

USE OF SYNCHROTRON RADIATION IN STUDIES OF PROTEIN STRUCTURE AND NUCLEIC ACID BINDING

U. Heinemann^{1,2*}, **B. König**¹, **E. Lanka**³, **A. Schütz**¹, **D. Carstanjen**⁴, and **K.E.A. Max**¹

¹ Crystallography, Max-Delbrück-Center for Molecular Medicine, Robert-Rössle-Str. 10, 13125 Berlin, Germany

² Institute of Chemistry and Biochemistry, Free University of Berlin, Takustr. 6, 14195 Berlin, Germany

³ Max-Planck-Institute of Molecular Genetics, Ihnestr. 73, 14195 Berlin, Germany

⁴ Leibniz-Institute for Molecular Pharmacology, Kraemerstr. 6, 12207 Berlin, Germany

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*) e-mail: heinemann@mdc-berlin.de

Interactions between proteins and DNA or RNA are not governed by simple lock-and-key mechanisms. Instead, the conformation of both the protein and the nucleic acid may be altered upon binding. In some cases, a defined three-dimensional structure non-existent in the apo state may be brought about by protein-nucleic-acid complex formation. In addition, a thermodynamic stabilization of the folded state may be observed. High-resolution crystal structures, accessible through synchrotron-based diffraction experiments, are indispensable for elucidating these structural principles which will be illustrated with three examples.

(i) Binding of Krueppel-like factor 4 (Klf4) to double-stranded (ds) DNA carrying the Klf4 target sequence leads to an induction of tertiary structure in the protein's zinc-finger domain. The spatial disposition of the three C2H2 zinc fingers of Klf4 is determined by a rigid dsDNA scaffold.

(ii) The plasmid RP4-encoded repressor KorB [1, 2] displays structural flexibility which facilitates the protein's functional cooperation with co-repressor KorA on RP4 promoters [3]. Flexible linkers between folded domains of KorB are important for its interaction with KorA over a fixed distance, but with variable geometry.

(iii) Cold shock domains interact with single-stranded (ss) DNA or RNA. The bacterial major cold shock proteins Bs-CspB [4, 5] and Bc-Csp [6, 7] bind ssDNA and ssRNA sequence-specifically and with high affinity to a conserved surface [8, 9]. The nucleic-acid single strands acquire a defined 3D structure upon protein binding. The same protein surface is used for ssDNA and ssRNA binding to the homologous cold-shock domain of the human transcription factor YB-1. YB-1 assumes spectral characteristics of a folded protein and is conformationally stabilized upon binding to a single-stranded nucleic acid.

References

- [1] H. Delbrück, G. Ziegelin, E. Lanka. U. Heinemann, "An Src homology 3-like domain is responsible for dimerization of the repressor protein KorB encoded by the promiscuous IncP plasmid RP4", *J. Biol. Chem.* **277** (2002) 4191–4198.
- [2] D. Khare, G. Ziegelin, E. Lanka. U. Heinemann, "Sequence-specific DNA binding determined by contacts outside the helix-turn-helix motif of the ParB homolog KorB", *Nature Struct. Mol. Biol.* **11** (2004) 656–663.
- [3] B. König, J. Müller, E. Lanka. U. Heinemann, "Crystal structure of KorA bound to operator DNA: insight into repressor cooperation in RP4 gene regulation", *Nucleic Acids Res.* **37** (2009) 1915–1924.
- [4] H. Schindelin, M. Herrler, G. Willimsky, M.A. Marahiel, U. Heinemann, "Overproduction, crystallization, and preliminary X-ray diffraction studies of the major cold shock protein from *Bacillus subtilis*, CspB", *Proteins Struct. Funct. Genet.* **14** (1992) 120–124.
- [5] H. Schindelin, M.A. Marahiel, U. Heinemann, "Universal nucleic acid-binding domain revealed by crystal structure of the *B. subtilis* major cold-shock protein", *Nature* **364** (1993) 164–168.
- [6] U. Mueller, D. Perl, F.X. Schmid, U. Heinemann, "Thermal stability and atomic-resolution crystal structure of the *Bacillus caldolyticus* cold shock protein", *J. Mol. Biol.* **297** (2000) 975–988.
- [7] D. Perl, U. Mueller, U. Heinemann, F.X. Schmid, "Two exposed amino acid residues confer thermostability on a cold shock protein", *Nature Struct. Biol.* **7** (2000) 380–383.
- [8] K.E.A. Max, M. Zeeb, R. Bienert, J. Balbach, U. Heinemann, "T-rich DNA single strands bind to a preformed site on the bacterial cold shock protein Bs-CspB", *J. Mol. Biol.* **360** (2006) 702–714.
- [9] K.E.A. Max, M. Zeeb, R. Bienert, J. Balbach, U. Heinemann, "Common mode of DNA binding to cold shock domains. Crystal structure of hexathymidine bound to the domain-swapped form of a major cold shock protein from *Bacillus caldolyticus*", *FEBS J.* **274** (2007) 1265–1279.