Selection and design of high affinity DNA ligands for mutant single-chain derivatives of the bacteriophage 434 repressor

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Abstract Single-chain repressor RR_{TRES} is a derivative of bacteriophage 434 repressor, which contains covalently dimerized DNA-binding domains (amino acids 1—69) of the phage 434 repressor. In this single-chain molecule, the wild type domain R is connected to the mutant domain R_{TRES} by a recombinant linker in a head-to-tail arrangement. The DNA-contacting amino acids of R_{TRES} at the −1, 1, 2, and 5 positions of the α3 helix are T, R, E, S respectively. By using a randomized DNA pool containing the central sequence -CATACAAGAAAGNNNNNNTTT-, a cyclic, *in vitro* DNA-binding site selection was performed. The selected population was cloned and the individual members were characterized by determining their binding affinities to RR_{TRES}. The results showed that the optimal operators contained the TTAC or TTCC sequences in the underlined positions as above, and that the Kd values were in the 1×10^{-12} mol/L—1×10^{-11}mol/L concentration range. Since the affinity of the natural 434 repressor to its natural operator sites is in the 1×10^{-9} mol/L range, the observed binding affinity increase is remarkable. It was also found that binding affinity was strongly affected by the flanking bases of the optimal tetramer binding sites, especially by the base at the 5′ position. We constructed a new homodimeric single-chain repressor $R_{TRES}R_{TRES}$ and its DNA-binding specificity was tested by using a series of new operators designed according to the recognition properties previously determined for the R_{TRES} domain. These operators containing the consensus sequence GTAAGAAARNTTACN or GGAAGAAARNTTCCN (R is A or G) were recognized by $R_{TRES}R_{TRES}$ specifically, and with high binding affinity. Thus, by using a combination of random selection and rational design principles, we have discovered novel, high affinity protein-DNA interactions with new specificity. This method can potentially be used to obtain new binding specificity for other DNA-binding proteins.

Keywords: binding site selection, bacteriophage 434 repressor, single-chain repressor, protein-DNA interaction, protein engineering.

The X-ray crystal structure of the bacteriophage 434 repressor and its N-terminal-DNA complex[1, 2] showed that 434 repressor binds DNA as a dimer specifically. The specific binding mediates the regulatory switch[3—5] between the lysogenic and lytic life cycles of the phage. The DNA binding domain (DBD) of 434 phage repressor has a Helix-Turn-Helix motif (HTH), and the amino acids Gln28, Gln29, Gln33 and Ser30 are critical for DNA recognition. Changing the binding specificity is challenging, there are several examples for mutant 434 repressors[6—9], but not all of these mutants represent new binding specificity. Creation of new, artificial dimers, for example