Their reproducibility depends on control of (a) the specific activity of the enzyme that is being wired; (b) the ratio of the wiring polymer to the enzyme; and (c) the thickness of the wired-enzyme film. Their selectivity depends on the redox potential of the electron relaying centers. The closer this potential is to the redox potential of the enzyme itself, the lesser the likelihood that a potentially interfering substrate will be spuriously oxidized. Fluctuations in current with partial pressure of oxygen, e.g., oxygen concentration in blood, depend on the ratio of the rate of direct electrooxidation of the $FADH_2$ centers to their rate of oxidation by molecular oxygen, and therefore on the rate of electron transfer to, and the electrical resistance of, the three-dimensional wired-enzyme structure. At high osmium-complex concentrations, and in sufficiently thin layers, the competition is won by electron transfer to the electrode via the osmium centers, and the electrodes are relatively insensitive to oxygen. The signal to noise ratio S/N is, in the absence of interfering substrates, proportional to the number of enzyme molecules that are effectively wired to the electrode surface per unit area. At a film thickness of ~ 1 μm, and at typical blood glucose concentrations ($\sim 10^{-2}$ M), a current density of $\sim 10^{-3}$ A cm^{-2} is achieved. With a low noise potentiostat and only unshielded leads to the biosensor, the noise is less than 10^{-7} A cm^{-2}, i.e., S/N is on the order of 10^4.

The output current stability depends on enzyme durability and on avoiding fouling of the electrodes, primarily by adsorbed proteins. Typical decay rates at 25 °C in the absence of proteins are $\sim 5\%$/day, but are much faster in whole blood. By designing redox polymers that form hydrogels, we are now improving the stability of the bioelectrodes. We are designing relays that are closer in their potential to those of the enzymes, with the objective of further reducing the residual interference by electrooxidizable species such as urate and ascorbate ions. We are also exploring the range of enzymes that can be electrically wired and are building sensors with these. Currently our list includes, in addition to glucose oxidase, the flavo enzymes D-amino acid oxidase, lactate oxidase, and glycerol-3-phosphate oxidase, as well as lipoamide oxidase, through which NAD^+/NADH requiring enzymes are coupled to the electrodes.

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Energetics of Interactions of Regular Structural Elements in Proteins

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Progress in understanding the principles governing the conformational stability and the folding of proteins requires elucidation of the nature of the interactions between the structural elements that occur in proteins.[1,2] The observed conformations of proteins generally exhibit a hierarchy of structural features.[2-5] At the lowest level of this hierarchy, short- and medium-range interactions give rise to "chain-folding initiation structures" that can form in local regions of the polypeptide chain in the initial stages of the folding process.[6] The same interactions are responsible for the preferences of parts of the polypeptide chain to fold into regular structural elements, such as α-helices and extended chains that form β-sheets. These regular elements, in turn, associate with each other as a result of long-range interactions[2,5] and, in some cases, form recognizable domains.[7] On the next level of structure, association of domains is also a resultant of long-range interactions.[2,8] Most proteins can be classified into

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Table I
Characteristic Properties of Computed Homopoly(amino acid) β-Sheets[a]

residue X	twist: δ, deg A	twist: δ, deg P	rel energy:[b] $E_P - E_A$, kcal/mol	obsd structure[c]	unit height: h, Å A	unit height: h, Å P	conformatnl region[d]
Gly	0.0	0.0	21.65		3.63	3.62	*E*
Ala	6.2	1.6	12.36	A	3.53	3.54	*E*
Abu	10.5	11.0	1.65		2.91	2.87	*C*
Val	32.2	29.8	-1.39	P	3.02	3.03	*C*
Ile	26.7	24.1	-1.97	P	3.05	3.04	*C*
Leu	-6.4	15.4	32.97	A	3.01	2.98	*C*
Lys	15.0	20.6	-5.62		2.96	2.95	*C*
Ser	-8.0	-7.2	-16.48		3.53	3.54	*E*
Thr	11.4	0.0	-7.44		3.57	3.55	*E*
Phe	7.0	18.2	-1.98	P	3.54	3.51	*E*
Tyr	7.8	16.4	-6.86	P	3.54	3.51	*E*

[a] From ref 30. Computed for minimum-energy regular β-sheets consisting of three $CH_3CO(X)_6NHCH_3$ chains. A = antiparallel, P = parallel. [b] Difference in the total energy of the β-sheets. [c] From refs 31 and 32. [d] Location of the minimum on a (ϕ,ψ) map, defined in ref 33, as described in the text.

various classes on the basis of the content and arrangement of α-helices and β-sheets.[3,9] The number and variety of stable arrangements of packed structures formed by α-helices and/or β-sheets is limited.[5,10]

In this Account, we demonstrate that many common features of these packing patterns can be explained in terms of local interaction energies, without having to take into account all of the interactions in the entire protein molecule.[10] Thus, insights gained from studies of the packing of regular structural elements provide useful generalizations for the analysis and the prediction of protein structure.

One of the most general structural features of proteins is the existence of preferential handedness of regular structures.[3,5] Thus, α-helices in proteins are right-handed; most β-sheets are not flat but twisted, and the twist always occurs to the right. Similar preferences are seen in intermediate-level structures that arise from the interaction of two or more of the regular structural elements. Thus, there is a definite preferred sense of handedness in the four-α-helix bundle, in the βαβ connection and in the β-barrel. These preferences arise because of energy differences between the various ways of packing the simple regular structures (i.e., the α-helices and/or β-sheets). The analysis of the favorable ways of packing and of the resultant preferences of handedness, in terms of the energies of noncovalent interactions, has been an active field of research in the Cornell Laboratory.

In the studies described here, polypeptide chains were generated, and their potential energies were determined by means of an algorithm developed in the Cornell laboratory, ECEPP (Empirical Conformational Energy Program for Peptides),[11,12] and by related programs for the generation of assemblies of polypeptides and the computation of the interchain energy.[13-18]

Handedness in Regular Structures

Handedness of the α-Helix. All observed α-helices are right-handed in proteins (with the exception of one very short left-handed α-helix in thermolysin) as well as in polymers formed by the natural amino acids, but left-handed helices exist for some poly(amino acid) derivatives. One of the first applications of conformational energy computations was the determination of interactions that result in the preference for a right- or left-handed helix sense. The presence of a β-carbon favors right-handedness of the α-helix for all the naturally occurring amino acids with an L configuration, because of the balance of nonbonded interactions involving this carbon atom and neighboring peptide groups.[19] On the other hand, the computations predicted that either right- or left-handed helices can occur for various polar side chains, such as differentially substituted poly(γ-benzyl-L-glutamate)s and poly(β-benzyl-L-aspartate)s. The preference arises from the balance of the interactions between the peptide dipoles of the backbone and dipoles in the side chains.[20,21] The computed helix senses have subsequently been verified experimentally.[22,23]

Sense of Twist of β-Sheets. All β-sheets observed in globular proteins have a right-handed twist.[24] In earlier analyses, it has been suggested that this exclusive preference may be due to energy and entropy factors operating at the level of the single residue[24] or to geometric constraints of the hydrogen bonds.[25-27] We have carried out a detailed analysis of the energy and geometry of strand packing in β-sheets of several poly(amino acid)s, in order to assess the energetic factors that

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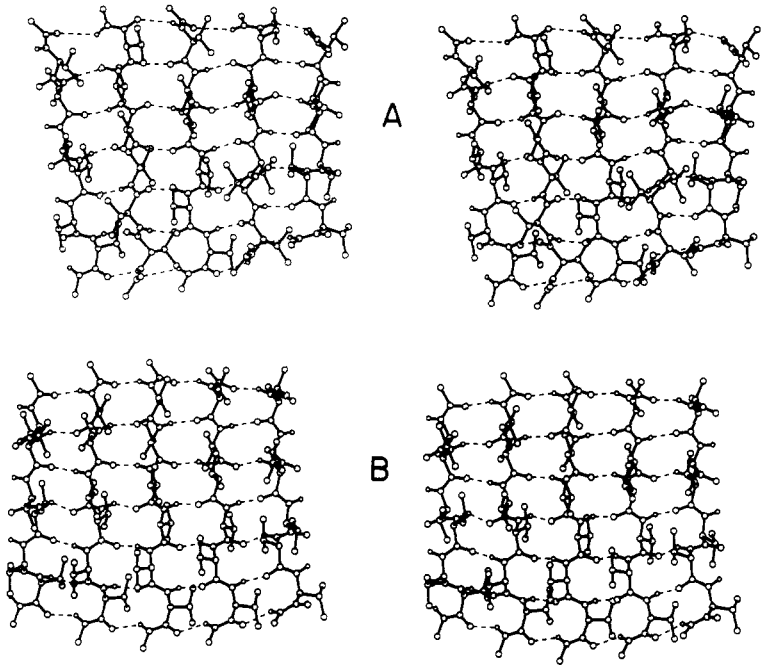

Figure 1. Stereodrawings of minimum-energy twisted β-sheets formed by five $CH_3CO(L\text{-}Val)_6NHCH_3$ chains. (A) Antiparallel structure. (B) Parallel structure. Hydrogen bonds are indicated by broken lines. (Reprinted with permission from ref 28. Copyright 1982 National Academy of Sciences.)

contribute to the direction and extent of twisting[13,28-30] and to the relative stabilization of parallel or antiparallel packing.[31,32]

The twist of β-sheets is expressed as an average of the twist of its individual strands. This quantity, in turn, is described in terms of δ, the angle between the projections of next-nearest-neighbor residues of the chain onto a plane that is perpendicular to the axis of the chain.[10,13] δ is a function of the helical parameter n, the number of residues per turn. For a strand with a regular conformation,

$$\delta = 360°(2 - |n|)/n$$

expressed as degrees per two residues. A corresponding average, $\langle\delta\rangle$, is used for nonregular chains.[13] Right-twisted, flat, and left-twisted strands are characterized by $\delta > 0°$, $\delta = 0°$, and $\delta < 0°$, respectively.

Almost all of the computed poly(amino acid) β-sheets have a right-handed twist (Table I), in agreement with the observations on proteins. The main exception is poly(Ser), with a predicted left-handed twist ($\delta < 0°$). A survey of dihedral angles in 34 globular protein structures indicated that Ser residues in β-sheets often have conformations that correspond to local left-handed twisting, i.e., to a local deformation, even though the overall twist of the entire β-sheet remains right-handed.[30]

A preference for a right-handed twist arises from intrastrand side chain–backbone interactions, as seen in poly(Ala) or poly(Val), but interstrand side chain–side chain interactions play an equally important role. For example, intrastrand interactions in an isolated extended strand of poly(Ile) would cause the left-handed twist of the chain to be energetically favorable, but interstrand interactions lead to the stabilization of the right-handed β-sheet.[29] On the other hand, backbone–backbone hydrogen bonding between strands does not result in a preference for either sense of twist, and it actually would favor structures without twist.[27,28] Increasing bulk of side chains leads to a larger twist, as seen from a comparison of Gly, Ala, Abu, and Val in Table I. The two residues causing the highest computed twists (Val and Ile), together with Leu, are the three most frequently occurring residues in β-sheets of proteins.[34] This explains why observed twists usually are high.

The residues of Table I fall into two distinct classes[30] [with the exception of poly(Gly), which is achiral].[28] In one class, containing Ala, Ser, Thr, and aromatic residues, the chains are nearly fully extended (conformation E),[33] with the translational repeat distance h near 3.52 Å, (ϕ,ψ) around (-150°,150°), and a generally small to moderate twist. The other class contains residues with large aliphatic side chains. The twist is generally larger, the chains are less fully extended (h = 2.9–3.0 Å), and (ϕ,ψ) is around (-90°,100°) (conformation C).[33] This conformation C region of the (ϕ,ψ) map generally has not been considered as a β-sheet although good hydrogen bonds are formed between strands in this region (Figure 1). The presence of two separate regions is related to the formation of coiled coils. Coiled-coil strands occur frequently in β-sheets.[25-27] Their structure has been analyzed by Chothia,[35] who pointed out that coiling requires the alternation of residues with dihedral angles falling into two distinct (ϕ,ψ) regions. These regions coincide approximately with the two classes described here. The same alternation is seen in

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the crystal structure of the transmembrane polypeptide gramicidin A.[36,37] In this molecule, the dihedral angles of the L-Ala and L-Trp residues fall into the more extended regions, while those of D-Val and D-Leu occur in the region corresponding to less extended chains, in agreement with the theoretical prediction.[30]

The extent of twisting is largely a function of the amino acid sequence of the strands, but it can be enhanced by interchain packing interactions that operate *within* the β-sheet. This has been demonstrated for the strongly twisted two-stranded β-sheet consisting of residues 14–38 in bovine pancreatic trypsin inhibitor.[38] Interactions with the rest of the protein molecule are not required for the maintenance of the strong twist. This result also implies that this β-sheet structure forms during an early stage of folding of the protein, preceding the completion of the disulfide-bond pattern. This example demonstrates how conformational energy computations can provide information about the probable pathway of folding of a protein.

Comparison of Parallel and Antiparallel β-Sheets. The relative stability of the two forms of the twisted β-sheet for each poly(amino acid) can be assessed from the computed energies. It was predicted[30] that the antiparallel β-sheet is favored for residues with a small unbranched (or γ-branched) side chain or no side chain, while the parallel form is favored for residues with β-branching, aromatic rings, polar groups near the backbone, or a very long side chain (Table I). All of these predictions agree with available experimental observations on oligopeptides.[31,32]

Arrangement of Strands in β-Sheets. A survey of observed β-sheets had indicated that strands with the greatest hydrophobic potential tend to occur in the center of β-sheets, while more hydrophilic strands tend to occupy positions on the edges.[39] A statistical analysis of the probability of contact formation between all pairs of amino acids has been used to predict the order of adjacency in which strands pack in a β-sheet in a protein.[40] The native strand arrangement of several proteins is among those arrangements for which the method predicts the highest probabilities. Other structural properties of β-sheets in proteins have been reviewed in detail by Salemme.[27]

Packing of Regular Structures

Preferred spatial arrangements in the packing of regular structural elements occur as the result of favorable interatomic interactions between these structures. Our conformational energy computations summarized here covered all classes of combinations of α-helices and β-sheets.

Much information about packing preferences can be obtained from considerations of the geometry of packing alone.[5,9,24-26,41] Geometrical modeling leads to useful insights, because a good fit of complementary surfaces generally implies a favorable set of nonbonded interactions. It is also necessary, however, to compute the energies of the various packed structures in order to assess their relative stabilities. In some cases, the computations based on energies have predicted structures that have not been found by a geometrical analysis.

Table II
Energy Parameters (in Kilocalories/Mole) and Orientations of Two Packed Poly(L-Ala) α-Helices[a]

orientatn angle: Ω_0, deg	tot. rel energy:[b] ΔE, kcal/mol	interchain energy, kcal/mol: tot. E	electrostatic	nonbonded	packing classification:[c] ij
−154	0.00	−17.23	−2.20	−15.02	34
170	0.76	−16.42	−2.24	−14.18	none[d]
146	1.74	−15.43	−1.45	−13.98	13
−36	3.35	−13.82	0.54	−14.36	13
127	3.86	−13.31	−1.20	−12.11	44
30	4.35	−12.84	0.95	−13.79	34
79	4.57	−12.54	0.15	−12.70	14
−136	4.92	−12.26	−1.29	−10.98	none[d]
−155	5.17	−12.05	−1.58	−10.47	34
−87	5.30	−11.89	−0.14	−11.74	33

[a] From ref 14. Minimum-energy packing arrangements computed for two $CH_3CO(L\text{-}Ala)_{10}NHCH_3$ α-helices. [b] $\Delta E = E - E_0$, where E_0 is the energy of the structure in line 1. [c] According to a geometrical "ridges into grooves" model (ref 42). The indices i and j refer to the interacting ridges on the two helices, as described in the text. Two packings with the same value of ij correspond to the reversal of orientation of one of the helices. [d] There is no "ridges into grooves" arrangement corresponding to this computed packing.

The relative orientation of two structures is expressed in terms of orientation angles,[14,15,41,42] denoted Ω. For two helices, the orientation angle Ω_0 is a measure of the tilting of the helix axes, with $\Omega_0 = 0°$ for parallel and $\Omega_0 = \pm 180°$ for antiparallel orientation. Analogous parameters, $\Omega_{\alpha\beta}$ and $\Omega_{\beta\beta}$, respectively, are used in helix/sheet and sheet/sheet packing to describe the orientation of the helix or of the second β-sheet, relative to the axis of a β-sheet used as reference.

α-Helix/α-Helix Packing. Previous studies of the packing geometry of two α-helices have suggested that there exist only a small number of preferred relative orientations of the helix axes. In most of these arrangements, the side chains of one helix intercalate into spaces between the side chains of the other helix. This concept of "knobs into holes", introduced by Crick[43] in 1953, or "ridges into grooves",[41,42] has been the basis of several geometrical models for the distribution of helix orientations.[5,41,42,44,45] On a given helix, a set of side chains with a mutual separation of j residues along the sequence (with j = 1, 3, and 4) forms a ridge, with a continuous groove between them.[41,42] The complementary packing of ridges and grooves in two adjacent helices results in preferred orientations of the two helices. These geometrical models cannot, however, distinguish between parallel and antiparallel arrangements.

Conformational energy computations on the packing of two $(L\text{-}Ala)_{10}$ α-helices have shown that only 10 stable packing arrangements occur within an energy range of 5.3 kcal/mol (Table II).[14] Several distinct values of the orientation angles have been found, but the three lowest energy packing arrangements are nearly antiparallel

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(occurring within ±35° of $\Omega_0 = \pm 180°$). The orientation angle for the energetically most favorable packing is $\Omega_0 = -154°$. This is the most favored observed arrangement in proteins, largely because it is strongly favored in the frequent four α-helix bundle structure (discussed below). The second most frequently observed helix packing in proteins[5,42] occurs in the range $-50° < \Omega_0 < -30°$, corresponding to the computed lowest energy packing that is not nearly antiparallel ($\Omega_0 < -36°$).

Both nonbonded and electrostatic contributions influence the packing of α-helices (Table II). The magnitude of the interchain nonbonded energy greatly exceeds that of the electrostatic energy, i.e., nonbonded interactions dominate the overall energy of stabilization of packed helices, as compared to separated helices. On the other hand, the computed contributions of these two terms to the *energy differences* between various ways of packing are comparable in magnitude, i.e., both are significant for the preferences of packing orientation.[14]

The computations summarized above have been carried out in the absence of solvation.[14] Solvent effects give rise to several additional energy contributions. As pointed out by Gilson and Honig,[46] the presence of water (or, in general, of a solvent with a high dielectric constant) has two destabilizing effects on electrostatic interactions. It reduces the magnitude of the interhelix dipole interactions (thereby decreasing their contribution to the energy differences between various arrangements), and it also destabilizes packed structures because of the desolvation of the helical dipoles upon helix association. On the other hand, the presence of water may also contribute to the stabilization of packed structures containing nonpolar side chains, as a result of hydrophobic interactions. Both sets of analyses[14,46] indicate that electrostatic interactions play a minor role in helix packing, in contrast to earlier estimates that were based on considerations of the dipole moment of the α-helix alone.[47,48]

The packing of α-helices may be influenced by the size and shape of the side chains in contact (as well as possible specific interactions between them, such as hydrogen bonds). A computation of the packing of a poly(L-Leu) and a poly(L-Ala) α-helix[15] indicated that the lowest energy near-antiparallel packings were not affected significantly by the substitution of Leu for Ala. The relative energies and the geometry of the packing in other, higher energy arrangements depend sensitively, however, on side-chain interactions. Sequence-specific interactions between α-helices may restrict significantly the number of probable ways of packing.[44]

The mutual influence of several α-helices on the packing was investigated for three helices (A, G, and H) in sperm whale myoglobin. Two nearly antiparallel tightly packed helices (G and H) are not affected by the introduction of a third, neighboring helix. On the other hand, a pair of nearby perpendicular helices with weak interactions (A and H) can pack in a variety of ways, but in this case, the presence or absence of a third helix (G) alters the relative energies of the A/H packings.[49]

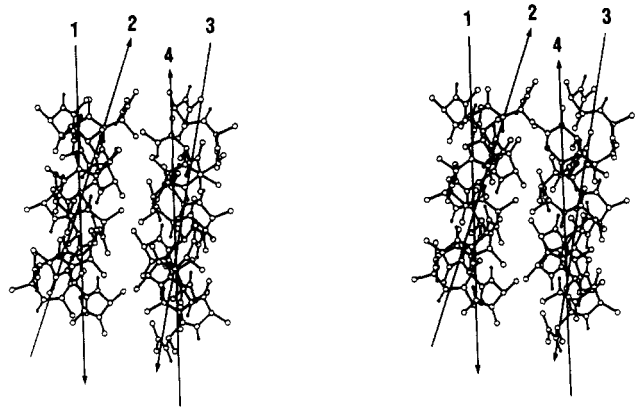

Figure 2. Stereodrawing of a minimum-energy four-α-helix bundle formed by four $CH_3CO(L\text{-}Ala)_{10}NHCH_3$ chains. The orientation angle of neighboring helices is $\Omega_0 = -168° \pm 7°$. The bundle is left-twisted. Only heavy atoms and amide hydrogens are shown. The arrows denoting the helix axes point from the N- to the C-termini. (Reprinted with permission from ref 53. Copyright 1988 National Academy of Sciences.)

Small shifts in the packing of neighboring α-helices can have important effects in the mechanisms of biologically important conformational changes.[50]

Four-α-Helix Bundle. The main structural features of this characteristic structural feature of many proteins[3,51,52] can be explained in terms of nonbonded interactions between the constituent helices.[53] In almost all of the observed bundles in proteins, neighboring helices are oriented nearly antiparallel to each other. This is an orientation that is also favored by electrostatic interactions between the dipoles of the helices.[48] It has been pointed out, however, that the presence of an aqueous solvent environment decreases the role of electrostatic interactions.[46] In the energetically most stable computed four-α-helix bundle,[53] the helices are slightly tilted, with an orientation angle of $\Omega_0 = -168°$ between neighboring helix axes (Figure 2). This is close to the most favorable angle of orientation computed for a pair of packed α-helices (Table II). The antiparallel orientation is a resultant of favorable nonbonded and electrostatic interactions in both the helix pair and the bundle, as discussed above. The tilting corresponds to a left-handed twisting of the entire helical bundle, as observed in many proteins.[51-53] The design and synthesis of a four α-helix bundle polypeptide[54] also has involved considerations of α-helix packing.

β-Sheet/β-Sheet Packing. As a result of the right-handed twisting of β-sheets, the overall shape of the sheet is saddle-shaped.[41,25-27] For the packing of two such β-sheets, two distinct classes of computed low-energy arrangements have been found.[55] In the class with lowest energies, the strands of the two sheets are aligned nearly parallel (or antiparallel) to each other, resulting in the complementary packing of the two

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saddle-shaped surfaces. The computed horizontal projected orientation angle lies in the range $-26° < \Omega_{\beta\beta} < 5°$. Observed values[41,56,57] fall into the range −20° to −45°. In the other class, the strands are nearly perpendicular to each other, with the computed $|\Omega_{\beta\beta}|$ between 93° and 107° and observed values[41,58] ranging from 71° to 99°. While the saddle-shaped surfaces are not complementary in this arrangement, their interactions are favorable because there is good packing between the corner of one sheet and the interior part of the other sheet. The intersheet energy is 1–4 kcal/mol higher than in the first class.

These two classes have been termed aligned and orthogonal packings in observed protein structures.[41,57,58] Orthogonal packings are usually formed by two β-sheets folded back on themselves, in such a manner that a strand passes from one sheet to the other sheet near their corner, forming a near-90° bend.[5,41,58] A covalent connection is usually observed, but the energy computation has shown that its presence is not necessary for the stabilization of the packed structure. Chothia and Janin[41,57] have proposed that the orientational angle $\Omega_{\beta\beta}$ for the aligned structures is related in a simple manner to the difference of twists of the two sheets.[57] In fact, the value of $\Omega_{\beta\beta}$ can be predicted[41,55] from the twists to within ±4°.

β-Barrels. Many β-sheets are rolled up into a cylinder, with hydrogen bonding between the first and last strands of the sheet. This structure has been termed a β-barrel.[3,27,59] Most of the β-barrels formed by a parallel sheet contain eight β-strands, and neighboring strands are almost invariably connected by an α-helix, resulting in a ...βαβαβ... arrangement[3,27] (see below). The structure of the parallel barrels is usually quite regular. β-Barrels formed from antiparallel sheets exhibit more variation in the number of strands and the overall shape of the structure.[27] They are usually not associated as closely with α-helices as are the parallel-sheet barrels.

As a result of the twisting of β-sheets, folding into a β-barrel results in the staggering of the hydrogen-bonding pattern and the tilting of the strands with respect to the axis of the β-barrel. Corresponding to the right-handed nature of the twist of β-sheets, the resultant β-barrel is also right-tilted, i.e., the strands follow right-handed helical paths about the axis.[3,25,60,61] Conformational energy computations on antiparallel β-barrels composed of eight strands, in which L-Val and Gly residues alternate, have shown that the barrel with right-handed tilt is much more stable than one with a left-handed or no tilt.[62] The relative energies are 0.0, 8.6, and 46.3 kcal/mol, respectively. Tilting of the strands is preferred over no tilting because it improves side-chain packing and therefore lowers the interstrand energy. In fact, the energy of the nontilted structure is high because unfavorable interactions between the side chains distort the barrel into a noncylindrical shape and result in the breaking of many of the hydrogen bonds. The same thing would happen with a β-barrel consisting entirely of poly(Val) strands, even in a tilted structure, because of the crowding of the side chains. Inside a stable β-barrel, it is necessary to have an appropriate alternation of large and small side chains in order to fill the cavity without steric crowding. This is observed in the β-barrels in proteins.[62] The preference for right- over left-handed tilting is related to the energetically preferred twist of the β-sheet, discussed above.

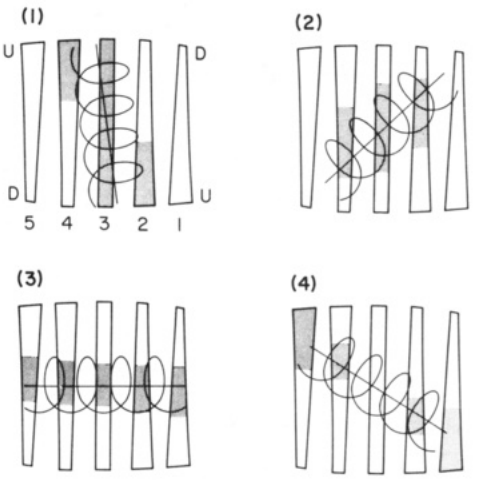

Figure 3. Schematic representation of the various classes of α-helix/β-sheet packing. The strands of the twisted β-sheet are shown schematically as rectangles drawn in perspective to indicate their tilting relative to the plane of the drawing. The letters U and D denote the corners of the sheet located above and below, respectively, the plane of the drawing. Shading indicates the region of the strands in contact with the α-helix. (Adapted from ref 16.)

α-Helix/β-Sheet Packing. α-Helices are often associated with β-sheets in globular proteins. A frequent structural motif is the βαβ or βαβαβ structure (termed Rossmann fold),[3,63] in which an α-helix connects two neighboring strands of a parallel β-sheet (see discussion below), but there are also many packing arrangements in which the helix and the sheet come from different parts of the sequence. The geometry of the packing of α-helices and β-sheets has been studied extensively in order to establish preferred chain orientations and residue contact patterns.[5,64,65] Observed preferences have been interpreted in terms of surface complementarity of the two regular structures[41,64] or in terms of the intercalation of side chains on the two surfaces.[65,66]

In a computational study, we have found four distinct classes of low-energy packing arrangements for a poly(L-Ala) α-helix packed against a five-stranded poly(L-Val) β-sheet.[16] They differ in the orientation of the helix axis with respect to the direction of the strands (Figure 3). In the lowest energy structures (class 1), the helix lies nearly parallel (or antiparallel) to the strands ($-10° < |\Omega_{\alpha\beta}| < 10°$). This is the class that occurs most frequently in proteins, and it has also been proposed from considerations of geometry[5,41,64] as the most favorable packing. In the second lowest energy

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packing (class 3), predicted by the energy computation but not by the geometrical analyses, the helix runs nearly perpendicular to the strands ($80° < |\Omega_{\alpha\beta}| < 100°$). Numerous packed structures of this type occur in proteins. Both classes are stable because the helix lies along the tangent of the curved surface of the β-sheet, so that residues along the entire helix can interact with those in the β-sheet. In another group with relatively low energy[16] (class 2), also suggested by geometrical analyses,[5,41,64] the α-helix lies along a diagonal of the β-sheet, but only its central part packs tightly against the saddle-shaped surface of the sheet ($-60° < \Omega_{\alpha\beta} < -40°$). This structure occurs frequently in proteins. Class 4 is energetically less favorable, because the extent of interresidue contacts is limited, when the α-helix is lying along the other diagonal ($\Omega_{\alpha\beta} \approx 60°$), but a few examples exist in proteins. The frequency distribution of observed orientation angles in proteins, $\Omega_{\alpha\beta}$, has maxima corresponding closely to the four classes of computed structures (cf. Figure 12 of ref 16).

Electrostatic (dipole) interactions are unimportant in α/β packing. Most of the interaction energy arises from nonbonded interactions.[16] Consequently, the orientation of the helix axis is not important here.

The detailed analysis of the interaction energies and of the shape of the β-sheet in the computed α/β structures has demonstrated that an antiparallel β-sheet is more flexible than a parallel β-sheet, because the former can be deformed more easily in response to packing interactions.[16] This difference in stability has also been indicated by computations on the structure of isolated β-sheets,[13,28] as well as by analyses of observed protein structures[3,66,67] and of hydrogen-bonding geometry.[26]

Handedness of the $\beta\alpha\beta$ Crossover. The crossover connection between two parallel strands in a β-sheet is almost always right-handed.[68-70] An α-helix occurs frequently in the crossover. The crossover can occur by itself ($\beta\alpha\beta$ structure), as part of the Rossman fold[63] ($\beta\alpha\beta\alpha\beta$), or as a constituent of parallel β-barrels. The preference for right-handedness has been interpreted on the basis of the preference for the right-handed twist of the β-sheet resulting in a lesser strain of the connecting piece between the strands than in a left-handed crossover,[71] or of favorable complementarity of the surfaces of the helix and the sheet,[66] as in α/β packing.

A comparison of right- and left-handed crossovers by means of conformational energy computations has established that the right-handed form is energetically much more favorable than the left-handed one, by at least 15 kcal/mol for a structure consisting of two (L-Val)$_6$ β-strands and an (L-Ala)$_{12}$ α-helix, connected by two flexible (L-Ala)$_4$ links.[72] The right-handed crossover is strain-free, as indicated by the high right-handed twist of the β-sheet (with a large positive value of δ, corresponding to δ in an isolated sheet[29]) and by the absence of high-energy conformations in the connecting links. In contrast, the left-handed crossover is strongly strained.[72] Thus, the computations confirm the qualitative explanation of Sternberg and Thornton.[71]

Extensions of the Analysis of Packing to Larger Structures

Domains. Domains constitute a higher level substructure of many proteins.[3,8] A domain often consists of an assembly of packed regular structures, of the type described in the preceding section. In order to predict protein structure, it is necessary first to locate the boundary of domains before investigating the packing within the domains. We have developed a procedure to identify domains of globular proteins from a knowledge of their amino acid sequence alone.[7]

Intermolecular Interactions. The association of protein subunits into oligomers frequently involves the packing of regular structures across subunit interfaces. Examples are the packing of four antiparallel pairs of α-helices in the dimer interface of citrate synthase,[73] the intersubunit four-α-helix bundle in the middle of the photosynthetic reaction center,[74,75] the β-sheet formed by two molecules in the crystal of the cyclic peptide gramicidin S,[76] and the two packed β-sheets, each containing strands from both subunits, in the dimeric structure of prealbumin.[77] Packing studies, similar to those summarized above, can be used to analyze the modes of packing of subunits.

Many intermolecular interactions in protein–ligand association (e.g., enzyme–substrate binding) also involve the docking of peptide chains of different molecules.[78] The packing programs could be used for the analysis and prediction of such docking arrangements as well.

Structure and Assembly of Collagen. We have analyzed the structure of the triple-helical collagen molecule[17] and the association of triple helices.[18] The theoretical prediction[17] of the most stable coiled-coil three-chain structure of poly(Gly-Pro-Pro) has subsequently been confirmed quantitatively by experiment.[79] The computations have elucidated the energetic reasons for many observed features, such as the sequence dependence of the triple helix structure, the enthalpy of melting of the triplet helix, and the preferred orientation of molecules in fibrils. They also have led to new predictions, such as the role of Hyp in stabilizing fibril structures. These studies have been reviewed in detail elsewhere.[80,81]

Conclusions

Most of the conformational energy studies summarized here have been carried out on model polypeptides, rather than on peptides with particular amino acid sequences. Therefore, the computations have yielded

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general conclusions about trends in some properties of packed regular polypeptide structures. These generalizations are not restricted by individual constraints that would arise from a specific amino acid sequence, yet they provide explanations for many observed features in proteins. For example, all observed preferences of handedness, as well as the general trends of chain orientation in packed regular structures, have been explained in terms of interaction energies.

Insights gained from studies of the energetics of packing are important for the prediction of protein folding. Information about preferred packing arrangements makes it possible to select for a given protein a small set of probable conformations, which then can be used as starting points for detailed energy computations. This potential application is significant because it helps to alleviate the multiple-minima problem, one of the greatest difficulties that remains in the prediction of three-dimensional structures of proteins.[82] By selecting likely structures at an intermediate level (between the levels of local conformational preferences of the amino acid sequence and the overall folding of the molecule), the number of probable conformations can be lowered considerably. Thus, the analysis of packing constitutes a link between conformational analysis of small peptides and the solution of the protein-folding problem.

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Rydberg States of H_3: Application of Neutralized Ion Beam Techniques

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Introduction

The possible existence and observation of the simplest triatomic molecule, H_3, has been a subject of scientific speculation for several years. Over the past decade, a substantial body of evidence has been obtained which shows that, rather than being a scientific curiosity, the H_3 molecule represents the simplest polyatomic example of an emerging new class of compounds referred to by Herzberg[1] as "Rydberg Molecules". In this Account, we will discuss evidence for the existence of H_3 in a metastable state and many of its spectroscopic properties, which have now been obtained in several types of experimental procedures, including neutralized ion beam techniques.[2] Several excellent reviews of the neutralized ion beam technique have recently been written,[3-6] and the reader is referred to these for a detailed discussion of the technique and its application to larger species.

Gregory I. Gellene was born in Paterson, NJ, in 1957. He received his B.S. in Biochemistry from Georgetown University in 1979 and his Ph.D. from Cornell University in 1983, where he studied with Richard F. Porter, developing neutralized ion beam techniques for the study of transient species. He spent an additional year at Cornell in postdoctoral studies, applying neutralization reionization mass spectrometry to hypervalent radicals. In 1984 he joined the faculty of Notre Dame, where he is currently developing combined optical/neutralized ion beam techniques.

Richard F. Porter has been a Professor of Chemistry at Cornell since 1964. For the past decade he and his students have been utilizing neutralized ion beam techniques to generate polyatomic radicals and metastables which are difficult, if not impossible, to prepare by conventional methods. His interest in this research on novel polyatomics began in 1978 while he was a visiting scientist with Drs. Lewis Friedman and Robert Beuhler in the Chemistry Department at Brookhaven National Laboratory.

Historical Background

In the late 1960s, Devienne[7,8] and his associates attempted to produce H_3 molecules in an experiment involving charge neutralization of a fast beam of H_3^+ ions followed by reionization of the neutral products. The sequence of steps was

$$H_3^+ + H_2 \rightarrow H_3^* + H_2^+ \quad \text{(neutralization)} \quad (1)$$

$$H_3^* + D_2 \rightarrow H_3^+ + e^- + D_2 \quad \text{(reionization)} \quad (2)$$

The appearance of a mass peak ($m/e = 3$) following reionization in his mass spectrometer was taken as evidence for long-lived H_3 molecules that had to survive transit from the point of formation to the point of reionization (~microseconds). In a separate measurement, the neutralized beam was converted into negative

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