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A novel super-secondary structure of β-proteins

A triple-strand corner

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A novel super-secondary structure of β-proteins, denoted here as a triple-strand corner, is considered in this paper. This structure can be represented as an antiparallel triple-strand β-sheet folded on itself so that the two β–β-hairpins are packed approximately orthogonally in different layers and the central strand bends by 90° in the right-handed direction when passing from one layer to the other. In all the triple-strand corners observed in proteins, the first β–β-hairpins are right-handed and the second ones are left-handed when viewed from the concave sides of the corners. Arrangement of other β-strands in the proteins involving the triple-strand corners is also examined.

β-Hairpin; β-Sheet; Folding; Packing; Right-handed structure

1. INTRODUCTION

β-Proteins have been the subjects of numerous investigations. A number of common features observed in this class of proteins and the principles governing β-sheet packings have been described previously [1–7]. Most of the β-proteins are layer structures and two classes of β-sheet packings can be distinguished; the so-called aligned β-sheet packing, and the orthogonal β-sheet packing. β-Proteins can also be grouped into several classes depending on a repetitive folding unit or a super-secondary structure occuring in each class. In most of the bilayer β-proteins with the aligned β-sheet packing there is a four-stranded super-secondary structure denoted as the abcd-structure [7]. Many β-proteins with the orthogonal β-sheet packing involve β–β-hairpin coiled coils and β–β-corners [8]. This paper considers features observed in the arrangement of β-strands in proteins with orthogonal β-sheet packing containing the triple-strand repetitive unit (denoted here as the triple-strand corner or the 3β-corner).

2. STRUCTURE OF TRIPLE-STRAND CORNERS

A triple-strand corner can be represented as an antiparallel triple-strand β-sheet folded on itself so that the two β–β-hairpins are packed approximately orthogonally in different layers (Fig. 1a). The central strand of the 3β-corner bends by approximately 90° when passing from one layer to the other and can be defined as a right-handed bend [6]. The triple-strand corners as well as the bends are right-handed in proteins of known structure, i.e. the central strand rotates about an imaginary axis in the right-handed direction when passing from one layer to the other. For comparison, Fig. 1b shows a left-handed triple-strand corner which is not observed in proteins.

The 3β-corner has both concave and convex surfaces. In the right-handed 3β-corner the N-terminal β–β-hairpin is right-handed (i.e. the second β-strand is located on the right relative to the first one) and the C-terminal β–β-hairpin is left-handed when viewed from the concave side. Such an arrangement of β-strands in the 3β-corner provides contacts between them and results in the formation of a small hydrophobic core. For comparison, Fig. 1c presents an imaginary structure in which the first β–β-hairpin is left-handed, the second one is right-handed while the central strand is a right-handed bend. As seen, its N-terminal and C-terminal β-strands are not in contact, and this structure is less compact than the 3β-corner and does not have a hydrophobic core. One may conclude that the right-handed 3β-corner is more stable than the other structures shown in Fig 1 and that the polypeptide chain has to fold into the right-handed 3β-corner rather than into the others.

Fig. 2 shows five examples of hydrogen-bonding patterns of the 3β-corners observed in γ-chymotrypsin [9], *Streptomyces griseus* protease A [10] and papain [11] when viewed from the concave sides (or from the hydrophobic cores). As seen, the N-terminal half of the central β-strand forms hydrogen bonds mainly with the

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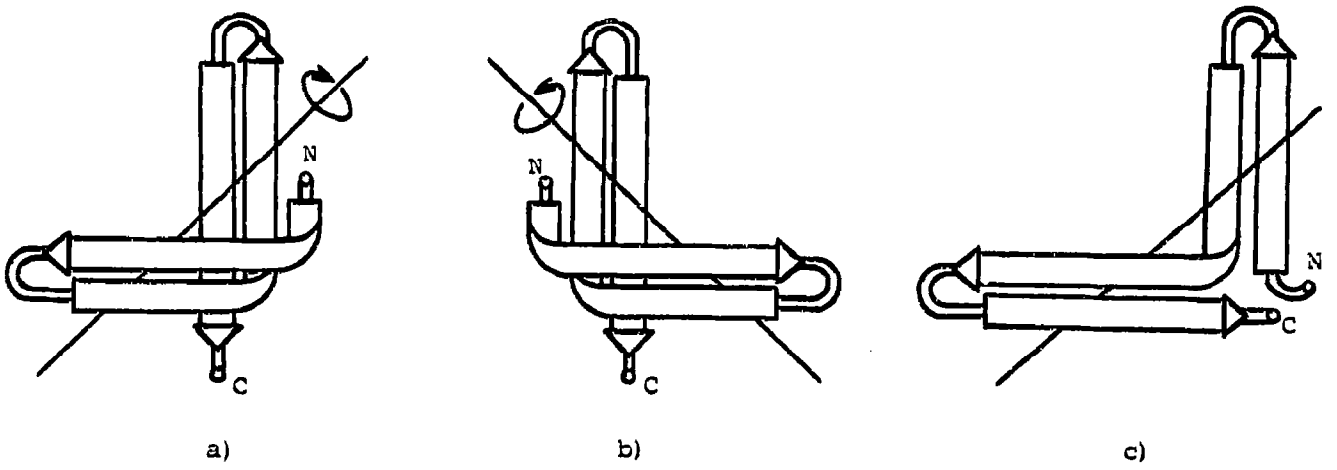

Fig. 1. A schematic representation of right-handed (a), left-handed (b) triple-strand corners and a less compact structure with a different arrangement of β-strands (c).

first β-strand and its C-terminal half forms hydrogen bonds with the third β-strand in each 3β-corner.

As mentioned above, the central strand of the 3β-corner can be defined as a right-handed bend described previously in ref. [6]. The bend in this strand can be produced by a classic β-bulge [12] or by a β-bend [6]. The conformation of a classic β-bulge can be described as the standard $\beta\alpha\beta\beta$-conformation in terms of shorthand nomenclature [13].

The conformation of the β-bend is very similar to that of the strongly twisted and coiled β-strand [6]. If one of the inner residues (residues located in the concave surface) of the β-bend is glycine it usually has a very extended or even an ε-conformation (for ε-conformations see [13]) having φ, ψ values in the bottom right quadrant of the Ramachandran map.

Fig. 3 shows some variants of 3β-corners having more complex structures than those described above. The β–β-hairpins in 3β-corners can have very long loops (Fig. 3a) with complex irregular structures (see, for example region 64–109 in γ-chymotrypsin [9]). The distant ends of long β–β-hairpins can be folded into β–β-corners similar to that shown in Fig. 3b (see also regions 163–183 of SGPA [10] and 134–162 of *Streptomyces griseus* trypsin [14]). The first β–β-hairpins can be folded into β–β-corners as shown in Fig. 3c (such 3β-corners are observed, for example, in β-lactoglobulin and related proteins) or into a coiled coil as shown in Fig. 3d. The coiled coil is a strongly twisted and coiled β–β-hairpin [8,15,16]. There are 3β-corners in which the bend in the central strand is produced by some residues in α-helical or in irregular conformations. Nevertheless the three-dimensional arrangement of the β-strands is very similar in all the observed 3β-corners.

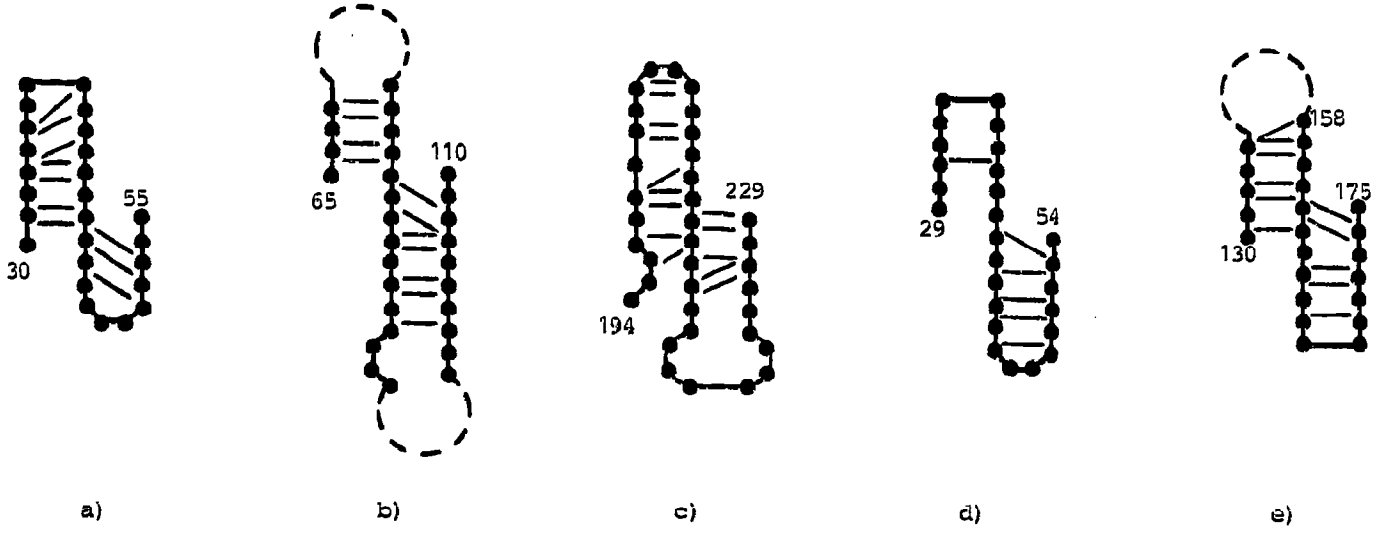

Fig. 2. Hydrogen-bonding schemes in the triple-strand corners observed in regions, 30–55, 65–110, and 194–229 of γ-chymotrypsin [9], 29–54 of SGPA [10] and 130–175 op papain [11].

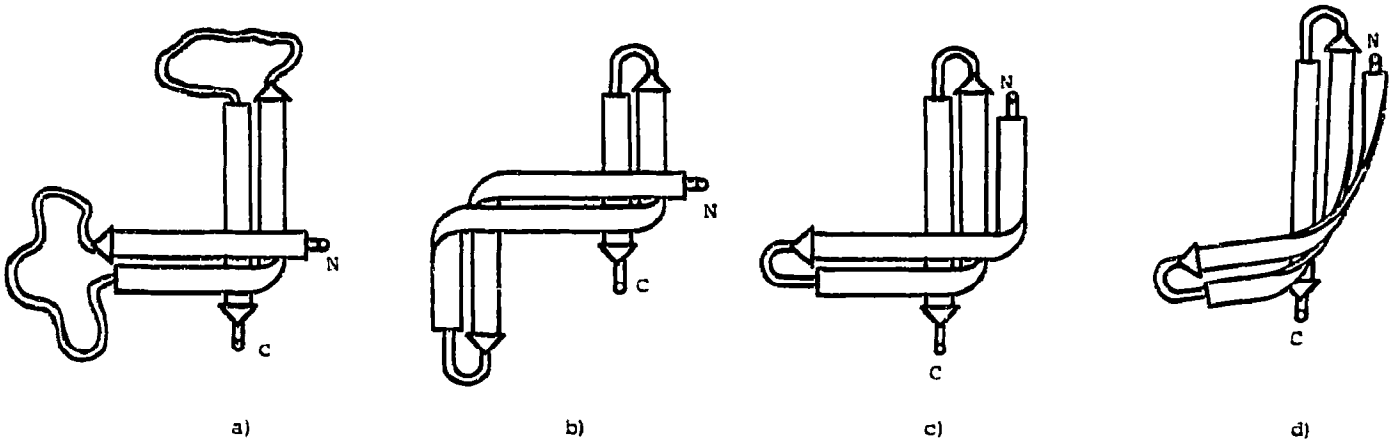

Fig. 3. Complex structures of 3β-corners.

3. ARRANGEMENT OF OTHER β-STRANDS RELATIVE TO 3β-CORNERS

Fig. 4 shows a schematic representation of protein molecules, domains and some incomplete structures involving 3β-corners. All the structures are oriented in a similar way so that 3β-corners are localized in their bottom right corners and the β-strands of the near β-sheets are directed horizontally and those of the far β-sheets vertically. The strands of the 3β-corner are

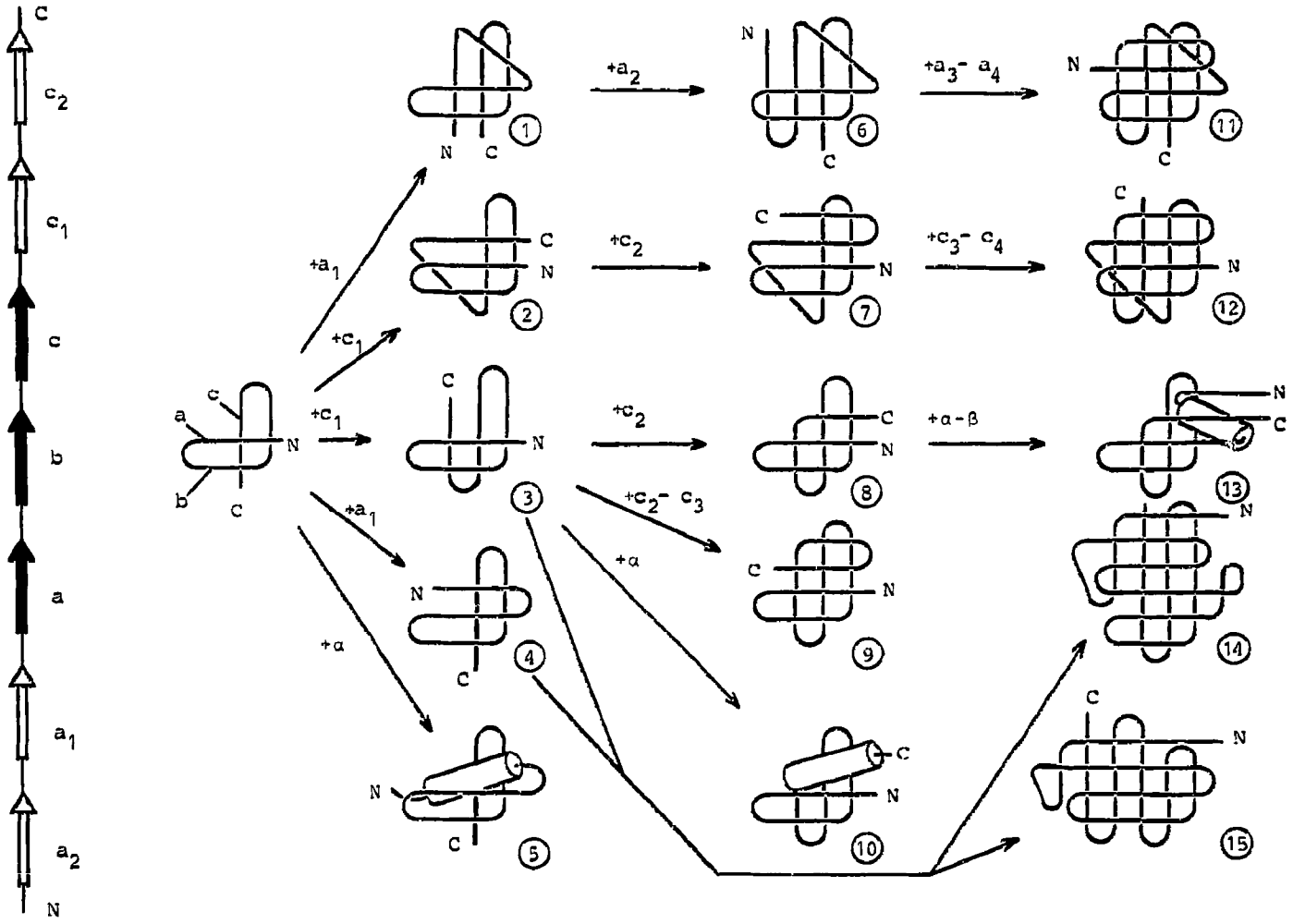

Fig. 4. A schematic representation of arrangement of β-strands in the protein structures involving 3β-corners. See also text.

labeled here as a, b and c, the strands joined to strand a as a_1, a_2, a_3, ... and the strands joined to strand c as c_1, c_2, ... as shown in the left part of Fig. 4.

To clarify the features in the arrangement of β-strands let us add the β-strands to the 3β-corner, step by step, taking into account only the structures observed in proteins. If strand a_1 is present in the protein, it is situated in the far β-sheet next to strand c or in the near β-sheet next to strand a to yield structures, 1 or 4, respectively. Similarly, strand c_1 is located in the near layer (see structure 2) or in the far layer (see structure 3) in proteins. It is noteworthy that structure 1 coincides with structure 2 and that structure 3 coincides with structure 4 when superimposed if the polypeptide chain direction is not taken into account. As described previously [7], structures 1 and 2 can be considered as complex variants of the abcd-structure. If there is an α-helix joined to the 3β-corner, it is packed into the concavity to form structure 5. A similar but very distorted structure is found in carboxypeptidase A inhibitor from potatoes [17]. CMTI-I, a trypsin inhibitor from squash seeds [18] has the polypeptide chain fold similar to that of structure 1.

Arrangement of the other β-strands joined to structures 1 and 2 is clearly seen in the upper part of Fig. 4. Structures 11 and 12 are essentially the same as they coincide when superimposed. Each structure consists of two 3β-corners related by a two-fold symmetry axis perpendicular to the plane of the figure. Domains of chymotrypsin [9], SGPA [10] and other serine proteases have such an arrangement of β-strands.

There are several ways of packing of the β-strands connected with structure 3. Strand c_2 can be situated in the near β-sheet next to strand a (see structure 8). A similar structure is found in the C-domain of bovine neurophysin [19]. Addition to structure 8 of an α–β-hairpin results in structure 13 observed in the C-terminal domains of papain [11] and other sulfhydryl proteases. Structure 9 is obtained if a c_2–c_3-hairpin is added to structure 3. This structure is found in the H-subunit of the photosynthetic reaction centre [20]. Packing of an α-helix joined to strand c_1 into the concavity of structure 3 results in structure 10 which occurs in the N-terminal domain of bovine neurophysin [19]. If all the strands a_1, a_2, a_3, ... form the near up-and-down β-sheet and all the strands c_1, c_2, c_3, ... form the far up-and-down β-sheet, this will result in formation of structures 14 and 15 observed in retinol binding protein [21] and β-lactoglobulin [22], respectively.

4. DISCUSSION

Thus, all the 3β-corners found in proteins of known structure are right-handed and situated at the edges of protein molecules and domains (see Fig. 4). In all the 3β-corners the first β–β-hairpins are right-handed and the second ones left-handed when viewed from the concave sides. As can be seen, all the 3β-corners wrap around hydrophobic cores of proteins. Consequently, most of the amino acid residues situated on the concave surface of a 3β-corner should be hydrophobic. The other β-strands of a molecule or a domain are arranged on the concave side of the 3β-corners to form a compact structure. Connections between β-strands do not cross as observed in other proteins [23]. Most of the β-sheets are antiparallel except the near β-sheet of the H-subunit of the photosynthetic centre (see structure 9 in Fig. 4), where strand c_3 is parallel to strand a. The two C-terminal domains of glutaminyl-tRNA synthetase also have a mixed β-sheet [24]. Their simplified structures can be obtained by arrangement of strand a_2 between strands a_1 and c of structure 1 in Fig. 4 so that strand a_2 is parallel to strand c.

An analysis of the three-dimensional arrangement of β-strands in the proteins and the features considered above suggests a hypothesis that the 3β-corners can fold independently of the remaining parts of molecules and can be nuclei for protein folding. Growth of the nucleus occurs through the step-by-step attachment of subsequent β-strands or β–β-hairpins on the concave side. At each step the next β-strand (or β–β-hairpin) along the chain is the first to be attached to the growing structure. Each subsequent β-strand can be situated in one or in the other layer of the structure. Apparently this is determined by the length and conformation of the connection region and by prohibition of crossing connections. We believe that such a folding pathway provides a limited number of the observed protein structures and elucidates their similarities.

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