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The β bulge: A common small unit of nonrepetitive protein structure

(β sheet/β barrel/secondary structure/tight turn/strand twist)

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ABSTRACT **A β bulge is a region between two consecutive β-type hydrogen bonds which includes two residues (positions 1 and 2) on one strand opposite a single residue (position *x*) on the other strand. Compared to regular β structure, a β bulge puts the usual alternation of side-chain direction out of register on one of the strands, introduces a slight bend in the β sheet, and locally accentuates the usual right-handed strand twist. Almost all β bulges are between antiparallel strands, usually between a narrow rather than a wide pair of hydrogen bonds. Ninety-one examples are listed. The two commonest types are the "classic" β bulge, with position 1 in approximately α-helical conformation, and the "G1" β bulge, with a required glycine at position 1 in approximately left-handed α-helical conformation. G1 bulges almost always occur in combination with a type II tight turn. The functional roles of β bulges probably include compensating for the effects of a single-residue insertion or deletion within β structure and providing the strong local twist required to form closed β barrel structures.**

One of the outstanding problems in globular protein structure is the task of describing the nonrepetitive "coil" regions that are neither helices nor β sheets. The one really major advance in this regard was identification of the tight turn (1), a loop in which the carbonyl oxygen of residue *n* hydrogen bonds to, or at least is close to, the NH group of residue *n* + 3. If all four residues are counted as part of the tight turn and the hydrogen bond is not required, then such turns account for up to a third of protein structure, and their occurrence can be predicted from the sequence with a moderate degree of success (e.g., ref. 2). Several other non-α, non-β conformations have been described, but except for the transition to 3_{10} or α_{II} conformation at the ends of α-helices (3), it happens that each of them has been identified for only one or a very few examples. The γ turn (4), which occurs in thermolysin (5), is a tighter turn involving only three residues and a highly bent hydrogen bond. Short pieces of polyproline II structure have been identified in pancreatic trypsin inhibitor (6) and in cytochrome c_{551} (7). An extended-chain "ε-helix" (rather similar to polyproline) has been identified in chymotrypsin (8). Two *cis*-prolines have been demonstrated in the Bence-Jones dimer REI, which form a distinct subclass of tight turns with a *cis*-proline in position 3 (9). Although, surely, additional examples of all these conformations could be found in other protein structures, each of them is apparently rather rare. The present paper describes a small unit of nonrepetitive structure which occurs almost as often as tight turns and which may also prove very useful for the description and understanding of protein structure.

Methods of procedure

Potential β bulges were identified by examination of backbone stereo drawings in the *Atlas of Macromolecular Structure on Microfiche* (10) and also from hydrogen-bond diagrams published in the following x-ray structure reports: chymotrypsin, EC 3.4.21.1 (11); Bence-Jones protein REI V_L (12); immunoglobulin Fab′ NEW (13); carboxypeptidase A, EC 3.4.12.2 (14); concanavalin A (15); carbonic anhydrase C, EC 4.2.1.1 (16); ribonuclease S, EC 3.1.4.22 (17); thermolysin, EC 3.4.24.4 (18); alcohol dehydrogenase, EC 1.1.1.1 (19); lactate dehydrogenase, EC 1.1.1.27 (20); flavodoxin (21); glyceraldehyde phosphate dehydrogenase, EC 1.2.1.12 (22); pancreatic trypsin inhibitor (23); egg white lysozyme, EC 3.2.1.17 (24); subtilisin, EC 3.4.21.14 (25); papain, EC 3.4.22.2 (26); cytochrome *c* (27); and cytochrome b_5 (28). Each bulge candidate was then examined on full-coordinate stereo drawings in the microfiche atlas to make sure that it had reasonable distances and angles for the hydrogen bonds and to observe any significant features of its environment in the structure. Assignment of a bulge as, for instance, wide or classic type, sometimes depends on imperfectly known peptide orientations.

General description of β bulges

Antiparallel β sheet has its side chains extending alternately above and below the sheet. There is a hydrogen-bonding pattern between any two strands in which closely spaced pairs of hydrogen bonds (using the NH and CO of a single residue) alternate with widely spaced pairs (using the CO of residue *n* and the NH of residue *n* + 2). The hydrogen bonding actually seen in antiparallel β sheets is typically less regular than the ideal pattern described above. Upon examination, these irregularities turn out to be almost all of one describable type, which we shall call a β bulge.

A β bulge is defined as a region between two consecutive β-type hydrogen bonds which includes two residues on one strand opposite a single residue on the other strand. Fig. 1 is a schematic representation of a β bulge. In more than 80% of the cases, β bulges occur between a closely spaced pair of hydrogen bonds rather than a widely spaced pair. β bulges are extremely rare in parallel β structure. Note on the left-hand side of Fig. 1 that an additional β strand cannot continue to bond past the bulge on the two-residue side, so that a bulge is always at either an edge or an end of the β sheet. The additional backbone length of the extra residue on the bulged strand is accommodated partly by bulging that strand up and out and partly by putting a slight bend in the β sheet. The distance between α-carbons *n* and *n* + 3 on either end of the two-residue bulged side averages 8.0 Å.

The β bulge can conservatively be taken to include the three residues inside its characteristic hydrogen bonds: two residues on the bulged strand and one on the opposite strand, identified as positions 1, 2, and *x*. Thus, a particular β bulge can be specified by listing those three residues: for instance, chymotrypsin A86,K87; K107. A wide bulge could be considered to include

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Abbreviations: The one-letter amino acid code is: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; Y, Tyr.

an extra half-residue on each end of each strand (positions 0, 3, $x-1$, and $x+1$), for a total of five residues. All three residues of narrow bulges are rather limited in possible ϕ,ψ conformational angles. Position x has ϕ and ψ within the normal range for antiparallel β structure but skewed to one side: the average for 109 "control" residues in antiparallel sheet gave $\phi = -122°$, $\psi = 143°$, while for position x of β bulges the average is $\phi = -97°$, $\psi = 127°$. In some bulges the hydrogen bond to the CO group of position x appears to be forked, coming from the NH groups of both position 1 and position 2. Most β bulges have considerable β structure on one or both ends, but bulges also occur in isolation.

Classic Type. Table 1 lists 91 β bulges which have been identified in proteins of known structure and specifies their type, while Fig. 2 plots ϕ,ψ angles for the narrow bulges, grouped by type. By far the most common type is the "classic" β bulge, which occurs between a narrow pair of hydrogen bonds and has residue 1 in approximately α-helical conformation (averaging $\phi = -100°$, $\psi = -45°$) and residue 2 in approximately normal β conformation (averaging $\phi = -140°$, $\psi = 160°$). All three side chains are on the same side of the β sheet. Fig. 3 shows a shaded, space-filling drawing of a classic β bulge.

A bulge causes the alternation of side-chain direction to be out of register on the two ends of the bulged, two-residue strand. The bulge can be thought of as turning over one half of the bulged strand; the classic bulge accomplishes this by changing the ψ angle of the residue in position 1 by about 180°, which moves it from the β conformational region to the α region. Except for a single case of an "inverted" β bulge in glyceraldehyde-*P* dehydrogenase (where there is a Glu-Arg salt bridge that presumably helps stabilize the unusual conformation of left-handed α in position 2), it is always position 1 that makes the 180° flip, so that for all narrow bulges position 2 remains quite close to normal β conformation. Once half of the strand has flipped over, it must shift by one residue along the other strand in order to hydrogen bond; that shift produces the bulge of two residues opposite one.

Examples of the classic β bulge all resemble each other fairly closely; in Fig. 4 five of them have been shown superimposed. In addition to the sideways bend seen in Fig. 1, classic β bulges also locally accentuate the normal right-handed twist (29) of the β strands, as can be seen in Fig. 3 or 4.

In the ϕ,ψ plot of Fig. 2, the subset of classic β bulges with points falling in the upper left corner of the cluster for position 1 also fall on the left side of the cluster for position 2, and five

Table 1. Ninety-one β bulges in proteins of known structure, with positions 1, 2, and x listed and type indicated

Chymotrypsin	
F41,C42; L33	Classic
I47,N48; W51	Classic
A86,K87; K107	Classic
G133,T134; L162	G1
G196,G197; V213	G1
V210,G211; L199	Classic
S214,W215; V227	Classic
Trypsin	
F41,C42; L33	Classic
I47,N48; W51	Classic
S86,K87; K107	Classic
G133,T134; I162	G1
G196,G197; V213	G1
Q210,G211; V199	Classic
S214,W215; V227	Classic
Elastase	
T44,C45; L33	Classic
I50,R51; W54	Classic
Q90,K91; R113	Classic
N139,S140; T167	G1
G204,G205; T221	G1
H218,G219; L207	Classic
S222,F223; V237	Classic
Subtilisin	
K265,G266; V198	Parallel
Carboxypeptidase A	
I38,G39; I47	Classic
G44,R45; S41	G1
R130,S131; G140	Gx NH
S197,Y198; L271	Parallel
S199,Q200; L271	Gx CO
Bence-Jones REI	
V_L	
G16,D17; L78	G1
K42,A43; T39	Classic
L47,I48; W35	Classic
S76,S77; R18	Wide
Immunoglobulin NEW	
V_L	
G15,Q16; L77	G1
T75,G76; R17	Wide
G102,T103; Y85	Classic
V_H	
Q16,T17; S83	Wide
I48,G49; W36	Classic
G55,T56; F52	G1
D106,V107; R98	Classic
C_H1	
P171,A172; L182	Wide
Immunoglobulin McPC 603	
V_L	
G16,E17; S77	G1
P43,P44; Q38	Wide
L47,I48; W35	Classic
G100,G101; Y87	Wide
V_H	
I48,A49; W36	Classic
D106,V107; R98	Classic
A110,G111; Y95	Wide
C_L	
N137,N138; S174	Wide
Ribonuclease S	
G68,Q69; C65	G1
T70,Q71; A64	Wide
V118,H119; A109	Classic
D121,A122; I107	Classic
Concanavalin A	
K35,K36; I27	Classic
G45,K46; I199	G1
L107,S108; A196	Classic
G152,N153; G149	G1
S164,D165; V159	Wide
T187,V188; K116	Classic
D208,G209; S96	Classic
Carbonic anhydrase C	
P45,L46; G80	Wide
Y50,D51; A76	Wide
L56,R57; E68	Classic
I90,Q91; V120	Classic
G169,K170; N60	G1
Prealbumin	
G22,S23; D18	G1
F44,A45; V32	Classic
G53,E54; S50	G1
Thermolysin	
G15,D16; G12	G1
N111,N112; G123	Gx NH
L202,R203; I188	Classic
Liver alcohol dehydrogenase	
H34,E35; G77	Gx NH
E74,S75; R37	Classic
G86,D87; V73	G1
Lactate dehydrogenase	
C266,R267; L293	G1
Gra-*P* dehydrogenase*	
V168,E169; R245	Inverted
D236,V237; A177	Gx NH
Pancreatic trypsin inhibitor	
A16,R17; G36	Gx CO
G28,L29; N24	G1
Rubredoxin	
G10,Y11; C6	G1
G43,I44; C39	G1
Staphylococcal nuclease	
I15,K16; K24	Classic
I18,D19; T22	Classic
Egg lysozyme	
G49,S50; N46	G1
R73,N74; W62	Wide
T4 lysozyme	
L33,V34; Y25	Classic
Papain	
V164,G165; L172	Classic
Cu,Zn superoxide dismutase	
L142,A143; V117	Classic
Flavodoxin	
G79,K80; S108	G1 (par)
Ferredoxin	
P52,E53; Y2	Wide
Cytochrome *c*	
G37,R38; W59	G1
Cytochrome c_{550}	
G35,P36; H19	Parallel
Cytochrome b_5	
I76,G77; V29	Classic

No bulges were found in erabutoxin, high-potential iron protein, triose phosphate isomerase, or rhodanese.

* Glyceraldehyde-*P*-dehydrogenase.

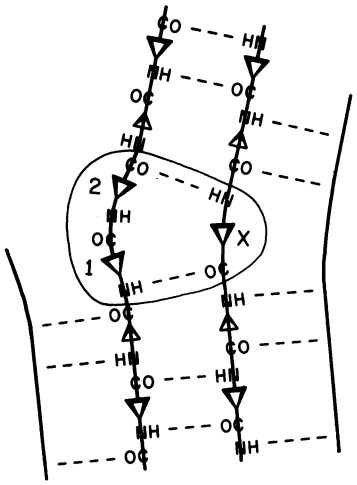

FIG. 1. A β bulge (outlined region) at the edge of an antiparallel β sheet. Smaller triangles represent side chains that are below the sheet, larger triangles those that are above it.

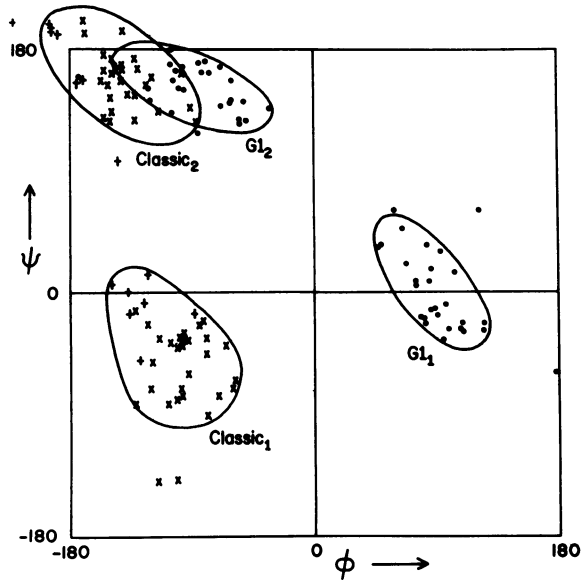

FIG. 2. Plot of ϕ,ψ conformational angles for positions 1 and 2 in β bulges of classic type (×), a subset of classic type (+), and G1 type (○).

of them have a glycine in position 2. However, they have been included within the classic type, since their conformations do not form a really distinct cluster.

G1 Type. The next most common type of β bulge is also narrow. It is called the G1 type, because 24 of the 26 examples have glycine in position 1. Position 1 has ϕ,ψ values centered around 85°, 0°, which is favorable only for glycine. Position 2 of G1 bulges is within the usual β region, but centered around $\phi = -90°$, $\psi = 150°$ (see Fig. 2).

Fifteen of the 26 G1-type bulges listed in Table 1 are found within an interlocking structure in which the glycine in position 1 of the G1 bulge is also the required glycine in position 3 of a type-II tight turn. The plane of the tight turn and its hydrogen bond is almost perpendicular to the plane of the G1 bulge. This combined structure has a consistent handedness which is constrained by the requirements of the three hydrogen bonds. A set of G1 bulges plus turns are shown superimposed in Fig. 5. Such an arrangement occurs next to the active site in chymotrypsin (G196,G197; V213). Two of the G1 bulges without coupled glycine turns occur in rubredoxin and involve all four of the cysteine iron ligands.

In most cases, the two strands of a β bulge are fairly far apart in sequence and can occur in either order (see Table 1), but almost half of the G1 bulges have short nearest-neighbor connections between the strands, with position 1 equal to either $x + 3$ or $x + 4$. These short G1 connections always involve at least one tight turn. Short connections occasionally occur between the strands of a classic bulge, but they are usually on the other end, with position 2 equal to $x - 3$.

Gx Type. This type of β bulge is represented by only six examples in Table 1, four with a glycine in position x. Either the carbonyls on both sides of the α-carbon in position x or else the NH groups on both sides of it are used to form the hydrogen bonds with the other strand. In this type, glycine is probably preferred because a side chain would point almost directly into the other strand.

Parallel Type. Bulges are very rare in parallel β sheet. There is a G1 bulge in flavodoxin, with the usual interlocked tight turn, but residue x is on a parallel strand. In carboxypeptidase, a short +2 connection between mixed sheet strands (see ref. 30 for terminology of connection types) has a single hydrogen bond between the intervening β strand and the connecting chain that crosses over it, forming a parallel bulge on one side of that hydrogen bond and a Gx bulge on the other (the bulges are between that central hydrogen bond and the first hydrogen bond of the normal β structure on each end of the connection). Rather similar +2 connections in glyceraldehyde-*P* dehydrogenase and in thermolysin use only the Gx bulge. There is a parallel bulge in cytochrome c_{550}, but although the conformation is very similar in the other cytochrome *c*s, one of the hydrogen bonds is either too long or in the wrong direction in all the other cases. Apparently parallel bulges are not favored even when the surrounding conformation suggests their suitability. Parallel β sheets are buried on both sides (30) so that hydrophobic–hydrophilic alternation does not occur, and also they are relatively unstable and more dependent on the cooperativity of extensive hydrogen bonding (30) so that perhaps they cannot tolerate the distortion produced by bulges. However, it is still unclear why bulges are so rare even at the ends of parallel β strands.

Wide Type. The remaining 14 bulges listed in Table 1 are between a wide pair of hydrogen bonds on antiparallel β strands. They differ considerably from one another in conformation, and no attempt has been made to classify them further.

Pseudo Bulges. In all examples considered so far and included in Table 1, it has been taken as a requirement that β-type hydrogen bonds be formed between the main chains on both ends of the β bulge. If only conformational angles along the bulged strand are considered, then numerous additional examples can be identified, although it seems misleading to call them β bulges. However, it does seem interesting to consider the fact that pseudo β bulges occur in which one or both hydrogen bonds from the bulged strand are made with a side chain or a water molecule instead of with the main chain of position x. Some examples are a pseudo classic bulge in the REI V_L dimer: Q92,S93 opposite Q90 side chain; a pseudo classic bulge in prealbumin: E89, H90 opposite V94 CO and opposite V94 NH with a water in between; and a pseudo G1 bulge in rubredoxin: G27,I28 opposite P15 CO and Y13 side chain. These cases underline the fact that although a bulge appears distorted when represented as part of a β-sheet hydrogen-bonding diagram, it is nevertheless an unstrained conformation and there seems no reason to assume it is much less stable than the β structure around it.

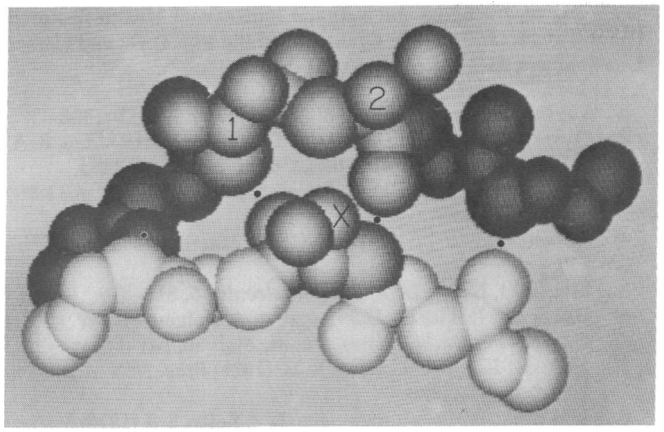

FIG. 3. Space-filling representation of the nonhydrogen atoms in a classic-type β bulge (staphylococcal nuclease I15,K16; K24), with the two-residue strand in black, the opposite strand in white, and bulge positions 1, 2, and x (labeled on their β carbons) in gray. Side chains are shown for the bulge residues out to the γ carbons, hydrogen bonds are dotted, and atom sizes are 0.44 times Van der Waals radii. This is the same view as in Figs. 1 and 4, but rotated 90° in the plane of the paper.

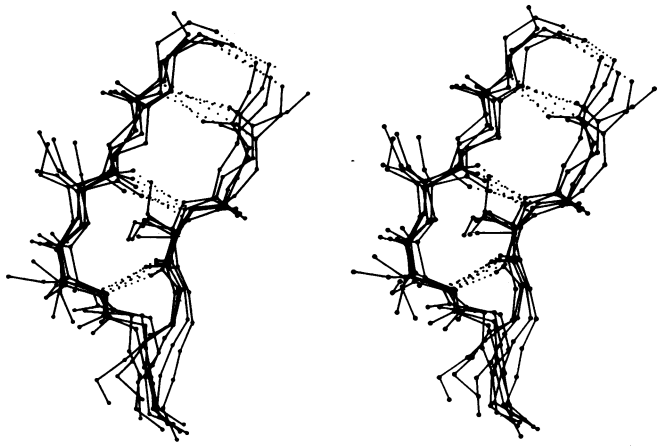

FIG. 4. Stereo drawing of five superimposed examples of classic β bulges: chymotrypsin, F41,C42; L33 and A86,K87; K107; concanavalin A, L107,S108; A196; carbonic anhydrase C, I90,Q91; V120; and staphylococcal nuclease, I15,K16; K24. Here and in Fig. 5 side chains are shown (out to Cγ) for just the three positions within the bulge. Carbonyl oxygens and hydrogen bonds are omitted in the very bottom of the drawing, where the two strands overlap in this projection.

Discussion

β bulges have two prominent sorts of features, when considered in contrast with the alternative of continuing the regular β structure. The first feature is their effect on which side chains extend to each side of the β sheet. The second feature is their effect on the directionality of the surrounding strands, due to the bend introduced into the β sheet, the accentuated local twist, and the direction from which a strand can enter a G1 bulge. Let us consider the consequences of these features with respect to prediction of β bulges, their formation during protein folding, and their possible functional roles in proteins.

There are some amino acid preferences for the residues making up β bulges. Glycine occurs most often in position 1 because it is required in the G1 type. In narrow bulges proline is forbidden in positions 1 and x, but in wide bulges proline is actually preferred in position 1. Position 2 shows a 10:1 preference for hydrophilic over hydrophobic residues, but positions 1 and x are apparently indifferent to hydrophobicity. In general, the residue preferences are not very dramatic, and there are only two sequence-consecutive residues to consider. For the purpose of predicting the occurrence of β bulges from the amino acid sequence, it would undoubtedly be helpful to consider also the sequence before and after the possible bulge. A β bulge causes the alternation of hydrophobic and hydrophilic residues typical of antiparallel β sheet to be out of register on the two ends of the bulge, and this effect is often quite evident in the sequence. Some examples (with an asterisk at the center of the bulge, hydrophobic residues underlined, and hydrophilic residues overlined) are:

$$\overline{K}\underline{L}\overline{K}\underline{I}\underline{A}^{*}\overline{K}\underline{V} \text{ (chymotrypsin),}$$

$$\underline{I}\overline{K}\underline{A}\underline{I}^{*}\overline{D}\underline{G} \text{ (staphylococcal nuclease),}$$

$$\underline{T}\overline{K}^{*}\overline{K}\underline{S}\overline{R} \text{ and } \overline{T}\underline{I}\underline{L}^{*}\overline{S}\underline{W}\overline{S}\underline{F}\overline{T} \text{ (concanavalin A),}$$

$$\text{and } \underline{G}\overline{K}^{*}\underline{A}\underline{P}\overline{K}\underline{L} \text{ and } \overline{T}\underline{F}\overline{T}\underline{I}\overline{S}^{*}\overline{S}\underline{L}\overline{Q} \text{ (REI } V_L\text{).}$$

The importance of the out-of-register alternation of side-chain direction, especially for classic bulges, suggests the possibility that they may take up the bulged conformation only at a relatively late stage in the folding process. Early during protein folding, when only a small piece of β sheet has formed, the correct alternation of hydrophobic and hydrophilic residues should not be important, since both sides of the proto-sheet would still be exposed to solvent. The same may be true of the detailed steric fit between side chains on neighboring strands, since there would still be a large degree of local flexibility. It may be, then, that a potential bulge first folds as regular β structure. Later, when a hydrophobic core has formed on one side of the β sheet the out-of-register strand end would become highly unfavorable, and it may actually perform the hypothetical 180° flip plus one-residue shift described above, producing the β bulge. If this sort of mechanism actually occurs, it would imply both relatively extensive readjustments at the final stages of protein folding and also the unfolding of some portions of the initial secondary structure. There have been previous suggestions that unfolding may occur for helices (2, 31). It is interesting that a kinetic intermediate has been observed in the folding of carbonic anhydrase whose circular dichroism indicates a larger amount of regular β structure than in the native protein (32). The five bulges in carbonic anhydrase could change the observed percentage of regular β structure by as much as 22% if the circular dichroism were significantly perturbed for all three residues of each bulge.

There are at least two quite different sorts of functional roles

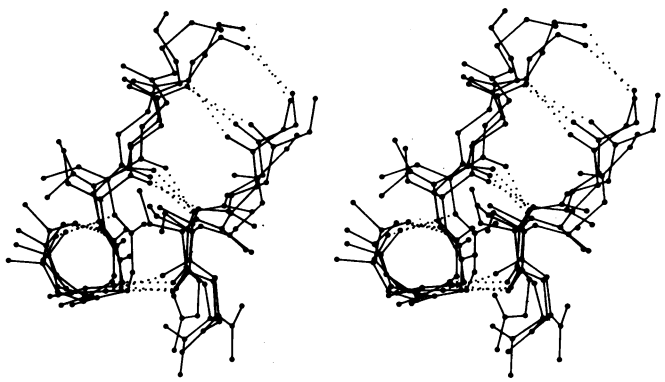

FIG. 5. Four G1-type β bulges superimposed on one another, with their associated type-II tight turns: trypsin, G133,T134; I162; elastase, G204,G205; T221; Bence-Jones REI V_L, G16,D17; L78; and cytochrome c, G37,R38; W59. The tight turn is the short hydrogen-bonded loop at the lower left. The α-carbon in the lower right corner of the loop is the required glycine in position 3 of the turn and position 1 of the G1 β bulge.

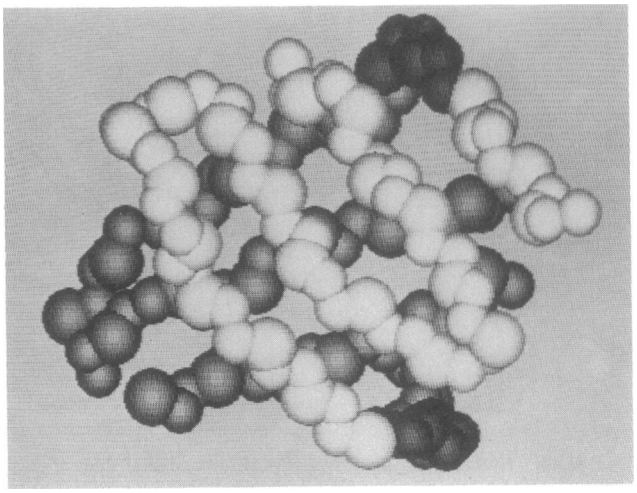

FIG. 6. Position of bulges in a β barrel: backbone N, Cα, and C atoms are shown (at 0.44 Van der Waals radii) for the six separate β strands that make up one of the β barrels in elastase, with the front β strands in white and the back strands in gray. Positions 1 and 2 are shown in black for the two bulges (T44,C45; L33 and Q90,K91; R113) that provide the strong local twist needed to close the β barrel. Each bulged strand forms one of the back (gray) strands on one end of the bulge and one of the front (white) strands on the other end of it.

that β bulges are suited to fill in a protein structure. The first sort of function would be to influence the direction in which a strand could leave a β sheet, to orient side chains in needed directions at an active or binding site, or to provide a bend in the β sheet which would help the overall structure to fit together better. β bulges occur in critical positions at active or binding sites for 14 of the proteins in Table 1. Bulges in the serine proteases, in staphylococcal nuclease, and probably in soybean trypsin inhibitor, are all strategically located at the sharpest corners within the β strands to help provide the very strong twist needed for forming a stable β barrel of only five or six strands. This is illustrated for one of the elastase β barrels in Fig. 6 (see also figure 6 in ref. 33). In the immunoglobulins the 47,48; 35 bulge in the V_L domain and the 48,49; 36 bulge in V_H provide a twist to complete closure around the V_L–V_H domain contact. Similarly, in prealbumin the F44,A45; V32 bulge helps "peel off" the extended hairpin loop that forms the proposed DNA binding site (34). For all the sorts of roles just discussed, it would be expected that β bulges should be highly conserved among related proteins. It is notable that the seven β bulges occurring in chymotrypsin are completely conserved in both trypsin and elastase (see Table 1); four of these bulges are at corners of the β barrels and two are at the active site.

A second possible sort of role for bulges is to provide a mechanism for accommodating a single-residue insertion or deletion mutation without totally disrupting the β sheet. There seem to be several such cases among the immunoglobulins, the clearest of which is a one-residue deletion in the C_H1 domain of McPC 603 relative to the sequence of Fab' NEW C_H1; the NEW structure has a bulge in the middle of a long pair of β strands, while in McPC 603 those strands form regular β structure all the way along past the deletion.

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