Keywords: homology modelling of proteins, protein-lipid interactions, structure-function relationships

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Relationship between sequence and structure of CDR-H3 in antibodies

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Antibody modeling is widely used for the analysis of antibody antigen interaction and for the design of potent antibody drug. Antibody combining site is composed of six complementarity determining regions (CDRs). The CDRs except for CDR-H3 is known to have limited numbers of canonical structures, and one can identify one of the canonical structures from the amino acid sequence. CDR-H3 lies in the center of antigen-binding site and shows significant variability in its length, sequence, and structure. Although it is not enough to accurate modeling, the method to classify CDR-H3 structure from the amino acid sequence was also proposed. However, after these methods to classify CDR structures were developed, many more antibody crystal structures were determined. It has enabled us to revise H3-rules, the method to classify CDR-H3 structure. In this work, we show recent progress of H3-rules based on systematic analyses of other five CDRs. As a consequence of the relative spatial positions in the CDRs, some basic residues on VL domain affect the conformation of CDR-H3. We also show the usefulness of whole structural feature of CDR-H3 prediction from the amino acid sequence. We can determine whether the hydrogen bond ladders or beta-turn are formed or not by H3-rules. Our revised H3-rules have the high accuracy of CDR-H3 structure prediction compared to the other methods. Since modeling the antibody structures is crucial for the design and analysis of potent antibody drugs for specific antigens, our empirical rules derived from large amount of structural data are expected to be used in antibody structure analysis and drug discovery. Structural analysis server, H3-rules 2007, can be accessed on the web: http://www.protein.osaka-u.ac.jp/rcsfp/pi/H3-rules/.

Keywords: antibodies structure, molecular modelling, sequence analysis

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Comparative analysis of putative NADPH- and NADH-dependent ketopantoate reductase

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The pantothenate (vitamin B_5) biosynthesis pathway has been proposed as a potential target for antimicrobials. Ketopantoate reductase (KPR, E.C. 1.1.1.169) is the second enzyme in the pathway and catalyzes the NADPH-dependent reduction of ketopantoate to pantoate. In an extensively studied *E.coli* KPR ternary complex (PDB code: 2OFP) the cofactor is bound in the active site cleft between the N-terminal Rossmann-fold domain and the C-terminal α-helical domain; a significant hinge bending encloses the active site around ketopantoate to provide a solvent-inaccessible environment in which catalysis occurs. Structural genomics projects have provided two more putative KPR crystal structures from *E.faecalis* (PDB code: 2EW2) and *P.gingivalis* w83 (PDB code: 2QYT). All three proteins adopt similar overall structures with conserved catalytically important residues, suggesting that their reaction mechanisms are similar. The putative NADPH 2'-phosphate binding-site of 2QYT (Arg42) is similar to that of *E.coli* KPR (Arg31) but differs in 2EW2 (Asp30). Comparative analyses of cofactor binding domains of homologous proteins suggest that 2EW2 is NADH-dependent and 2QYT is NADPH-dependent. We have predicted cofactor and substrate binding sites and their binding mode using docking studies. Based on the sequence analysis of KPR family members including Methicillin-resistant *S.aureus* (MRSA) we have hypothesized that both NADPH- and NADH-dependent KPR are widely distributed in different organisms. As a case study, we have modeled the putative KPRs Q2FV20 and Q2FVH3 from *S.aureus* (strain NCTC 8325) and our analysis suggest that they are NADPH- and NADH-dependent, respectively. These models could contribute in understanding their reaction mechanisms and eventually to designing novel inhibitors.

Keywords: computer-aided molecular modelling, docking, cofactors

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Dynamics of EcoO109I studied by small-angle X-ray scattering and molecular dynamics simulation

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EcoO109I is a type II restriction endonuclease, which forms homo dimer. Upon DNA-binding, the two subunits rotate counter-clockwise relative to each other and the two catalytic domains undergoes a large structural change to capture the DNA. Using 150 ns of molecular dynamics simulation of the DNA-free form, we investigated intrinsic dynamics of EcoO109I in solution and its relation with the structural change. In the simulation, the overall structure fluctuated largely, which led to large fluctuation in the radius of gyration. The small-angle X-ray scattering profile calculated from the simulation, in which the scattering from explicit water molecules were taken into account, has shown an excellent agreement with the experimental profile. We performed a principal component analysis and found that the main dynamics was the counter-clockwise motion of the two subunits, which is observed in the structural change. We also found that the dynamics of the catalytic domains correlates well with the structural change. These strong correlations between the intrinsic dynamics and the structural change indicate that the structure of EcoO109I is very flexible in the direction of its functional movement intrinsically, and therefore can effectively achieve its structural change upon binding.

Keywords: small-angle X-ray scattering, intrinsic dynamics, structural change