Side-chain Conformations Cooperatively Restricted in Protein Secondary Structure

II. Side-chain Configurational Entropies of α -helices in the Folding Nuclei

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Abstract : Protein side-chain configurational entropies and their correlations were analyzed for α -helices in several proteins. The conformations of several bulky side-chains in helices were found to be cooperatively fixed by the restricted side-chain conformations of the neighboring β -branched amino acids. Such helices correspond well to the experimentally observed nuclei in the folding procedures, so that the folding rates should be significantly accelerated. This phenomenon may be a reasonable answer to the Levinthal paradox.

Key words : Protein structure; side-chain conformation; secondary structure; configurational entropy; folding nucleus; Levinthal paradox.

In our preceding article,¹⁾ we have developed a novel method for exhaustively searching for the side-chain conformations with energies near the global energy minimum. The partition function and the side-chain configurational entropy can be directly obtained with correctly evaluated correlations among the side-chains, even for strongly cooperative systems. Using this advantage, the side-chain configurational entropy was examined for α -helices and a β -sheet in proteins.

Consequently, the conformations of several bulky side-chains in α -helices were determined to be fixed through the restricted conformation of the neighboring β -branched amino acid in space, depending upon the local backbone structure. These mechanisms are one of the origins of the structural uniqueness of native proteins, and forbid structural diversity.¹⁾ Moreover, it suggests an important role for the side-chain configurational entropy in protein folding. Bulky side-chains often have a key role in the unique packing of

the hydrophobic core.^{2),3)} If the conformations of such bulky side-chains are determined by the local backbone structure, the process to find the correct side-chain rotamers becomes much more rapid than that with the non-restricted side-chain conformations that are independent of the local backbone structure. Since the rate-determining step in protein folding is considered to be finding the correct side-chain rotamers,⁴⁾ the restriction of the conformation of the side-chain should be associated with the construction of the folding nucleus.

This mechanism of fixing the side-chains on an α -helix may generally accelerate the protein folding process. By pulsed hydrogen exchange with NMR measurements and site-directed mutagenesis, the folding procedures have been precisely revealed for several proteins such as cytochrome-c,⁵⁾ ribonuclease A,⁶⁾ barnase,⁷⁾ hen lysozyme,⁸⁾ ubiquitin,⁹⁾ T4-lysozyme,¹⁰⁾ apomyoglobin,¹¹⁾ and chymotrypsin inhibitor 2.¹²⁾ For these proteins, particular helices are formed in the early stage of the refolding procedure.

Here, using the method for exhaustive structure searching, we have examined the side-chain conformations of α -helices in the above proteins, the folding procedures of which have been well revealed.

The side-chain configurational entropies were calculated for individual α -helices at T=300 K with

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(e) 2LZM

h5	h6	h7	h8	h9	h10
(12.4/12.6)	(4.2/4.3)	(7.4/7.9)	(4.4/5.4)	(5.3/6.4)	(9.1/9.1)

(f) 5MBN

hF	hG	hH
(8.8/9.8)	(12.8/13.3)	(13.8/14.3)

Fig. 1. Apparent residual entropies S_i^{app} of individual helices in several proteins, the folding kinetics of which have been analyzed experimentally. The former and the latter numbers, in the parentheses under the bars indicating each helix, are the correct entropy for each helix and the sum of the apparent residual entropies for all the residues composing the helix, respectively. The black bars correspond to buried residues, with solvent accessibilities less than 30%. The white bars correspond to exposed residues with solvent accessibilities greater than 60%. The gray bars are the other residues. The ranges of the helices were taken from DSSP.¹⁷⁾ ○ and ▲ indicate amino acid residues with aromatic and β -branched side-chains, respectively. (a) ubiquitin (PDB code; 1UBQ),¹⁸⁾ (b) chymotrypsin inhibitor 2 (2CI2),¹⁹⁾ (c) cytochrome-c (3CYT),²⁰⁾ (d) barnase (1BRN),²¹⁾ (e) T4-lysozyme (2LZM),²²⁾ (f) apomyoglobin (5MBN),²³⁾ (g) hen lysozyme (6LYZ),²⁴⁾ and (h) ribonuclease A (5RSA).²⁵⁾

$\Delta E_{\text{offset}} = 30$ kcal/mol, as described previously.¹⁾ The N- and C- termini of each peptide were capped with an acetyl and an N-methyl group, respectively. First, the global energy minimum conformation with the energy E_{min} was found by the DEE algorithm^{13),14)} using the small-size rotamer library developed by Tanimura *et al.*,¹⁴⁾ which is similar to that of Ponder & Richards.¹⁵⁾ Second, the exhaustive structures with the energies less than $E_{\text{min}} + \Delta E_{\text{offset}}$ were searched for using the same rotamer library. The probability of the i 'th side-chain taking the r 'th rotamer p_{ri} was calculated, and the apparent residual entropy S_i^{app} was computed for each i 'th residue from p_{ri} :

$$S_i^{\text{app}} = -R \sum_r p_{ri} \ln p_{ri}. \quad [1]$$

The interaction energies used in the calculations were given by the all-atom parameters of the AMBER force field,¹⁶⁾ excluding the 1–5 electrostatic interactions. All computations were performed on a DEC Alpha Station 600 5/266.

The side-chain conformations of all of the helices in the above eight proteins were calculated, and the resulted residual entropies are shown in Figs. 1a–h.

In ubiquitin and chymotrypsin inhibitor 2, which have only one helix each, the helix includes β -branched

amino acids with fixed conformations in the buried side of the helix, as shown in Figs. 1a and b. In the initial folding phase of ubiquitin, the backbone amide protons in the α -helix become 80% protected.⁹⁾ The structure of the transition state for the folding of chymotrypsin inhibitor 2 is around the nucleation site composed of the α -helix, except for the C-terminal end.¹²⁾

In cytochrome-c, barnase, T4-lysozyme, and apomyoglobin, the helices of {h1 and h4}, {h1}, {h1, h3, and h5}, and {hA, hB, hG, and hH}, respectively, have been experimentally observed to be formed at the early folding stage.^{5),7),10),11)} The current analysis indicates that the side-chain entropies of these helices are all small, mainly due to the positioning of buried Val and Ile residues, which could cooperatively restrict the conformations of aromatic, bulky side-chains (see Figs. 1c–f).

In hen lysozyme, the helices have been confirmed to be formed in the early folding stage.⁸⁾ Fig. 1g indicates that {h2 and h3} of hen lysozyme also have small side-chain configurational entropies. In ribonuclease A, many amide protons in the β -sheet and in the C-terminal half of h3 are strongly protected.⁶⁾ Among the three helices, the side-chain entropy of h3 is the smallest, due to the buried Val residues, as shown in Fig. 1h.

Therefore, it is concluded that the β -branched amino acids, Val and Ile, participate in the formation of most of the experimentally observed, local secondary structures at the beginning of the refolding process. Moreover, several bulky side-chains are also restricted cooperatively with the β -branched amino acids. This phenomenon should greatly increase the rate of the folding procedure, and so it may be a reasonable answer to the Levinthal paradox,²⁶⁾ which proposed that a native protein must not inspect all possible peptide conformations during the folding procedure.

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