Six- β -Strand Bundle Structure. A New Super Secondary Structure Constructed by Anti-Parallel Packing of Six β -Strands

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(Received August 28, 1995)

KEY WORDS Super Secondary Structure / Six- β -Strand Bundle Structure / Polypeptide / Artificial Protein / Molecular Mechanics / ECEPP /

In native proteins, super secondary structures formed by specific spatial arrangements of the α -helix and β -strand are essentially important structural elements to construct the suitable three-dimensional arrangement of the side-chain group for exhibiting molecular functions. However, it is estimated that a total number of basic super secondary structures is limited up to the maximum number 1000.¹ From the viewpoints of molecular design of artificial proteins which have explicit molecular functions exceeding those of native proteins, it is very important to construct new super secondary structures by extending the extent of amino-acid residues to the outside of 20 naturally occurring amino-acid residues, especially D-amino-acid residues. In this communication, a 6- β -strand bundle structure constructed by anti-parallel packing of 6- β -strands are proposed as a new super secondary structure for artificial proteins composed of L- and D-amino-acid residues.

THEORETICAL

As the first step, theoretical analysis is carried out based on the Ala-residue approximation^{2,3} for the model polypeptides which are constructed by six β -strands having opposite optical sense, *i.e.*, three of them are composed of n Ala residues and others are composed of n D-Ala residues. These six β -strands are alternately connected in optical sense by two Gly residues which have the largest conformational flexibility in conformational space among 20 naturally occurring amino-acid residues. That is, the amino-acid sequence of the model polypeptides are Ac-(Ala) _{n} -Gly-Gly-(D-Ala) _{n} -Gly-Gly-(Ala) _{n} -Gly-Gly-(D-Ala) _{n} -Gly-Gly-(Ala) _{n} -Gly-Gly-(D-Ala) _{n} -NHMe ($n=6,10$). Conformation energy of the model polypeptide is optimized by increasing number of β -strands step by step using the energy functions of ECEPP⁴ and by searching all energy minima for the whole conformational space of Gly-Gly portions. Then, as the second step, for the purpose of analyzing the effect of side-chain groups on the relative stability of each super secondary structure, further calculations were carried out for two model polypeptides, Ac-(Ala-Xaa)₃-Gly-Gly-(D-Ala-D-Xaa)₃-Gly-Gly-(Ala-Xaa)₃-Gly-Gly-(D-Ala-D-Xaa)₃-Gly-Gly-(Ala-Xaa)₃-Gly-Gly-(D-Ala-D-Xaa)₃-NHMe, where Xaa = Val and Nle (norleucine)

are used as model cases for the β -branched and unbranched amino-acid residues, respectively.

RESULTS AND DISCUSSION

The lowest-energy conformation is a new super secondary structure (Figure 1), which is named 6- β -strand bundle structure, is constructed by anti-parallel arrangement of 6- β -strands with types II' and II β -bend at (Ala)-Gly-Gly-(D-Ala) and (D-Ala)-Gly-Gly-(Ala) portion, respectively. This conformation is stabilized by the strong network of hydrogen bonds between adjacent β -strands and zipper structure of methyl group situating inside of the bundle structure. This super secondary structure is completely different from already-known super secondary structures which have been found in native proteins such as β -sheet and β -barrel. Many energy minima were found for this model polypeptide, *e.g.*, 2167 and 648 minima for $n=6$ and 10 within $\Delta E_{\text{res}} < 3 \text{ kcal mol}^{-1}$, respectively. (ΔE_{res} is energy difference per residue from the lowest-energy minimum.) However, ΔE_{res} of the second low-energy conformation, which is well-known anti-parallel β -sheet structure, is very large, *e.g.*, $\Delta E_{\text{res}} = 1.03$ and $1.19 \text{ kcal mol}^{-1}$ for $n=6$ and 10, respectively. The large value of ΔE_{res} indicate that the 6- β -strand bundle structure is dominantly stable structure as assembled structures of β -strands, and also that 6- β -strand bundle structure has a tightly bundled structural feature which is remarkably distinct from those of 3- or 4- α -helix bundle structures fluctuating among many energetically close and structurally resemble minima.^{5,6} The difference in stiffness between β -strand bundle structure and α -helix bundle structure caused by the difference in interaction mode between them. That is, the former is mainly stabilized by favorable hydrogen bonds between adjacent β -strands, but the latter is generally stabilized by von der Waals interactions. Above structural feature suggests that the 6- β -strand bundle structure is a important structure as one of rigid backbone structures in artificial proteins.

Moreover, further calculated results for Xaa = Val and Nle indicate that the 6- β -strand bundle structures are also stable structures in spite of the branched or non-branched at β -carbon in side-chain group. It means that designing suitable hydrophilic residues for Xaa portion could further stabilize the 6- β -strand bundle structure in aqueous solutions by the suitable distribu-

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Figure 1. A new super secondary structure named 6- β -strand bundle structure which was found as the lowest-energy conformation of Ac-(Ala)₁₀-Gly-Gly-(D-Ala)₁₀-Gly-Gly-(Ala)₁₀-Gly-Gly-(D-Ala)₁₀-Gly-Gly-(Ala)₁₀-Gly-Gly-(D-Ala)₁₀-NHMe by theoretical conformational analysis using the energy functions of ECEPP. This conformation is stabilized by the strong network of hydrogen bonds between adjacent β -strands and zipper structure of methyl group situating inside of the bundle structure. This super secondary structure is completely different from already-known super secondary structures which have been found in native proteins such as β -sheet and β -barrel. (a) a stereo side-view; (b) a top-view.

tion of amino-acid residues that the hydrophilic Xaa residue and the hydrophobic Ala residue situate outside and inside of the 6- β -strand bundle structure, respectively. The rigid rod-like structural character of the 6- β -strand bundle structure is resemble to that of the single α -helix. However, spatial arrangement of side-

chain groups along the rod axis of the former structure is not helical but parallel and stratified arrangement with 6 fold symmetry, suggesting that this spatial character of 6- β -strand bundle structure is very useful as backbone structure of the new artificial proteins with molecular functions surpassing those of native proteins.

Previous results for Ac-(Ala)₁₀-Gly-Gly-(Ala)₁₀-Gly-Gly-(Ala)₁₀-Gly-Gly-(Ala)₁₀-Gly-Gly-(Ala)₁₀-Gly-Gly-(Ala)₁₀-NHMe indicate that a typical anti-parallel β -sheet structure is the most energetically favorable conformation and also that no 6- β -strand bundle structures are found as minima.⁷ These results suggest that 6 fold symmetrical packing of β -strand found in this work is essentially induced by the co-existence of L- and D-amino-acid residues. Similar results were also obtained for the 4- α -helix bundle structures.^{5,6} That is, for the case composed of only L-Ala residue, its packing motif corresponds to those found in native proteins. However, for the case composed of L- and D-Ala residues, it forms unusual packing motif that four helical axis hold in the same direction. These results indicate that it is very important to use both of L- and D-amino-acid residues

for designing backbone structures of artificial proteins in the meaning of extending the region of super secondary structures of them.

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