Investigation of DNA-binding properties of the UxuR and ExuR proteins, regulators of hexuronate metabolism in gammaproteobacteria.

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Besides glycolysis that converts glucose into two molecules of pyruvate relieving free energy in the form of ATP and NADH, gammaproteobacteria can get energy from several alternative metabolic pathways induced in stress conditions. For example, *Escherichia coli* (E. coli) can use the hexuronates D-glucuronate and D-fructuronate as the sole carbon sources. These sugars are metabolized by the Ashwell pathway, which generates intermediates that are converted to pyruvate via the Entner–Doudoroff pathway. The hexuronate metabolism in *E. coli* is regulated by two related transcription factors from the GntR family, UxuR and ExuR, which have 46% identity. Using various genomic approaches the binding sites of ExuR and UxuR proteins on *E. coli* chromosomal DNA have been determined and a number of targets for the proteins, including autoregulation sites, have been identified. Bioinformatics analysis of the obtained results allowed to determine DNA consensuses which are specifically recognized by ExuR and UxuR.

To measure the binding constants of selected oligonucleotides to the proteins the surface plasmon resonance has been chosen. The affinity of the UxuR protein for DNA fragments containing the region that is supposed to be specifically recognized by the UxuR protein is in the nanomolar range. The ExuR protein forms a less stable complex with a DNA fragment containing the putative region specifically recognized by the ExuR protein. A DNA fragment containing a nucleotide sequence that can be recognized by both proteins binds to proteins with different affinities. These results allow us to obtain stable DNA-protein complexes suitable for crystallization.

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