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Structural studies of the avidin complexes with ligands

Avidin is a tetrameric glycoprotein present in the egg-white of amphibians, reptiles and birds, which is capable of binding biotin with exceptionally high affinity. The interaction between avidin and biotin, its natural ligand, is one of the strongest non-covalent interaction known in nature and that is why became useful in many laboratory techniques. Currently various biotin derivatives with potentially weaker affinities are being synthesized to modulate the strength of interaction or to obtain desire conjugates, which can be used in the form of complex with avidin in the analytical techniques and as drug carriers.

In doctoral dissertation crystal structures of avidin complexes with ligands were solved using X-ray crystallography. The main aim of the project was to investigate the interactions between avidin and fluorescent ligands or metallocene biotin derivatives. The ligands had been synthesized by Damian Plażuk, Ph.D., D.Sc., from Department of Organic Chemistry, Faculty of Chemistry, University of Lodz. The experimental work included: purification of protein, complexes formation, crystallization, X-ray data collection and processing, structure determination, refinement and validation. The analysis of the obtained structures of the avidin complexes with ligands contributed to overall knowledge about the interactions of avidin with biotin conjugates.

In the first stage of research, the crystal structure of avidin-biotinylruthenocene complex was determined. Biotinylruthenocene (RuBiot) is a biotin derivative, which consists a carbonyl group located close to ruthenocene moiety. This carbonyl group plays a significant role in stabilization of the ligand in the avidin binding pocket. The important interactions of biotinylruthenocene in the pocket are also the π-electron, involving contacts between both cyclopentadienyl rings of ruthenocene and two residues: Ser73 and the guanidinium moiety of Arg114. In order to investigate, how the change of the atom in the metallocene (Ru→Fe) will affect the binding of the ligand to the protein, the structure of the avidin-FcBiotOH complex was solved. FcBiotOH is a biotin derivative, which consists a hydroxyl group next to the ferrocene moiety. The ferrocene moiety is stabilized in the binding pocket by π-electron interaction, involving contact between the cyclopentadienyl ring and Ser101, in which the polar hydroxyl group of the Ser101 residue is a proton donor and the π-electron system of the cyclopentadienyl ring serves as an acceptor. The structure shows that one of the significant interactions, that stabilizes ligand in the cavity, is the hydrogen bond between the hydroxyl group, which is located close to the ferrocene moiety, and two residues: Ser73 and Ser75. A set of hydrogen bonds stabilizing the bicyclic ring system of both ligands in the deepest part of the binding pocket is retained but the rest of the bonds differ between structures as well as in comparison to those occurring in the avidin-biotin complex.

In the next stage of research, the nature of the interaction of two ferrocene biotin derivatives with avidin were investigated. The ligands contained an additional methylene

group (FcHomoBiot-en) or an aliphatic linker (FcHexHexBiot). Both ligands are anchored in the binding cavity of avidin by hydrogen bonds and the ferrocene moiety does not interact directly with the protein, but is stabilized by the amino acids of the loops surrounding the binding site.

Comparison of the determined structures with the structure of unbound form of avidin showed that the main differences occur in the CD, DE and FG loop regions. The most interesting was the conformational change of the CD loop, which can regulate access to the binding pocket. Superposition of C_α atoms of the structures shows that the position of the CD loop is correlated with the presence of the ligand in the binding pocket. The crystal structures of avidin complexes with metallocene biotin derivatives allowed to determine the effect of individual functional groups on the position and stability of the ligands.

In the last stage of study, three crystal structures with fluorescent ligands (pyrene biotin derivative, pyrene desthiobiotin derivative and HABA) were determined. The structures allowed to characterize the protein-ligand interactions and to determine the effect of the process of binding fluorescent derivatives on the conformation of the loop surrounding the binding site.

All solved structures were deposited in the Protein Data Bank (PDB codes: 4I60, 4JHQ, 5HLM, 5MYQ, 5IRU, 5IRW). Structural studies confirmed that, despite the hydrophobic nature of the binding site, hydrogen bonds play an important role in stabilizing ligands in the β-barrel of avidin. The protein-ligand stoichiometry in crystal structures is 1:1. The obtained structures for the first time demonstrated the possible way of interaction of the metallocene or pyrene moiety with avidin. They have also shown that a relatively narrow protein binding site can accommodate large aromatic moieties.