

## Abstract

Understanding protein function highly benefits from the knowledge of its three-dimensional structure, especially in the case of protein-ligand complexes. Structural biology methods such as X-ray crystallography, SAXS and NMR are therefore widely used for structural studies of protein-ligand interaction. In this work, these methods were used to understand two biological processes involving protein interactions: X-ray structural analysis was used to study binding of effector molecule to a prokaryotic transcription factor. NMR and SAXS techniques were used to study interaction of a monoclonal antibody with its protein antigen.

Transcriptional regulator DeoR negatively regulates the expression of catabolic genes for the utilization of deoxyribonucleosides and deoxyribose in *Bacillus subtilis*. DeoR comprises an N-terminal DNA-binding domain and a C-terminal effector-binding domain (C-DeoR), and its function is regulated by binding of a small-molecular effector deoxyribose-5-phosphate. We determined crystal structures of C-DeoR both in the free form and in complex with deoxyribose-5-phosphate. Structural analysis revealed unique covalent binding of effector molecule through a reversible Schiff-base double bond with an effector-binding-site lysine residue. The physiological nature of this binding mode was confirmed by mutational analysis and mass spectrometry of the Schiff-base adducts formed in solution. Comparison of the free and effector-bound structures of C-DeoR explained on a molecular level the mechanism of DeoR function as a molecular switch.

CD44, the cell receptor for hyaluronate, is involved in cell adhesion, migration, and tumor metastasis. CD44 binds hyaluronate through the hyaluronate-binding domain (HABD), which triggers an extensive conformational rearrangement in HABD that induces CD44 shedding. We constructed a single-chain variable fragment (scFv) of antibody MEM-85. MEM-85 is of therapeutic interest, recognizes a poorly characterized epitope in HABD, blocks hyaluronate binding, and induces CD44 shedding. Antibody scFv fragments exhibit poor homogeneity caused by scFv oligomerization. We developed a novel optimization approach, which we used for the preparation of a homogeneous scFv sample for NMR-based mapping of the epitope of MEM-85 in HABD. This mapping together with mutational analysis identified epitope residues in the C-terminal tail of the HABD. Additionally, we built a rigid-body model of the HABD – scFv MEM-85 complex based on the SAXS data. These biophysical methods provided a detailed insight into the mechanism of MEM-85 action: MEM-85 binds to an epitope located outside the hyaluronate-binding site and induces a conformational change in the HABD tail, similar to that induced by hyaluronate. The mechanism of MEM-85 cross-blocking of hyaluronate binding is not a direct physical competition, but rather an allosteric, relay-like effect.