contributions, since incorporation of every peptide unit into the helical sequence is accompanied by the same change in backbone entropy, the widely recognized[15,25] backbone entropy contribution to σ is manifest in the factor s^2 required to interconvert ω and σ. The present results suggest that previously postulated[18,20,23] enthalpy contributions may have an important influence on the magnitude of σ. Initial experimental data[20] suggest $\Delta H^\circ_\sigma < 0$. This is not necessarily contrary to the present results which ignore the interaction of side chains; side-chain interactions which work to favor helix–coil junctions are easily imagined. That σ may depend upon amino acid side chain, as suggested by some experimental results,[20] is evident from the prior discussion. For short chains, *i.e.*, DP < 20, the asymptotic conformational energy of a helical unit cannot be realized. Taking m^* = DP for these short chains $\Delta H'_\omega$ and $\Delta H'_s$ may be evaluated as before using the previous values for $\langle V\rangle_{coil}$ and $\langle V_c\rangle_{coil}$. The intramolecular enthalpy contribution $\Delta H'_\sigma$ to the lumped initiation parameter σ is thus evaluated and is presented as a function of chain length in column 9 of Table I along with dipolar contributions to $\Delta H'_\sigma$ in column 10. The inference that σ should increase with decreasing chain length for DP < 20 is consistent with recent data on the chain length dependence of σ[19]. Finally it is clear that the cooperative character of the helix–coil transition may be accounted for on the basis of intramolecular interactions alone. One must therefore expect the high-temperature normal transition anticipated for polybenzyl-L-glutamate in mixed solvent systems and the observed normal transition of polybenzyl-L-aspartate in two-component systems to be cooperative in contrast with recent supposition.[20]

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