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Mechanism of Selection of Side-Chain Rotamers in α-Helices

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Abstract—In this study, a possible mechanism of selection of side-chain rotamers based on the rotamer distributions in known coiled-coil proteins is suggested. According to this mechanism, interhelical hydrophobic, polar, and packing interactions bring α-helices closer to each other and this effect squeezes side chains out of the helix–helix interface. As a result, in dimeric coiled coils and long α-α-hairpins where α-helices are packed in a face-to-face manner, most side chains occupying the a-positions have t-rotamers and those in the d-positions g^--rotamers. In tetramers, where α-helices are packed side-by-side, most side chains in the a-positions adopt g^--rotamers and those in the d-positions t-rotamers.

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Structural uniqueness is a characteristic feature of native proteins. The unique packing of side chains in the protein core is essential to attain the overall structural uniqueness of proteins. When a native protein folds, the side chains in the hydrophobic core are generally restricted to a single conformation. The observed side-chain dihedral angles χ_1 cluster around ideal values, such as the –60°, +60°, and 180°, that are often referred to as *gauche* minus (g^-), *gauche* plus (g^+), and *trans* (t), respectively, which is confirmed statistically [1-8]. The rotamers of most side chains in proteins are limited to a smaller number that depends on the secondary structure. For example, in α-helices the C_β-branched hydrophobic side chains of Val and Ile have a single allowed rotamer, and those of Leu, Phe, and Tyr (these are the Cγ-branched side chains) can have two, g^-- or t-rotamers [1-8]. In this study, the analysis was restricted to residues Leu, Phe, and Tyr as their stereochemical properties are similar and they can have two, t- and g^-- rotamers of side chains in the α-helical conformation.

Most papers mentioned above describe the distribution of side-chain rotamers in proteins and the frequency of occurrence of each rotamer in the PDB. In the present study, the problem of selection of side-chain rotamers is considered in order to find a possible mechanism of their selection in α-helical coiled-coil proteins.

MATERIALS AND METHODS

In this study, coiled-coil proteins and long α-α-hairpins have been analyzed. The coiled-coil structures are usually formed by long α-helices packed at angle $\Omega = 20°$ into parallel or antiparallel dimers, trimers, tetramers, or pentamers. Coiled-coil-forming amino acid sequences share a characteristic heptad repeat, $(abcdefg)_n$, with predominantly hydrophobic residues in the a- and d-positions and polar residues generally elsewhere. In many coiled-coil proteins and their designed analogs called "leucine zippers", the a- and/or d-positions are occupied by Leu residues only. These structures are of great interest as such a homogeneous set of hydrophobic side chains facilitates the finding of features of the rotamer distributions. To reduce the influence of the heterogeneity on the results of the analysis, coiled coils in which the a- and d-positions are most often occupied by Leu, Phe, and Tyr have been selected for this study. These side chains have similar stereochemical properties, i.e. they are similar in size and they are C_γ-branched and can have two, t- or g^--rotamers in α-helices. Three sets of proteins have been compiled and used: 1) dimers having parallel packing of α-helices (their PDB codes are 1D7M, 1DH3, 1GD2, 1ZII, 1UIX, 1KDD, 1CZ7, 1KQL, 1S9K, 1LLM, 1CE9, 1T6F, 1P9I); 2) long α-α-hairpins and antiparallel

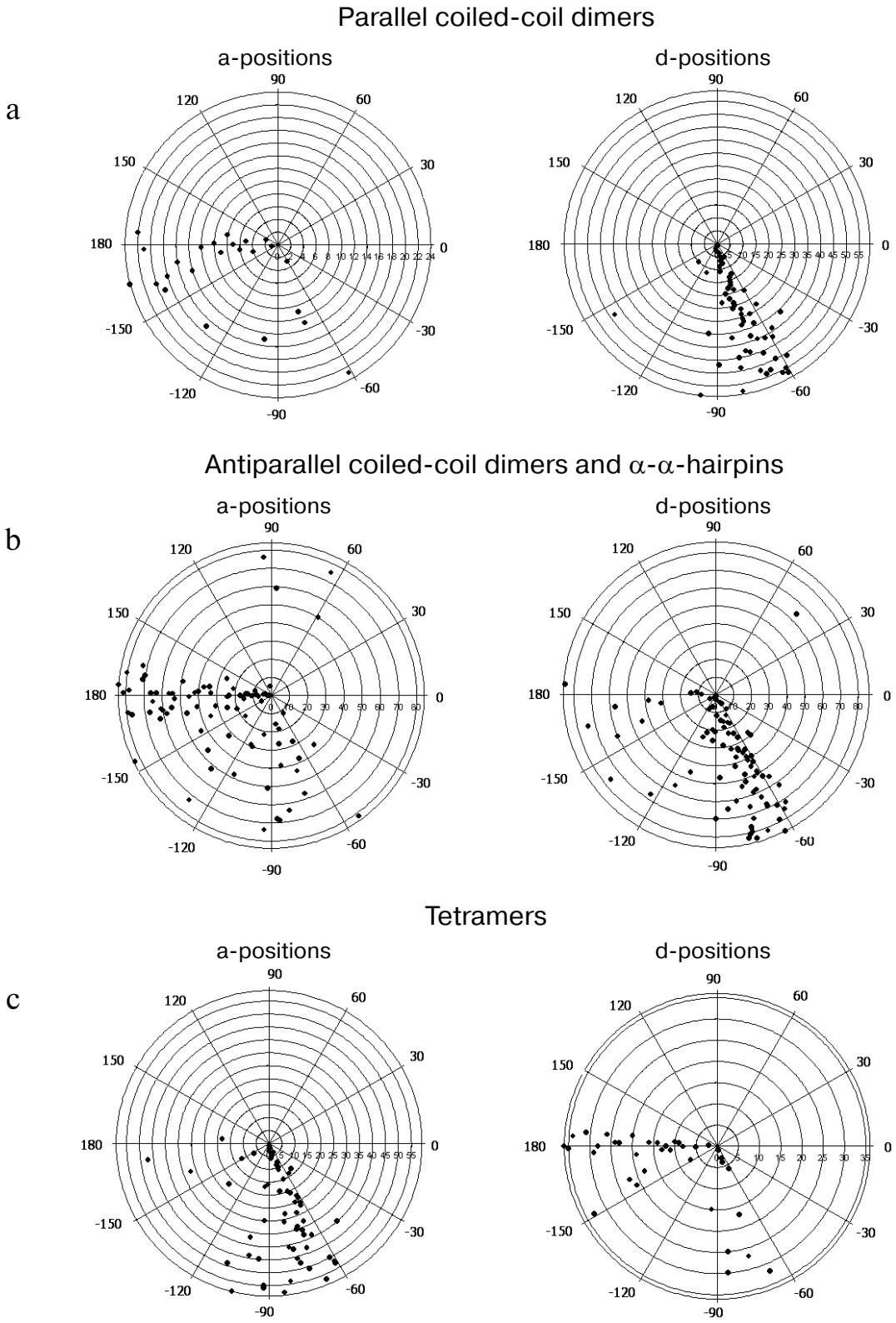

Fig. 1. Distribution of torsion angles χ_1 of hydrophobic side chains found in a- and d-positions of parallel coiled-coil dimers (a), antiparallel dimers and long α-α-hairpins (b), and tetramers (c) shown with circular diagrams. The bottom half of each circle shows negative values (0-(−180°)) and the upper half positive values (0-180°) of angles χ_1. Numbers on the horizontal axis show the number of experimental dots (χ_1 values) falling into the corresponding circle.

dimers (1A32, 1A36, 1AQT, 1CXZ, 1DG3, 1E79, 1FKA, 1FXK, 1GRJ, 1IDS, 1QOJ, 1QSD, 1SRY, 1CII, 1L8D, 1FPO, 1A92, 1GMJ, 1QO5, 1YF2, 2C2A, 1AYU, 1ZHC, 1K1F, 1ENV, 1JAL, 1QVR); and 3) tetramers (2BNI, 2B1F, 1W5K, 1C94, 4HB1, 1GCL, 1SFC, 1GL2, 1HVV, 1VDF, 1NHL, 1JTH, 2B1F, 2HB1, 1GCL, 1G1I, 1HTM). The atomic coordinates have been obtained from the Protein Data Bank (http://www.rcsb.org/pdb). Torsion angles χ_1 have been calculated with the program MOLMOL [9]. Assignment of the heptad positions (a-g) in the selected coiled coils was carried out manually by inspecting their structures with the program RasMol [10].

RESULTS AND DISCUSSION

In this work, we have taken into account our previous results concerning the same problem: i) the rotamer distribution in the a- and d-positions depend on the type of α-helix packing [11]; ii) close packing of hydrophobic side chains on the surface of α-helices is obtained for definite combinations of their rotamers [12, 13]. There are two main ways that amphipathic α-helices pack against each other, "face-to-face" and "side-by-side" packing [12-14]. In the first case, two α-helices are packed so that their hydrophobic side chains form a double layer in the helix-helix interface, and 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. As seen in Fig. 1a, in the coiled-coil dimers in which the α-helices are packed face-to-face and parallel, leucine side chains found in the a-positions have t-rotamers ($\chi_1 = 180 \pm 30°$) and those in the d-positions have g^--rotamers ($\chi_1 = -50 - (-100°)$). The rotamer distribution of residues Leu, Phe, and Tyr found in the a- and d-positions of coiled-coil dimers and long α-α-hairpins where α-helices are packed face-to-face and antiparallel is similar to that observed in parallel dimers, although there are some deviations (Fig. 1b). The main reason for this is that antiparallel dimers have heterogeneous sets of residues in the a- and d-positions (Leu, Phe, Tyr) and the parallel dimers have only leucines. The rotamer distributions in coiled-coil tetramers are shown in Fig. 1c. As seen, the rotamer distributions in tetramers are quite different from those in dimers. Here most side chains found in the a-positions have g^--rotamers and those in d-positions t-rotamers.

The features of the rotamer distribution and the rotamer preferences described above can be explained taking into account the principle of close packing and interhelical interactions. Interhelical hydrophobic, polar, and packing interactions bring α-helices closer to each other, and this effect squeezes side chains out of the helix–helix interface. Figure 2 shows that in order to obtain a closer packing of α-helices in a coiled-coil dimer, leucine side chains should change from g^-- to t-rotamers in the a-positions and from t- to g^--rotamers in the d-positions. It looks like squeezing of side chains out of the helix–helix interface. Analysis shows that essentially the same is observed if the a- and d-positions are occupied by residues Phe or Tyr as well as by Leu-Phe, Phe-Leu, and Leu-Tyr combinations. In the long α-α-hairpins and coiled-coil dimers with antiparallel packing of α-helices, the overall picture of the squeezing is similar, but side chains are packed in ad- and da-layers instead of the a- and d-layers in the parallel dimers.

Figure 3 demonstrates the similar transitions of leucine side chains occupying the a- and d-positions in the coiled-coil tetramers having parallel packing of α-helices. The transition of leucine side chains occupying the a-positions from t- to g^--rotamers allows the α-helices in the tetramer to be closer to each other, and this makes the overall structure more compact (Fig. 3a). It looks like each pair of adjacent α-helices squeezes side chains out of the interface to the hydrophobic core (those in a-positions) or outside (side chains occupying the g-positions). If leucine side chains (shown with dashed lines in Fig. 3a) or other side chains of similar size occupy g-positions of α-helices, they should preferably adopt g^--rotamers, as there is a definite similarity of the α-helix packing in tetramers and dimers. Let us compare the packing of side-chain in the d-layer of the dimer (Fig. 2b) and, for example, in the bottom pair of α-helices in the tetramer (Fig. 3a). As seen, leucine side chains in a- and g-positions of the tetramer have g^--rotamers and are packed as well as the side chains in the d-positions of the dimer (Fig. 2b).

The models shown in Fig. 3b demonstrate that rather compact tetramer structures can be obtained when leucines occupying the d-positions have both t- and g^--

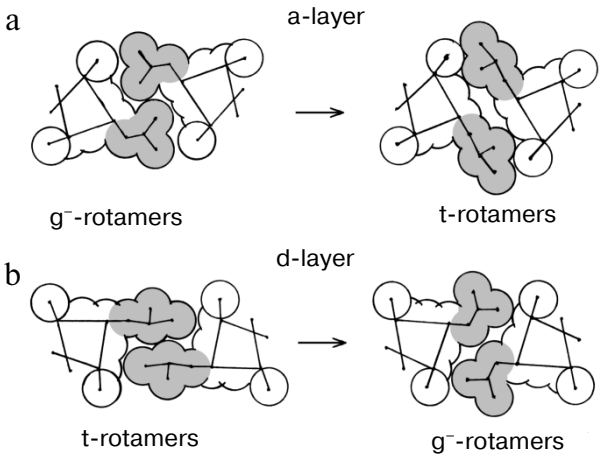

Fig. 2. Schematic drawing showing transitions of leucine side chains from g^-- to t-rotamers in the a-positions (a) and from t- to g^--rotamers in the d-positions (b) when two α-helices in a coiled-coil dimer move closer to each other.

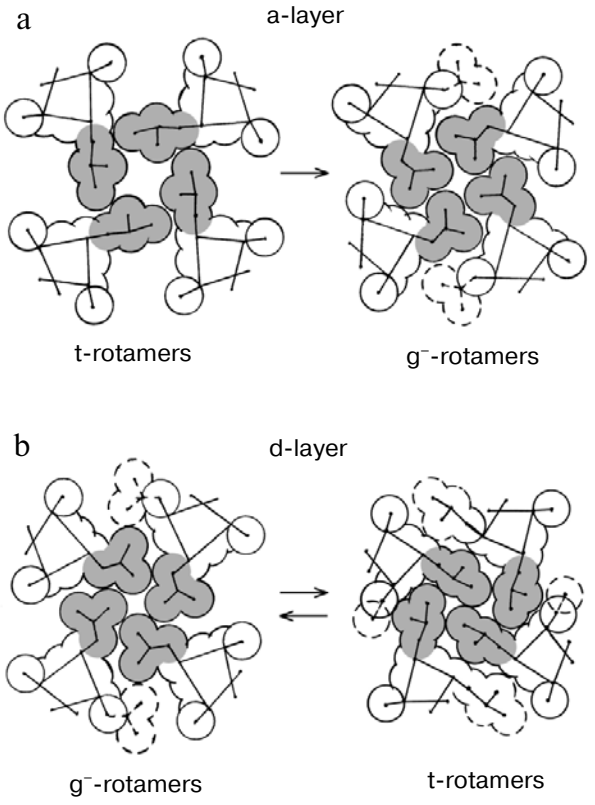

Fig. 3. Models of packing of leucine side chains in the a- and d-layers of tetramers with parallel packing of α-helices. a) A schematic transition of a less compact tetramer with leucine t-rotamers in the a-positions to a more compact tetramer structure having leucine g^--rotamers in the a-layer. b) A scheme of the transition of leucine side chains from g^-- to t-rotamers in the d-layer of the tetramer.

rotamers. Nevertheless, a detailed analysis of the α-helix packing in the tetramer and comparison with dimers show that the packing of side chains having t-rotamers is preferable. As can be seen, the packing of side chains in the a-layer of the dimer (Fig. 2a) is very similar to the packing of leucine side chains occupying the d- and e-positions of the tetramer (see, e.g., the upper or bottom pair of α-helices in Fig. 3b). In the e-positions, both hydrophobic (Leu, Phe, Tyr) and polar (Arg, Lys, Glu, etc.) side chains should adopt t-rotamers, because g^--rotamers could result in steric "clashes" of their C_γ-atoms and side chains of the neighboring α-helix.

Thus the side chain conformation depends on both the residue position and the type of α-helix packing. The observed features of the rotamer distributions in coiled-coil structures can be explained by the squeezing mechanism suggested in this study. All this allows us to suggest that a similar mechanism works in globular proteins, which will be analyzed in the near future.

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