EFFECT OF THE STRUCTURAL CONTEXT ON SPECIFICITY OF INTRA- AND INTERHELICAL INTERACTIONS IN PROTEINS

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SUMMARY

Motivation: In proteins, α-helices can be packed in some different ways and each type of the α-helix packing forms a specific structural environment (or structural context) of side chains forming the interface.

Results: A stereochemical analysis of intra- and interhelical side chain–side chain and side chain–main chain interactions in different α-helical packings enable us to show that the specificity of these interactions is dependent not only on the physico-chemical character of residues but also on their structural context.

INTRODUCTION

Hydrogen bonding, ionic and hydrophobic interactions play important roles in stabilizing the native structure of a protein as well as in protein folding. It is widely believed that, whereas nonspecific hydrophobic interactions contribute to protein stability, the polar interactions can impart specificity to protein folding. An analysis of the frequency of occurrence of interhelical polar side chain–side chain pairs connected by hydrogen bonds or salt bridges in proteins shows that some of them occur frequently, others rarely, and there are those not to occur at all (Efimov, Kondratova, 2003). It is reasonable to assume that higher frequencies of occurrence of some interhelical pairs show that interactions are favorable for these side chain–side chain pairs compared to others. This does not mean that one side chain “recognizes” the other. The specificity appears at the level of higher order structures, for example, in pairs of closely packed α-helices.

METHODS AND ALGORITHMS

For this study, a data set of 120 non-homologous globular α-proteins and 45 coiled coils was used. Interhelical hydrogen bonds were determined using WHAT IF (http://www.cmbi.kun.nl:1100/WIWWWI/). Interhelical salt bridges were determined with the use of our own software.
IMPLEMENTATION AND RESULTS

In proteins, α-helices can be packed in some different ways and each type of the α-helix packing forms a different structural environment (or structural context) of side chains forming the interface and taking part in interactions. There are two main ways that amphipathic α-helices pack against each other. In the first case, two α-helices are packed so that their hydrophobic side chains form a double layer in the packing interface. Here, hydrophobic stripes of the α-helices interact in a face-to-face manner and hence this is referred to as the face-to-face packing of α-helices. In the case of a side-by-side packing of α-helices, their hydrophobic stripes associate in a side-by-side manner and form a common hydrophobic surface on the bihelical structure. In each case, α-helices can be packed either parallel or antiparallel. Two α-helices neighboring in the chain, packed side-by-side and antiparallel can form either a right-turned or left-turned α-α-hairpin (for details, see Efimov, 1979, 1999).

Figure 1. Comparison of distances between backbone surfaces forming the interface of pairs of α-helices packed in α face-to-face (A) or side-by-side manner (C). The $C\alpha-C\alpha'$ distance (the prime denotes belonging to another helix) is an average distance between $C\alpha$-atoms of closest residues forming the interface. N is the number of α-helical pairs having the corresponding distance. Circular diagrams on the right show the frequency of occurrence of sidechain-sidechain pairs forming interhelical H-bonds in the corresponding sets of α-helical pairs. Donors and positively charged groups of one helix are laid of on the horizontal axes, while ordinates show acceptors and negatively charged groups of the other helix. The radius of the circle is directly proportional to the frequency of occurrence of the corresponding sidechain-sidechain pair.

A stereochemical analysis of these α-helical packings in a data set of 120 non-homologous globular α-proteins and 45 coiled coils has shown that:

i) On average, backbone surfaces forming the interface are arranged closer in the side-by-side packings than in face-to-face packings of α-helices (Fig. 1A, C).

ii) In pairs of α-helices packed face-to-face, the interhelical H-bonds and salt bridges are formed, as a rule, between long side chains (most often Lys-Glu, Lys-Gln, Arg-Glu...
and Arg-Gln pairs, see Fig. 1B), and those in the side-by-side packings are formed by both long and short side chains (Fig. 1D). This appears to be one of the most important determinants of specificity of the α-helix packing in proteins. For example, if two interacting α-helices have no long side chains in the corresponding positions, they can not be packed face-to-face but can be packed side-by-side.

iii) Each type of the α-helix packing has its specific set of rotamers of hydrophobic side chains in a- and d-positions. In other words, selection of side chain rotamers in a- and d-positions of α-helices depends on the type of the α-helix packing and consequently on the structural context. In order to demonstrate this feature we used a representative set of 13 coiled-coil dimers in which α-helices are packed face-to-face and parallel: 1D7M, 1DH3, 1GD2, 1ZII, 1UIX, 1KDD, 1CZ7, 1KQL, 1S9K, 1LLM, 1CE9, 1T6F, 1P9I. Fig. 2 shows that in these proteins most side chains of leucines found in a-positions have trans-isomers (χ1 ≅ 180°) and those in d-position – gauche-isomers (χ1 ≅ -60°). This is a characteristic of α-helices packed face-to-face and parallel. Other packings of α-helices have different sets of side-chain rotamers (Efimov, 1979; Brazhnikov, Efimov, 2006, in preparation). For example, in α-helices packed side-by-side and parallel (as found, e.g., in coiled-coil tetramers) most of Leu residues occupied a-positions have side-chain g-rotamers and those in d-positions t-rotamers. It should be noted that these strong rotamer preferences depending on the residue position and the structural context have been demonstrated for the first time. Earlier computational studies have described the identification and classification of side-chain rotamers as well as the frequency of occurrence of different rotamers depending on the local secondary structure (see, e.g., Dunbrack, Cohen, 1997; Lovell et al., 2000).

Figure 2. Distribution of torsion angles of leucine side chains found in a-positions (A) and d-positions (B) of 13 coiled-coil dimers.

DISCUSSION

A principal prerequisite of bonding is known to be certain proximity of the partners to each other. For H-bonding, the donor-acceptor distance should be less than 3.5 Å (according to WHAT IF criteria) and in salt bridges the distance between heavy atoms of oppositely charged groups should be less than 4 Å. In face-to-face packings of α-helices, bulky hydrophobic side chains are located in the interface and this results in larger interhelical distances as compared with side-by-side packings, where hydrophobic side chains are
arranged on the surface. In our opinion, this is the main reason that only long polar side chains form interhelical H-bonds and salt bridges in face-to-face packings of α-helices (Fig. 1A, B). In side-by-side packings, the interhelical distance is such that short side chains are able to form interhelical H-bonds (Fig. 1C, D). On the other hand, intra- and interhelical interactions between hydrophobic side chains also differ in face-to-face and side-by-side packings of α-helices thus resulting in different sets of hydrophobic side chain rotamers.

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REFERENCES