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Relative orientation of close-packed β-pleated sheets in proteins

(twisted β-sheets/protein secondary and tertiary structure)

CYRUS CHOTHIA*† AND JOËL JANIN‡

*William Ramsay, Ralph Foster and Christopher Ingold Laboratories, University College London, Gower Street, London WC1E 6BT, England; †Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, England; and ‡Unité de Biochimie Cellulaire, Département de Biochimie et Génétique Moleculaire, Institut Pasteur, 28 rue du Docteur Roux, 75724 Paris Cedex 15, France

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ABSTRACT **When β-pleated sheets pack face to face in proteins, the angle between the strand directions of the two β-sheets is observed to be near −30°. We propose a simple model for β-sheet-to-β-sheet packing in concanavalin A, plastocyanin, γ-crystallin, superoxide dismutase, prealbumin, and the immunoglobin fragment V_{REI}. This model shows how the observed relative orientation of two packed β-sheets is a consequence of (*i*) the rows of side chains at the interface being approximately aligned and (*ii*) the β-sheet having a right-handed twist. The special amino acid composition of residues at the β-sheet-to-β-sheet interfaces makes the contact surfaces essentially smooth and hydrophobic.**

The three-dimensional structure of most globular proteins can be described as an assembly of close-packed α-helices and β-pleated sheets. It is therefore important to understand the rules that govern the packing of secondary structure (1–3). We have previously analyzed the packing of α-helices on α-helices (4) and on β-sheets (5). In this paper, we concentrate on β-sheet-to-β-sheet packing in a family of proteins that are formed by two β-pleated sheets packed face to face.

Concanavalin A (6), plastocyanin (7), γ-crystallin (8), superoxide dismutase (9), prealbumin (10), and the immunoglobulin domains (11) are representative members of this family, which also contains the coat proteins of tomato bushy stunt virus (12) and of southern bean mosaic virus (13), azurin (14), and other proteins. All these proteins have two β-pleated sheets packed face to face and oriented so that the direction of the strands in one sheet is at an angle of about −30° to that in the other sheet, the minus sign indicating a left-handed screw rotation (Fig. 1). We show here that this characteristic orientation is a natural consequence of the right-handed twist of normal β-pleated sheets and allows the close packing of side chains between the two β-sheets in contact. In other proteins formed by β-pleated sheets, such as trypsin or staphylococcal nuclease, the β-pleated sheets are packed with the strand directions making an angle of about 90° (1). The principles applying to these structures are different and will be discussed elsewhere.

A model for packing twisted β-pleated sheets

The β-pleated sheets observed in protein structures are not flat but twisted in a right-handed direction (16). In this section we describe the effects of introducing this twist into an idealized model of two β-sheets packed face to face (Fig. 2). This model will be useful in understanding the structural descriptions presented in later sections.

Fig. 2*a* illustrates one effect of the right-handed twist on a single polypeptide chain. On twisting of the main chain, the side chains that point up curve in the opposite direction to those that point down. As one looks down the chain, those that point up curve from left to right, while those that point down curve from right to left.

Fig. 2 *b* and *c* illustrates some of the effects of twisting two β-pleated sheets that are packed together. We show, on the left of the figures, two hypothetical untwisted β-sheets packed with their rows of side chains aligned: those in the top β-sheet are parallel to those in the bottom. On the right of the figures we show what happens when these packed β-sheets are twisted about an axis that is in the plane of their interface. We see that for the twisted close-packed β-sheets: (*i*) The ends of the *side chains* that form contacts across the interface maintain their alignment. (*ii*) The *main chains* of the two sheets now point in different directions.

As one looks down the twist axis, the main chains of the top β-sheet go from left to right, while those of the bottom go from right to left (Fig. 2*b*). As one looks from the top (Fig. 2*c*), the main chains make an angle, which is negative because it represents a left-handed screw rotation. This angle, Ω, would be −35° if the top and bottom main chains are 10 Å apart and the twist along the chains is 4°/Å.

This angle of −35° would be increased or decreased by the rows of residues being not exactly aligned, by there being a different amount of twist, or both. If the side chains are not exactly aligned, and if the direction of the twist axis is closer to the direction of the strands in one β-sheet than to that in the other, the extent of the twist in the two β-sheets will differ somewhat.

Viewed from the end, the twisted β-pleated sheets *in projection* take a cylindrical shape that Fig. 2*b* shows clearly. Note that the "barrel effect" (17) is observed even in the absence of a continuous network of hydrogen bonds joining the two β-sheets into a single one. In our model, top and bottom strands have their side chains pointing to each other and their main chains cannot hydrogen bond. The actual shape of the structure is not a "barrel" or a cylinder, but a twisted prism with a rectangular cross-section that rotates around the twist axis.

The model described here supersedes a previous model of Chothia *et al.* (1). In the remainder of the paper, we shall discuss its relevance to real protein structures.

β-Sheet-to-β-sheet packing in six proteins

We used computer graphics to examine the detailed atomic structures of the six proteins or protein subunits illustrated in Fig. 1: concanavalin A, plastocyanin, γ-crystallin domain I, superoxide dismutase, prealbumin, and immunoglobulin fragment V_{REI}. For each protein, we determined the twist and the relative orientation of the β-sheets and we analyzed the packing of amino acid residues at the β-sheet to β-sheet interfaces.

β-Sheet Twist. The twist is conveniently expressed by the dihedral angle between pairs of adjacent strands. This angle is negative for all but three of 44 pairs of strands in the twelve β-sheets, indicating a normal right-handed twist of the strands,

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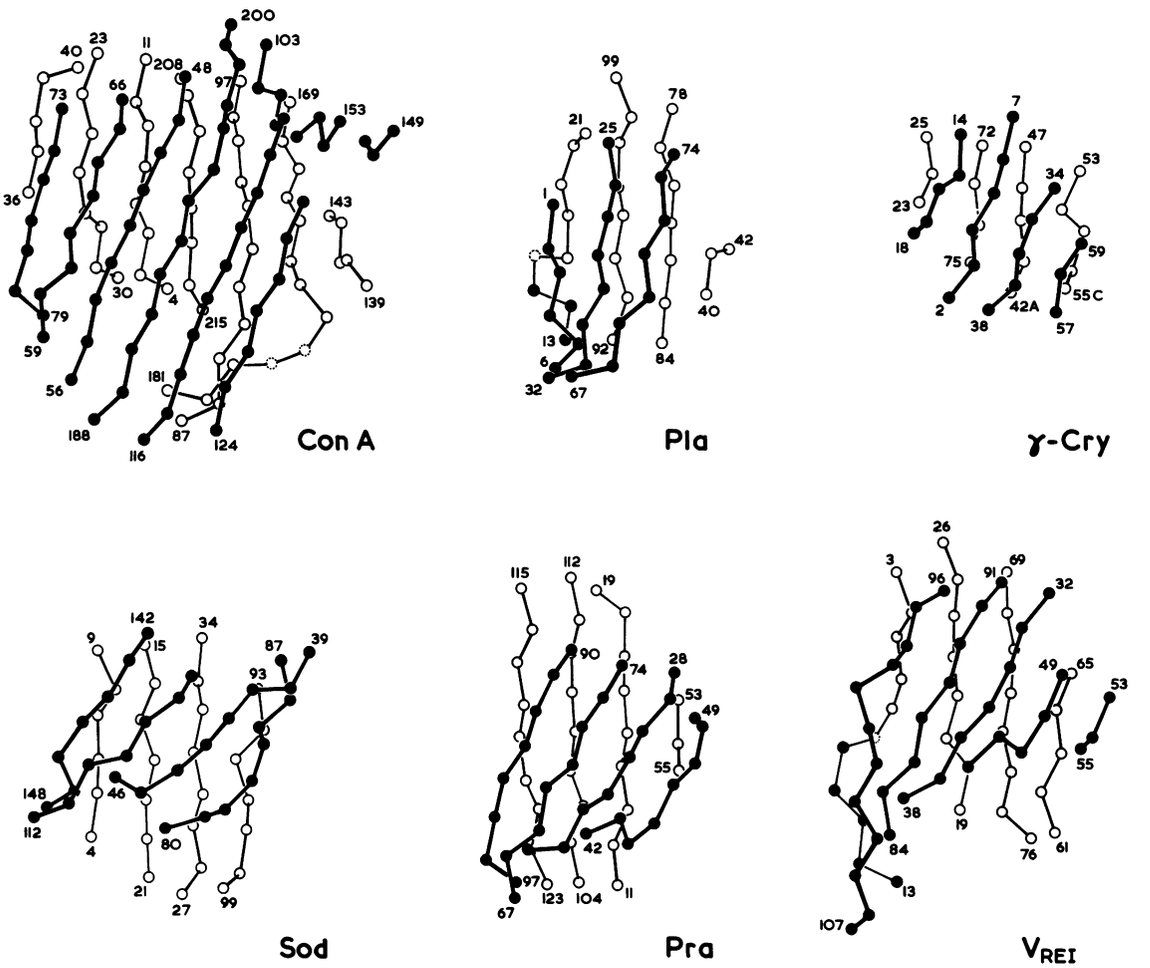

FIG. 1. The β-pleated sheets in concanavalin A (Con A), plastocyanin (Pla), γ-crystallin (γ-Cry), superoxide dismutase (Sod), prealbumin (Pra), and the immunoglobulin fragment V_{REI}. ●, Cα atoms in the "top" β-sheet; ○, Cα atoms in the "bottom" β-sheet. Atomic coordinates are from the Cambridge Protein Data Bank (15), except for plastocyanin and γ-crystallin, gifts of J. M. Guss, H. C. Freeman, and T. Blundell (7, 8).

which leads to a left-handed twist of the β-sheets viewed from the edge. The mean of the distribution (Fig. 3) is −17° and the standard deviation is 9°. The distribution is not significantly different from that found in α/β proteins, in which the mean dihedral angle between adjacent strands is −19° with a standard deviation of 7° (4).

The dihedral angle between the right-most and left-most strands of each β-sheet is a measure of its total twist. It varies between −30° over six strands in the top β-sheet of concanavalin A and −65° over four strands for the top β-sheet of prealbumin (Table 1). Thus, despite the rather narrow distribution in Fig. 3, the β-sheets can differ widely in their total twist.

Relative Orientation of the β-Sheets. Relative orientation is fixed by the Ω angle of Fig. 2*c*. The values found for the six proteins are listed in Table 1. The values are in the range −30° ± 15°.

Residue Packing at the β-Sheet-to-β-Sheet Interface. Packing of atoms inside a protein structure may be studied by drawing sections through a space-filling model of the protein, generated by a computer from the atomic coordinates. We show such sections in Fig. 4, for the immunoglobulin fragment V_{REI} and for the prealbumin subunit. The sections are cut perpendicular to the mean direction of the strands, so that the viewpoint is the same as in Fig. 2*b*, which makes comparison with the model easier. The sections in Fig. 4*a* are made through the front part of the interface, the sections in Fig. 4*b*, through the middle, and the sections in Fig. 4*c*, through the back. A complete representation of the structures would involve stacking a number of such sections on top of each other.

We see in Fig. 4 that: (*i*) The description of the prealbumin and V_{REI} structures as made of two β-pleated sheets packed face to face is essentially correct. Residues not belonging to the β-sheets are marked in dotted lines. They make little contribution to the internal packing. (*ii*) The β-sheet-to-β-sheet interface is made up of side chains pointing directly to each other. In each section, a line may be drawn that separates one β-sheet from the other. Side chains from either side hardly cross this line. Thus, intercalation of side chain from top and bottom is the exception rather than the rule in these interfaces. In the six sections shown, it occurs once with Trp-35 in V_{REI} (Fig. 4*b*), which fits its side chain into a hole in the bottom β-sheet. Yet, the interface is not a plane. Due to the twist, the dividing line between the β-sheets rotates clockwise from the front to middle to back sections. (*iii*) The rows of side chains are approximately aligned even though the main chains are not. Thus, we can follow the contacts between residues 12, 14, and 16 from strand 11–19 in the prealbumin bottom β-sheet with residues 69, 71, and 73 from strand 67–74 of the top β-sheet. Fig. 1 shows that the main chains of these two strands are at an angle of −30° or so. In V_{REI} residues 75, 73, and 71, from the bottom β-sheet strand 69–76, are in contact with residues 86, 35, and 33 from the two adjacent strands, 84–91 and 32–38, in the top β-sheet and the angle between the main chains in the top and bottom

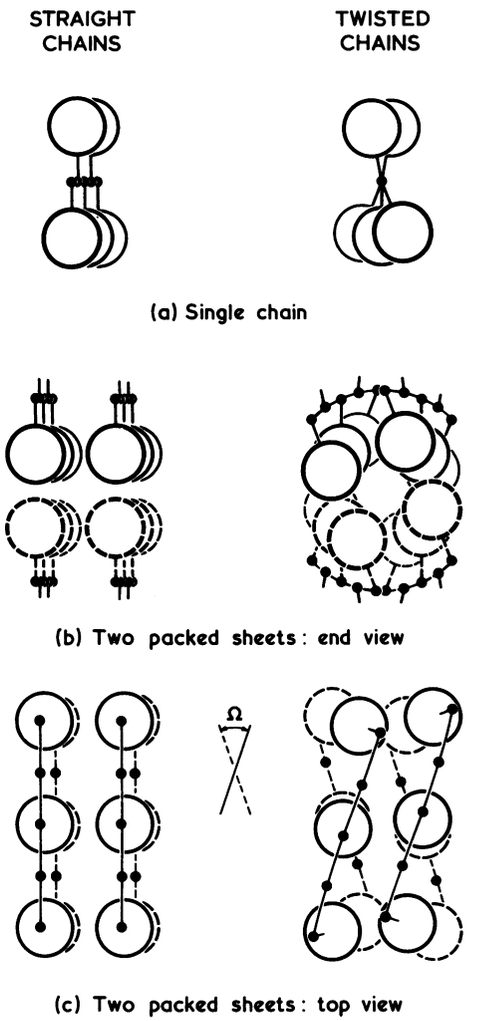

FIG. 2. A model for the packing of β-pleated sheets. The main chain is represented by one small filled circle per residue, the side chains being large open circles. In *a* we show a single strand, untwisted or twisted in the normal right-handed direction. In *b* and *c* we show views of packed β-sheets with two strands each. Only the side chains pointing into the interface are drawn.

β-sheets is again −30°. The contact between the side chains is maintained over more than 15 Å through the twist of the β-sheets, in accordance with the model described in Fig. 2.

Sections cut through concanavalin A, γ-crystallin, plastocyanin, and superoxide dismutase are similar in their general appearance to those shown here for prealbumin and the V_{REI} fragment. The dihedral angle made by the main chain of pairs of strands in contact across the interface varies from −10° to −55° around a mean value of −33°. The variation results in part from the variable twist of the strands, and in part from an imperfect alignment of the rows of side chains. The largest misalignment occurs in plastocyanin and is responsible for its small Ω value.

Packing at the Edges of the β-Sheets. All but 4 of the 24 strands that form the edges of the 12 β-sheets pack in a normal way, that is, like internal strands, even though their main chain is hydrogen bonded on one side instead of two. The four exceptions are of two types: (*i*) Residues 13–21 in plastocyanin and residues 3–13 in V_{REI} form two sections of β-strand each, joined by a non-β-strand residue. The first section is part of the top β-sheet, the second, part of the bottom β-sheet (see Fig. 1). Though strand 13–21 in plastocyanin, or 3–13 in V_{REI}, cannot pack as a whole in accordance with the model, each of the two sections does. This can be seen in Fig. 4 for V_{REI}. (*ii*) In the

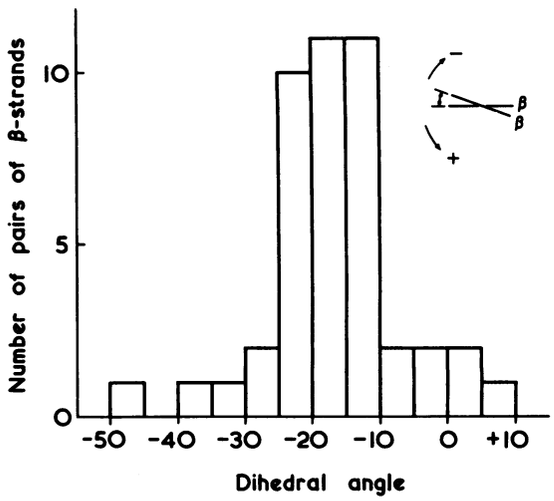

FIG. 3. Histogram of the twist angle (in degrees) between adjacent strands. The dihedral angle between adjacent strands in a β-sheet expresses the twist of the β-sheet. Ten values out of 44 are outside the range −25° to −5°. They all involve strands at the edge of their respective β-sheets.

superoxide dismutase subunit, the edge strands 4–9 and 142–148 hydrogen bond to each other: Val-5 to Gly-148 and Lys-9 to Cys-144. Residues 9 and 148 are at the corners of their β-sheets (Fig. 1) and local sharp twists in the β-sheets turn their peptides so they are in a position to hydrogen bond to residues 144 and 5, respectively.

Amino acid composition of the β-pleated sheets and of their contact residues

The β-pleated sheets of concanavalin A, plastocyanin, prealbumin, superoxide dismutase, and the V_{REI} fragment contain a total of 355 residues, of which 119 are involved in the β-sheet-to-β-sheet interfaces through their side chain. The proportion of contact residues, 33%, is less than the 50% expected from a model in which half of the side chains in the β-sheets point into the interface and half away from it. The discrepancy results in part from the β-sheets being of unequal size and not overlapping exactly, and in part from non-β-sheet residues packing between the ends of the β-sheets.

Table 2 gives the amino acid composition of the β-sheets and that of the contact residues in the β-sheet-to-β-sheet interfaces.

Table 1. Twist and relative orientation of the β-pleated sheets

Protein	β-Sheet twist, °		Relative orientation of the β-sheets Ω, °
	Top	Bottom	
Concanavalin A	30 (6)	80 (7)	−30
γ-Crystallin	40 (3)*	45 (3)*	−25
Plastocyanin	40 (3)*	45 (3)*	−20
Prealbumin	65 (4)	45 (4)	−35
Superoxide dismutase	55 (4)	35 (4)	−45
V_{REI}	60 (5)*	40 (4)	−30

The angles were calculated by fitting vectors to the main chain atoms of the strands. Previously we only fitted the Cα atoms (1) and in some cases the different fits give slightly different values for the angles. The top β-sheet is that nearer to the reader in Fig. 1 and the bottom β-sheet the one farther away. The values are given as $\theta°$ (n), in which θ is the angle between n strands. The strands are those at the edges of the β-sheets except in the cases marked *, for which we have excluded strands of three residues that have an unusually large or small twist and would therefore create a false impression of the extent of the overall β-sheet twist.

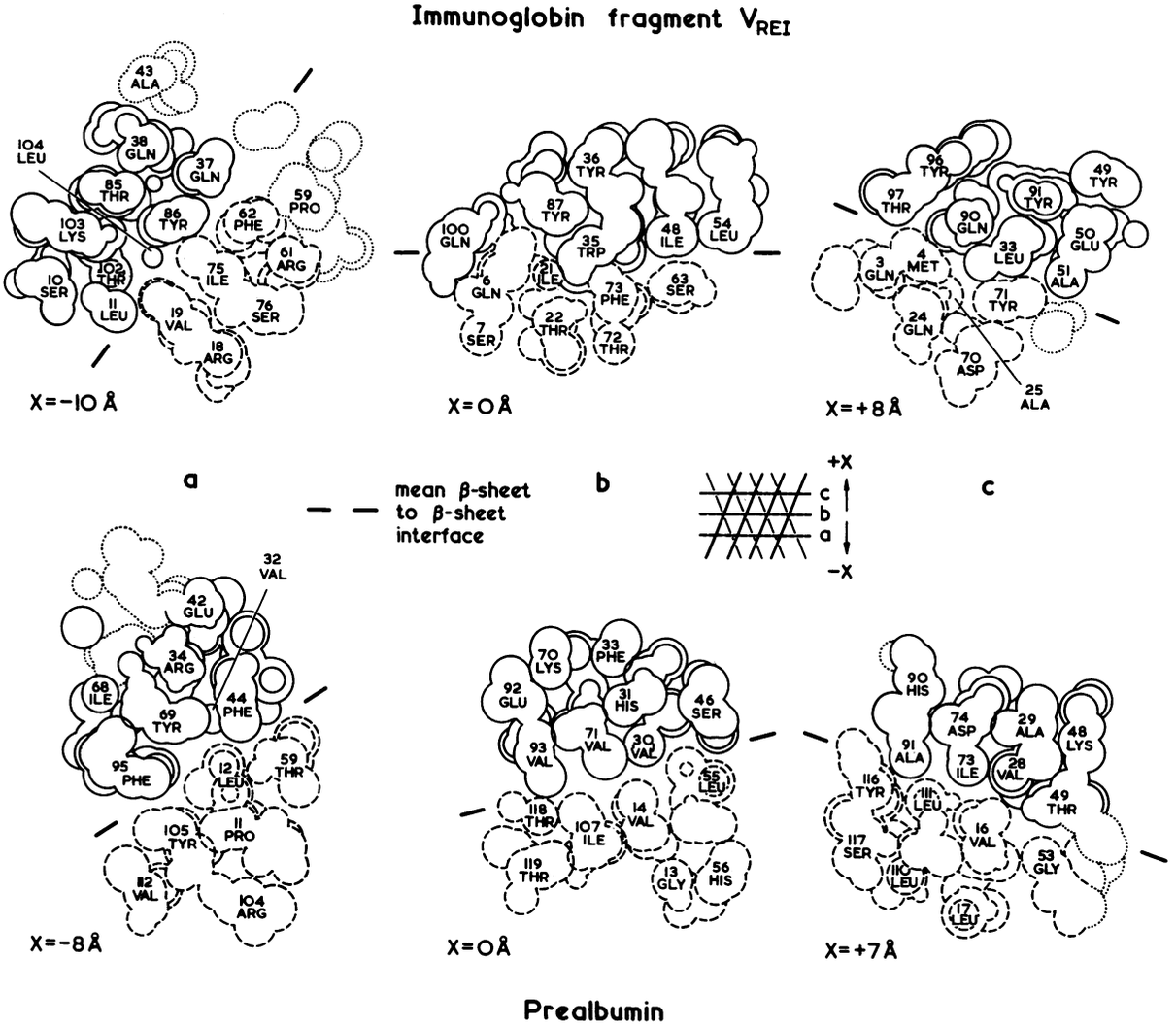

FIG. 4. The β-sheet-to-β-sheet residue packing in prealbumin and V_{REI}. Space-filling models of the proteins were generated in the computer. Sections were cut perpendicular to the mean direction of the strands in the β-sheets—see the small schematic *Inset*. Broken lines represent the edges of the van der Waals envelopes of the residues in one β-sheet and full lines the residues in the other β-sheet. Bar lines mark their mean interface. Dotted lines represent residues not in either β-sheet. Three sections separated by 1 Å are superimposed, so that each picture describes a 2-Å-thick slice. The *x* value gives the relative position of the middle section. The viewpoint of the proteins is the same as that in Fig. 2*b* and the two should be compared. The sections usually cut through two residues from each strand, one pointing into the interface and one away. Strand affiliation of the residues can be seen in Fig. 1.

The composition of the β-sheets in our sample is not significantly different from average, taking the much larger sample of β-sheets of Levitt (18) as a reference. *Yet, the amino acid composition of the contact residues is unusual, with four amino acids, Leu, Val, Ile, and Phe, forming 61% of the total.* This proportion is much greater than in the average β-sheet, where it is 31%, or in the average protein interior, where it is 38% (19). The branched side chains of Val, Ile, and Leu make the surface of the β-sheets approximately smooth (20). The most common configuration of aromatic residues has the side chain lying flat over the main chain. As a consequence, there is no regular intercalation of side chains in the β-sheet-to-β-sheet interfaces, a point that Fig. 4 illustrates. Like α-helix-to-β-sheet interfaces (5), but unlike α-helix-to-α-helix interfaces, in which intercalation of side chains is the rule (4), β-sheet-to-β-sheet interfaces tend to be formed by the assembly of essentially smooth surfaces.

β-Sheet residues forming contact with α-helices in a sample of eight α/β proteins (5) consist of 10% Leu, 24% Val, 14% Ile, and 6% Phe, which makes them similar to the residues involved in the β-sheet-to-β-sheet contacts studied here. Most probably, the large excess of branched hydrophobic residues in α-helix-to-β-sheet and in β-sheet-to-β-sheet contacts reflects the need to produce a well-packed interface. As a result of α-helices packing on both sides of the β-sheets in α/β proteins, 67% of the β-sheet residues are involved in the interfaces, against 33% here. The influence of the contact residues on the amino acid composition of their β-sheets is therefore more evident. Lifson and Sander (20, 21) have noted that parallel and antiparallel β-pleated sheets differ in their amino acid compositions, and especially that the former are enriched in Val and Ile. We do not think that this indicates a preference of these types of residues for the parallel association of strands, but that parallel β-sheets are found almost exclusively in α/β proteins, where the α-helices make the necessary connections between the strands, whereas all β-proteins have mostly antiparallel strands (22, 23).

Conclusion

In this paper we have described how, in the class of protein structures illustrated in Fig. 1, β-pleated sheets pack together. Other publications have been concerned with their strand to-

Table 2. Amino acid composition of the β-sheets and of the β-sheet-to-β-sheet contact residues in five proteins

Residue	Composition, mol %		
	Average β-sheet	β-Sheets in the five proteins	Contact residues in the five proteins
Val	11.9	12	17
Leu	7.0	8	16
Ile	7.8	7	15
Phe	4.6	6	13
Ala	7.6	8	10
Thr	7.7	9	6
Tyr	4.8	5	6
Gly	8.7	7	4
Cys	1.6	1	3
Met	1.5	1	3
Gln	2.5	3	2
Trp	1.7	2	2
Lys	5.4	5	1
Asp	4.3	4	1
Glu	3.8	4	1
Asn	3.3	3	1
Ser	7.6	8	0
Arg	3.0	2	0
His	2.6	4	0
Pro	2.5	1	0
Total	99.9	100	101

The percentage amino acid composition of the 355 β-sheet residues in concanavalin A, plastocyanin, prealbumin, superoxide dismutase, and V_{REI} is compared with (*i*) the 1555 residues representing an average β-sheet (18) and (*ii*) the 119 residues forming β-sheet-to-β-sheet contacts in the five proteins.

pology (22–24) and with the patterns formed by their contact residues (3). Taken together, these papers give us a clear picture of the general principles that govern the three-dimensional structure of these proteins.

The reason for the rows of side chains being approximately aligned at the interface is that it allows the β-sheets to be twisted *and* have a large contact surface. If two twisted β-sheets are put face to face at a large value of Ω they splay apart. This occurs in the other class of structures formed by β-sheets in which $\Omega \approx 90°$. The packing principles that determine these structures are discussed elsewhere.

The forces that determine the three-dimensional structures of proteins are too complex to be described in exact detail. Yet, the elaborate architecture of proteins obeys rules that can be revealed by an empirical analysis of available structural data. We have attempted to draw such rules for the assembly of proteins made of α-helices packed on α-helices (4), or on β-sheets (5), and now, for proteins made of two β-sheets packed face to face. In all three sorts of structures, the basic geometry of the association is fixed by that of the elements. Thus, the right-handed twist of β-pleated sheets is the prominent feature in α-helix-to-β-sheet and β-sheet-to-β-sheet association. The β-sheets appear as essentially smooth twisted surfaces, their smoothness resulting from a restricted amino acid composition of the regions involved in the association. On these smooth surfaces α-helices with their axis parallel to the strands, or a β-sheet turned by −30°, form a complementary surface that fits with little need for rearrangement of the side chains at the interface (24, 25). The association is stabilized by the removal of hydrophobic surfaces from contact with the solvent, which yields a favorable release of hydrophobic free energy.

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