Local densities orthogonal to β -sheet amide planes: Patterns of packing in globular proteins

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ABSTRACT We have investigated the efficiency of packing by calculating intramolecular packing density above and below peptide planes of internal β -pleated sheet residues in five globular proteins. The orientation of interest was chosen to allow study of regions that are approximately perpendicular to the faces of β -pleated sheets. In these locations, nonbonded van der Waals packing interactions predominate over hydrogen bonding and solvent interactions. We observed considerable variability in packing densities within these regions, confirming that the interior packing of a protein does not result in uniform occupation of the available space. Patterns of fluctuation in packing density suggest that the regular backbone-to-backbone network of hydrogen bonds is not likely to be interrupted to maximize van der Waals interactions. However, high-density packing tends to occur toward the ends of β -structure strands where hydrogen bonds are more likely to involve nonpolar side-chain groups or solvent molecules. These features result in internal protein folding with a central low-density core surrounded by a higher-density subsurface shell, consistent with our previous calculations regarding overall protein packing density.

The details of three-dimensional molecular architecture are now known for numerous proteins that have been studied by x-ray crystallography. This information has allowed analysis of the final results of protein folding. Comparison of the folding patterns of a number of structures has been used to generate algorithms for prediction of secondary structural elements with some success. However, the folding of individual domains to create a final tertiary protein structure has remained a more elusive problem. The current study was undertaken to investigate packing densities near β -pleated sheets in the interior of globular proteins, focusing on regions where nonbonded interactions predominate.

Protein structures are folded compactly (1). By analyzing 75 nonhomologous protein structures, Williams *et al.* (2) found an average of only one buried water molecule for every 27 residues. Klapper (3) calculated the packing fraction—the ratio of the volume occupied by a protein's constituent groups of atoms to the protein's molar volume—for a number of proteins and found values of 0.75 ± 0.03 . Dividing ribonuclease into groups of 5–25 atoms, Richards (4) reported packing fractions in small intramolecular units ranging from 0.66 to 0.84. Less densely packed areas were observed near the active site groove, which was surrounded by a more densely packed shell. We previously calculated internal packing densities of proteins to be 1.05–1.23 g/cm³, considerably lower than the observed values of 1.33–1.42 g/cm³ (5). Kuntz (6) also found the density within carboxypeptidase to be inhomogeneous. In 17 predominately hydrophobic regions (10–120 atoms), the calculated average density was 0.93 g/cm³; the density of the

rest of the protein would need to be 1.55 g/cm³ to equal the observed overall density of 1.39 g/cm³.

To further evaluate regional density and intramolecular packing within the interior of protein molecules, we focused upon submolecular areas of β -structure in five globular proteins. Cylindrical regions were centered at the peptide bonds linking β -pleated sheet residues in the interior of each protein. Packing density was determined for the spaces above and below the peptide planes—regions approximately orthogonal to the curved surface of the β -sheet itself. These areas allow analysis of packing in regions primarily determined by interaction of nonbonded atom groups.

MATERIALS AND METHODS

Protein Coordinates. The coordinates of all nonhydrogen atoms of the following five globular proteins at 2.0-Å resolution were used in this study: ribonuclease S (7), hen egg white lysozyme (8), α -chymotrypsin (9), subtilisin BPN' (10), and carboxypeptidase A (11). Each of the coordinate sets used in this study had been subjected to refinement by Diamond's model building computer program (12). In addition, the coordinates of chymotrypsin and lysozyme underwent a real space refinement (13) and Levitt's and Lifson's energy minimization program (14). While the Diamond program constrained the peptide group to a planar conformation, the refinements of ribonuclease S and lysozyme included nonrigid constraints on bond angles and lengths that allowed deviation from planarity of about 10°. The final coordinates used were estimated to be accurate within 0.2 Å for the internal atoms of these proteins.

Choice of Internal Pleated Sheet Residues. The β -pleated sheet peptides situated in the interior of the molecules were identified by measurement of Watson–Kendrew models for chymotrypsin and ribonuclease (ribonuclease model built in the laboratory of F. M. Richards) or by interactive computer graphics for lysozyme, subtilisin, and carboxypeptidase (graphics programs written by S. Harbison and R. Langridge). Peptides closer than 8.5 Å to the van der Waals surface of the molecule were excluded. One hundred twelve internal β -residues met these criteria in the five proteins.‡

Location of Atoms Near the Peptide Group. The midpoints of the peptide C—N bonds were chosen as the centers of cylindrical submolecular regions defined at the chosen amide groups. All atoms located within 10.5 Å of a chosen peptide center were identified. The position of each atom within this range was described in relation to the peptide group trans-

‡Amide planes identified as occurring in internal β -pleated sheets include: subtilisin BPN' ($n = 20$; amides with the carbonyl group of residues 27–31, 89–94, 121–124, and 147–151), carboxypeptidase A ($n = 31$; residues 47–52, 61–65, 103–110, 190–196, and 265–269), α -chymotrypsin ($n = 38$; residues 30–33, 42–45, 51–54, 102–106, 138–140, 154–156, 181, 197–200, 208–212, and 225–229), lysozyme ($n = 8$; residues 50–54, 56, 57, and 60), and ribonuclease S ($n = 15$; residues 45–48, 71–74, 82–84, and 106–109).

FIG. 1. Submolecular cylindrical regions centered about amide planes. Zones designate the cylinder or cylindrical ring 0–1, 1–2, . . . , 5–6 Å from the z axis. Regions designate division into smaller volumes 0–2, 2–4, and 4–6 Å above or below the amide plane.

posed to the right-handed Cartesian coordinate system shown in Fig. 1.

Densities in Cylindrical Submolecular Regions. The density in the space including and immediately surrounding each internal peptide group was computed. This calculation was based on an assigned shape, occupied volume, and atomic mass for each constituent atom or group of atoms. The occupied volume of each atom within a protein molecule (van der Waals volume) was assigned based upon a hard sphere atomic model, with the submolecular volume of a region defined by the intersection of spheres each of radius equal to a van der Waals radius.[§] Hydrogen atoms were considered to form part of the sphere of the atom to which they are bound. The van der Waals radii used in this study were those employed by Bondi (15) and by Richards (4). The atomic mass of each atom or group of atoms (including hydrogen bound to carbon, oxygen, nitrogen, or sulfur) was considered to be uniformly distributed over the volume of a sphere of a radius equal to the van der Waals radius for each atom or group of atoms.

A series of cylinders and cylindrical rings were defined centered at the midpoint of the peptide C—N bond and having as an axis a line perpendicular to the peptide plane passing through this point—the z axis in the standard orientation (see Fig. 1). The series consisted of the cylinder with a radius of 1 Å (zone 1) and the cylindrical rings with radii of from 1 to 2 Å (zone 2), from 2 to 3 Å (zone 3), . . . from 5 to 6 Å (zone 6). (Only zones 1, 2, and 3 are shown in Fig. 1.) Each zone was divided into six regions by passing planes perpendicular to the z axis at $z = 6, 4, 2, 0, -2, -4,$ and -6 Å. The regions are designated by the code Z_i, m to n , where i is the zone and m and n are the z coordinates of the planes at the top and bottom

of the region. Thus $Z5, 2$ to 4 , refers to the region in zone 5 bound by planes intersecting the z axis at $+2$ and $+4$ Å. On occasion, mirror image regions above and below the plane were combined. Such combined regions were denoted by $Z_i, \pm m$ to n .

The packing density was calculated for each of the cylindrical zones. First, all atom spheres intersecting the cylinder were identified. The fraction of the volume of each such atom situated within the cylinder was calculated by iterative numerical integration of the volume of intersection of the atom sphere and the cylinder (0.01-Å intervals). The same fraction of the mass of the atom or group was assigned to the volume within the cylinder. The masses of all atoms or fractions of atoms within each cylinder were added. The ratio of this sum (expressed as g/cm³) was designated as the “density” in that particular submolecular region. Such densities were computed for the six zones, each with six different heights (36 regions) about each of the chosen 112 internal β -structure peptides. The density calculated, although expressed in g/cm³ to facilitate comparison with the experimental and calculated density of the molecule as a whole, is actually an approximate measure of electron density or packing. This results from the fact that for the major elements involved (C, O, N, and S, but not H),

$$\text{number of electrons} = \frac{\text{atomic mass}}{2}.$$

The fact that this is not true for hydrogen introduces only a small error.

RESULTS

Average Density near β -Peptides in Each Protein. The average values of density in the 36 cylindrical regions surrounding internal β -peptide groups are listed in Tables 1–3 for the three largest individual proteins. The highest average densities were in the regions including the five atoms of the peptide group— $Z1, \pm 0$ to 2 (densities of 2.17–2.28 g/cm³), and the lowest average densities were at $Z1, \pm 2$ to 4 (0.56–0.62 g/cm³). These extremes result from choosing the amide plane that includes five atom centers as the center of the regions of interest. The arbitrary selection of 2-Å region heights placed the neighboring region in an area relatively devoid of electron density. Nevertheless, the very low densities in this region, $Z1, \pm 2$ to 4, indicate inefficient packing directly above and below the peptide group. This results from the difficulty in interdigitating other groups between the nearby side chains on the β -pleated sheet. The submolecular region of this study that most accurately reflects packing against the surface of the β -sheet is $Z1, \pm 4$ to 6, since the regions ± 0 to 2 Å and ± 2 to 4 Å from the peptide plane are influenced by the presence of the peptide group itself.

Zone 6, 4 to 6 Å above and below the peptide plane, has an average density similar to the average density in the interior of

Table 1. Average density near 20 internal β -peptides in subtilisin BPN'

Region height, Å from peptide plane	Average density, g/cm ³					
	1 Å	2 Å	3 Å	4 Å	5 Å	6 Å
4 to 6	0.95	1.04	1.08	1.06	1.02	0.99
2 to 4	0.58	1.18	1.17	1.19	1.14	1.24
0 to 2	2.26	1.92	1.55	1.39	1.30	1.35
-2 to 0	2.22	1.90	1.47	1.38	1.31	1.30
-4 to -2	0.62	0.84	1.18	1.25	1.22	1.26
-6 to -4	0.93	0.97	1.04	1.06	1.08	1.08
Zone average	1.26	1.31	1.25	1.22	1.18	1.20

$\rho_{\text{int}} = 1.15$ g/cm³ is from ref. 5. The average density for zones 1–6, indicated as the outer radius in Å, is shown.

[§]Van der Waals radii used in this study are as follows: main-chain C or NH, 1.7 Å; main-chain O, 1.4 Å; main-chain C, 2.0 Å; aliphatic CH, CH₂, or CH₃, 2.0 Å; C of COOH, CONH₂, or ring atom of His, Phe, Tyr, or Trp, 1.7 Å; OH of Ser, Thr, or Tyr, 1.6 Å; O of COOH, 1.5 Å; O or N of CONH₂, 1.6 Å; NH₂ of Lys, 2.0 Å; S or SH, 1.8 Å; solvent H₂O, 1.4 Å.

Table 2. Average density near 38 internal β -peptides in α -chymotrypsin

Region height, $\text{\AA}$ from peptide plane	Average density, g/cm^3					
	1 $\text{\AA}$	2 $\text{\AA}$	3 $\text{\AA}$	4 $\text{\AA}$	5 $\text{\AA}$	6 $\text{\AA}$
4 to 6	1.12	1.14	1.03	1.04	1.09	1.08
2 to 4	0.56	0.84	1.09	1.20	1.27	1.29
0 to 2	2.24	1.92	1.53	1.43	1.39	1.43
-2 to 0	2.17	1.80	1.39	1.33	1.34	1.39
-4 to -2	0.62	0.86	1.12	1.27	1.25	1.22
-6 to -4	1.06	1.11	1.08	1.10	1.12	1.10
Zone average	1.30	1.28	1.21	1.23	1.24	1.25

ρ int = 1.15 g/cm^3 is from ref. 5. The average density for zones 1–6, indicated as the outer radius in $\text{\AA}$, is shown.

globular proteins. Overall average internal densities for these proteins have been calculated by the method of expanding spheres (5); these values are included in Tables 1–3 as ρ int. The average densities of regions, Z6, ± 4 to 6, are less than ρ int by only 0.08 g/cm^3 (weighted average deviation). The similarity provides reassurance that the selection process used here has chosen internal peptide groups and excluded surface regions as intended and indicates that the region above and below the peptide plane has an average density similar to that of the interior of globular proteins. There was no significant difference between the average density of Z1, ± 4 to 6, and Z1, -4 to -6 , indicating that the peptide group is not consistently asymmetric with respect to packing in the direction orthogonal to the peptide plane.

Densities in Zone 1, 4–6 $\text{\AA}$ Above and Below the Individual Peptide Planes. Density values in each of the 36 submolecular regions about each chosen internal β -structure peptide revealed marked variation in density. In a given submolecular region, densities in the region Z1, ± 4 to 6, ranged from 0.12 to 3.08 g/cm^3 . The variation existed, but was less marked, in zone 6 regions (e.g., 0.64–1.78 g/cm^3) for which the density was determined in a larger volume (69.1 $\text{\AA}^3$) than zone 1 (6.3 $\text{\AA}^3$).

To simplify comparison of the densities near individual peptides, the calculated packing densities of Z1, ± 4 to 6, were categorized as either low (<1.0 g/cm^3), medium (1.0–1.4 g/cm^3), or high (>1.4 g/cm^3). The midpoint of these categories was chosen to be 1.20 g/cm^3 , the average between the mean values for the density Z6, -6 to $+6$ (1.24 g/cm^3), and ρ int (1.16 g/cm^3). The range of values for the medium density classification (1.0–1.4 g/cm^3) includes all calculated average internal densities (1.05–1.23 g/cm^3) and most experimentally observed densities in these globular proteins (1.33–1.42 g/cm^3) (16). Therefore, any low and high density values represent submolecular regions with packing significantly different from the molecule as a whole.

One possible explanation for the wide variation of Z1, ± 4 to 6, density values is that too small a volume was sampled resulting in spuriously high values occurring next to spuriously low densities. However, we found that it was more likely for a

Table 3. Average density near 31 internal β -peptides in carboxypeptidase A

Region height, $\text{\AA}$ from peptide plane	Average density, g/cm^3					
	1 $\text{\AA}$	2 $\text{\AA}$	3 $\text{\AA}$	4 $\text{\AA}$	5 $\text{\AA}$	6 $\text{\AA}$
4 to 6	1.07	1.16	1.14	1.09	1.04	1.00
2 to 4	0.59	0.85	1.13	1.20	1.23	1.33
0 to 2	2.28	1.93	1.55	1.44	1.34	1.35
-2 to 0	2.18	1.89	1.47	1.38	1.33	1.32
-4 to -2	0.61	0.80	1.11	1.24	1.24	1.24
-6 to -4	1.17	1.11	0.95	0.99	1.11	1.17
Zone average	1.32	1.29	1.22	1.22	1.22	1.24

ρ int = 1.21 g/cm^3 is from ref. 5. The average density for zones 1–6, indicated as the outer radius in $\text{\AA}$, is shown.

high (or low) density of Z1, 4 to 6, to be accompanied by similarly high (or low, respectively) density of Z2, 4 to 6 (70% of cases), than to be different (30% of cases). Furthermore, it was not uncommon to find a consecutive sequence of two to four peptide planes about which the density in Z1, 4 to 6, was uniformly high or low (e.g., peptides 91–94 in subtilisin and 105–107 in carboxypeptidase). We conclude, therefore, that the observed high- and low-density regions represent an actual variation in packing density in regions with volumes on the order of 10–20 $\text{\AA}^3$ in the protein molecule. The packing of a region of this volume would be contributed to by several atoms. Richards (4), studying packing fractions in ribonuclease and lysozyme, reported a similar variation in packing throughout the molecule. We did not determine whether any of our low-density regions contained holes large enough to accommodate a solvent molecule, although any internal solvent molecules observed by x-ray crystallography were included in our calculations.

Analysis of Individual Proteins. Subtilisin BPN'. The β -residues studied in subtilisin BPN' are all part of one twisted sheet with five parallel chains. The high-, medium-, and low-density values of Z1, ± 4 to 6, are shown in Fig. 2. A low-density region in the center of the twisted β -sheet is seen. In this protein, α -helices pack on both sides of the β -sheet beyond the central low-density regions. High-density peptides occur in two groups—one at each end of the β sequences. At one end, near the molecule's surface and at residues 27, 89, 121, and 147, for which the average density value in Z1, 4 to 6, is 1.52 g/cm^3 . This value approaches that which we calculated (5) (1.58 g/cm^3) for the exterior shell density required to reconcile the experimentally observed density with ρ int. To our knowledge, this is the first evidence indicating that inhomogeneous intramolecular packing may be an important factor in accounting for unusually low calculated internal density (ρ int) in globular proteins and that denser packing occurs near the surface of the protein as has been proposed (5).

At the other end of the β sheet are the high-density discs of peptides 31, 124, and 151, situated immediately internal to the active site. X-ray diffraction difference studies have reported that motion of the catalytic residues (Asp-32, His-64, and Ser-221) (17) and the substrate binding region (residues 125–127) (18) accompany the binding of inhibitors and substrate analogs. The low-density sides of residues 31(–) and 151(+) are directed toward these "breathing sites."

Carboxypeptidase A. Of the proteins included in this study, carboxypeptidase has the most extensive β -pleated sheet—eight chains with four parallel and three antiparallel contacts. The large twisted sheet is situated in the central core of the molecule. The spatial arrangement of the high- and low-density regions in relation to the molecular interior and to the active site is illustrated in Fig. 3. When the Z1, ± 4 to 6, densities were viewed in three dimensions, it was clear that packing patterns were different for the two sides of the β -sheet. Fig. 3A illustrates the more interior or concave aspect of the sheet. A large low-density area in the center of the sheet is surrounded by higher density. A linear group of peptides at one end (away from the active site) of the β -sheet (peptides 52, 61, 104, 190, and 265) have an average density of Z1, ± 4 to 6, of 1.36 g/cm^3 . This value approaches, but does not reach, the outer shell density of 1.47 g/cm^3 , which we have calculated (4) to account for the low value of ρ int (1.21 g/cm^3) in carboxypeptidase. Fig. 3B reflects the packing pattern over the convex surface of the β -sheet. Helices A, B, F, and H pack roughly around the periphery of this side of the β -sheet that is directed toward the active site region. More dense packing was found on this side of the sheet. One peptide near the catalytic site that was included in the calculation, peptide 269, however, has a low density in Z1, -4 to -6 . The side chain of Glu-270 near this amide group has been shown in electron difference studies to undergo a motion of ≈ 2 $\text{\AA}$ on binding of

FIG. 2. (A) Ribbon diagram of subtilisin BPN' indicating high (red)-, medium (green)-, and low (yellow)-density regions above and below the amide planes in internal β -pleated sheets. The active site is in the lower center of the figure. (B) View perpendicular to A with the protein rotated 90°. The active site is in the lower center of the figure.

substrate (11). One might speculate that the adjacent low-density facilitates this motion.

α -Chymotrypsin. The β -pleated sheet structure of α -chymotrypsin forms two distorted cylinders (9). Densities of the Z1, ± 4 to 6, submolecular regions are shown Fig. 4. The center of the cylinders are low-density regions that contain a core of hydrophobic side chains (9). On the outside of the cylinders, the region of cylinder 1 to cylinder 2 contact is in a more densely packed environment. Five water molecules are buried in this intercylinder region, three of which contribute density to our submolecular regions. The particularly high-density regions near peptides 225–229 occur internal to the active site region. High-density packing internal to the active site residues was observed in all proteins included in this study.

Lysozyme and ribonuclease. Similar findings were observed upon analysis of the two smaller proteins (lysozyme and ribonuclease) included in this analysis. The general patterns of a central core of low density, low density near the active site groove, and subsurface higher density were also noted in these

FIG. 3. (A) Ribbon diagram of carboxypeptidase A indicating high (red)-, medium (green)-, and low (yellow)-density regions above and below the amide planes on the concave side of the internal β -pleated sheet. The active site is behind this surface of the sheet. (B) View of the opposite surface of the β -sheet of that shown in A. The active site is on the right of this side of the β -sheet.

proteins, although the trends were less obvious since fewer residues met the criteria for inclusion in the calculations.

DISCUSSION

We have developed a new method for calculating local packing density in globular proteins. The approach taken differs from previous methods (3, 4, 19, 20) because (i) it does not require assumptions regarding the structure of a solvent shell at the surface of the molecule, (ii) it is less dependent on the correct assignment of van der Waals radii, and (iii) it allows us to direct our analysis to regions of the molecule where packing interactions are likely to predominate over bonded interactions.

A major conclusion from the results of our calculations is that the density near β -pleated sheet peptides is found to be inhomogeneous when submolecular regions with volumes on the order of 6–69 Å³ are compared. Kuntz (6) and Richards (4) have also reported density variations within globular proteins. Chothia (19) and Finney (20) have calculated the

FIG. 4. Ribbon diagram of α -chymotrypsin indicating high (red)-, medium (green)-, and low (yellow)-density regions above and below the amide planes in internal β -pleated sheets. The active site is on the right.

volume occupied by various groups of atoms in proteins and concluded that the interior of a protein is densely packed with little variation in the volumes occupied by a chosen group throughout the molecule. The standard deviation of the volume occupied by a given amino acid residue was 8% of the mean value. However, amino acid residues represent a comparatively large volume (66.4 Å³ for Gly to 237.6 Å³ for Trp) compared to our Z1, 4 to 6, regions (6.3 Å³), and short range forces generally govern the configuration of side chains in proteins (21). The general shape of the amino acid itself would be relatively constant, while only that portion of the occupied volume representing packing interactions would vary. Thus, Chothia's results (19) reflect not only long range packing but also include standard bonded and short range forces.

Finney (20) reported that the occupied volumes for atom groups (one heavy atom with its attached hydrogens) have standard deviations of 10–15% of their mean values. These deviations are lower than variations of 20–25% reported by Richards (4), resulting from a different description of the surface of the molecule. Our regions do not include the surface. Atom groups (20–50 Å³), described by Finney (20), were also larger than our Z1, ± 4 to 6, regions. However, the variation he reported (10–15%) was even less than we found for our 69.1 Å³ Z6, ± 4 to 6, regions (standard deviation = 23% of the mean). A portion of the occupied volume of his chosen regions was constant, resulting from the bonded interactions of the group. In fact, groups with two bonds to the rest of the protein (e.g., main chain C, N, -CH, or -CH₂) tend to have a lower standard deviation (<15% of the mean) than those groups with only one bond (CH₃, NH₃⁺; 20%). Our regions 4–6 Å above and below the peptide plane do not include any specific bonded groups of atoms.

With regard to the overall folding of the molecule, we found some more densely packed regions near the surface of the molecule. This laboratory has reported (5) an unusually low average density in the interior of globular proteins. We suggested a densely packed subsurface shell of the molecule as one possible explanation for the difference between the internal density, ρ_{int} , and the experimentally observed protein density, ρ_{expt} . We report here the first indication that such a dense shell does exist in some globular proteins. This observation does not rule out contributions from other possible explana-

tions for the difference between ρ_{int} and ρ_{expt} : that the protein is surrounded by a jacket of extremely dense water or that numerous unidentified water molecules exist in the interior of the protein. Electrostriction from charged surface groups contributes to the discrepancy in density values to some extent but could not account for more than 10–20% of the difference (5). Our results indicate that increased density toward the exterior may be responsible for a portion of the difference. Richards (4) found that the surfaces of lysozyme and ribonuclease had low packing fractions. However, our dense shell does not extend to the outer edge of the molecule, since all our chosen regions are internal. This may account for the apparent discrepancy between Richards' results (4) and our results.

We also documented the trend reported by Richards (4) that the active site groove is a loosely packed region, consistent with motion of local groups in facilitating substrate binding. We also found densely packed regions immediately internal to the catalytic residues. Efficient packing at this location may ensure proper arrangement of the side chains involved in catalysis.

High- and low-density regions occur in patterns with respect to the β -pleated sheet structure of the molecule. High-density regions were found toward the ends of β -structure sequences. At these locations, the regular backbone-to-backbone hydrogen bonding pattern of the sheet is interrupted. Although all potentially available main chain groups participate in hydrogen bonds, those near the surface are likely to involve side chains or solvent molecules. These pairs are less constrained geometrically than are the main chain hydrogen bonding groups at the center of a β -sheet. Low-density areas tend to occur in the central regions of the large β -sheets where interstrand hydrogen bonding is maximized. In these regions, improved van der Waals packing interactions might strain or break hydrogen bonds; hence, the smaller energy stabilization from potential van der Waals interactions is apparently sacrificed. In some cases, a well packed structure would not have to compete with formation of good hydrogen bonds, but maintaining an intact network of hydrogen bonds may limit opportunities for close packing within the β -structure.

We have found that an isolated β -pleated sheet inside typical protein molecules is an inhomogeneously packed structure. Effective van der Waals stabilization generally involves interactions with groups distant in the polypeptide sequence. Conformational energy calculations on the regular β -sheet structure of poly(L-alanine) (22) indicated that intersheet van der Waals interactions contribute approximately one-third of the total stabilization energy. Packing of the irregular sheets in globular proteins is less efficient and the potential van der Waals stabilization is not as great. Recent studies by Bromberg and Dill (23) suggest that protein side-chain packing is analogous to the imperfect packing of nuts and bolts in a jar. We found regions with dense packing in subsurface locations where regular patterns of secondary structure were interrupted. In the interior of proteins, van der Waals interactions and packing density were maximized whenever possible, but the regular pattern of hydrogen bonding that stabilizes β -structure introduces constraints on optimum van der Waals interactions.

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