Holo-ZitRMG binding to dsDNA fragments by ITC

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MATERIALS TEXT

The interaction of fully purified $ZitR_{MG}$ protein with dsDNA of different sizes (19- and 20mers), and each containing one DNA-binding domain was explored. Complementary oligonucleotides containing an imperfect **TTAAC**YR**GTTAA** palindrome overlapping either the -35 or the -10 box of the ZitR-controlled promoter region (see Table S1 for forward oligonucleotide sequences) were purchased (SIGMA, Eurogentec and Invitrogen), and purified by SDS-PAGE or desalting. 5' overhang nucleotides (adenine in the forward ssDNA oligonucleotide and thymine on the reverse one) were added to the sequence in order to make the dsDNA stickier, which might help in crystallization. Annealing of complementary forward and reverse oligonucleotides were carried out by incubation at 95 °C during 5 min in a 20 mM Tris-HCl (pH 8) and 150 mM NaCl buffer, followed by incubation on ice to slowly decrease the temperature.
Isothermal Titration Calorimetry (ITC) was performed on a Microcal ITC200 (GE Healthcare) (calorimetry platform, IBBMC/IMAGIF). Purified $ZitR_{MG}$ protein was dialyzed against a 20 mM Tris-HCl (pH 7.0), 150 mM NaCl and 100 µM $ZnSO_4$ buffer. Duplicate titration of approximately 20 mM $ZitR_{MG}$ protein, while stirring at 1000 rpm, was carried out by 20 injections of 2 ml of each dsDNA at 270 mM in the same buffer as the protein. The heat generated by DNA dilution was determined from the peaks measured after full saturation of the protein. Experimental data were fitted to the theoretical titration curves using the Origin software (OriginLab, Northampton, MA) according to the relationship between the heats generated by each injection. The following values were calculated: AH_{cal}, enthalpy change in kcal.mol^{-1}; K_a, association-binding constant in M^{-1}; n, number of binding sites. The binding constant of each interaction is expressed as $1/K_a = K_d$ (in mol.L^{-1}).