Supplementary Material (ESI) for Molecular BioSystems

Supplementary Information

Temperature and salt concentration alter base-sequence selectivity of a duplex DNA–binding protein

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Experimental

Preparation of *Pho*TBP

Genes encoding TATA-box-binding protein from *Pyrococcus horikoshii* (*Pho*TBP) were amplified by means of polymerase chain reaction (PCR) with KOD-Plus DNA polymerase (Toyobo, Japan) and *Pyrococcus horikoshii* genomic DNA as a template. The PCR products were inserted into the pET26b vector (Merck, Germany). The correctness of the DNA sequence was confirmed by use of an ABI 3100 Genetic Analyzer (Applied Biosystems, Japan). Using the *Escherichia coli* Rosetta2(DE3) expression system (Merck), we obtained *Pho*TBP containing a C-terminal (His)6-tag as soluble protein. Rosetta2(DE3) cells harboring the appropriate expression plasmid were precultured overnight with shaking at 28 °C in 3 mL lysogeny broth (LB) medium with 30 mg L^{-1} kanamycin and 34 mg L^{-1} chloramphenicol. The precultured cells were inoculated into 200 mL LB medium containing 30 mg L^{-1} kanamycin and 34 mg L^{-1} chloramphenicol and shaken at 28 °C until the optical density (OD) at a wavelength of 600 nm reached approximately 0.6. Isopropyl β-D-thiogalactopyranoside (final concentration, 0.5 mM) was added, and the mixture was shaken overnight at 28 °C. Cells were harvested by centrifugation at 7000 × g for 10 min and then suspended in 20 mM sodium phosphate buffer. The suspension was sonicated and then centrifuged at 40000 × g for 30 min. The soluble fraction was incubated at 70 °C for 30 min and then centrifuged at 40000 × g for 30 min. The resulting supernatant was purified by immobilized metal affinity chromatography using His-bind resin (Novagen, Japan), and then further purified on a HiLoad 26/60 Superdex 75 prep grade column (GE Healthcare Biosciences, Japan). The purity of the *Pho*TBP was confirmed by sodium dodecyl sulfate – polyacrylamide gel electrophoresis. The concentration of *Pho*TBP was determined from the molecular absorption coefficient at a wavelength of 280 nm (11085 M^{-1} cm^{-1}) and from the molecular mass (22.6 kDa).

In vitro selection

As a DNA pool, a 68-nucleotide (nt) DNA template containing a 24-nt randomized sequence of centrally positioned oligonucleotides (5'-CGGAATTCCGATAAGTCGAGTC -N24- CAATGTACTAATCGGGATCCCG-3') was amplified to DNA duplexes via the action of rTaq DNA polymerase (Takara Bio Inc., Japan) using the forward primer (5'-CGGAATTCCGATAAGTCGAGTC-3') and the reverse primer (3'-GTTACATGATTAGCCCTAGGGC-5'). First, the PCR fragments of the DNA duplex were incubated with Ni-NTA superflow resin (Qiagen Inc., Japan) only in the binding buffer (20 mM Tris•HCl [pH 7.7], 1 M or 150 mM NaCl, 10 mM imidazole) for 30 min at room temperature to remove nonspecific DNAs that had interacted with the resin. The treated DNAs (50 pmol) were incubated in the binding buffer for 30 min at 50 or 25 °C with resin-bound *Pho*TBP (5 nmol) containing a C-terminal (His)6-tag. After being washed for about 30 seconds (5 times) with the binding buffer, the *Pho*TBP–DNA complexes were eluted with elution buffer (20 mM Tris•HCl [pH 7.7], 1 M or 150 mM NaCl, 500 mM imidazole). The bound DNA fragments were extracted from the eluted solution by conventional phenol–chloroform extraction and ethanol precipitation. Extracted fragments were amplified by PCR using the forward and reverse primers described above, and the PCR products were used as a DNA pool for the next cycle of selection. This process was repeated to a total of 7 cycles. DNA fragments selected after these cycles were cloned into pGEM-T Easy Vector (Promega, USA), and the DNA sequences of their random regions were analyzed.

Estimation of binding constants [1,2]

Binding constants (K_a) of the interactions between *Pho*TBP and selected DNAs were determined with a ITC instrument (VP-ITC, MicroCal). Under the condition of NaCl at various concentrations (400, 600, 800, 1000, 1200, 1400, 2000 mM) in 20 mM Tris•HCl (pH 7.5) and 1 mM EDTA at 50 or 25 °C, each degassed DNA solution (5 – 70 μM) was titrated with *Pho*TBP (75 – 665 μM) in the same buffer. Control experiments were carried out to calculate the heat of dilution for *Pho*TBP into the buffer containing 20

mM Tris•HCl (pH 7.5) and 1 mM EDTA under the condition of NaCl at various concentrations (400, 600, 800, 1000, 1200, 1400, 2000 mM) at 50 or 25 °C. The thermogram for the interaction between *Pho*TBP and each DNA was determined by subtracting the heats of the control experiment. Each experiment was repeated at least three times. The thermograms were analyzed with Origin7 software (MicroCal) to determine the K_a values. The thermodynamic parameters for the interaction between *Pho*TBP and each DNA were estimated by using the standard relationship:

$\Delta G^o = - RT \ln (K_a)$

$\Delta G^o = \Delta H^o - T\Delta S^o$

where ΔG^o is the binding free energy change, ΔH^o is the binding enthalpy change, ΔS^o is the binding entropy change, R is the gas constant, and T is the temperature.

Reference

[1] P. Giri and G. S. Kumar, *Mol. BioSyst.*, 2008, **4**, 341–348.

[2] P. Giri and G. S. Kumar, *Arch. Biochem. Biophys.*, 2008, **474**, 183–192.

ATTATAGCTTGCTTTTAAGTATGT
ACGTAAGGGGCTTTTAAGTATTAT
ACGTAAGGGGCTTTTAAGTATTAT
ATTAAAAGCCTGTTTTTATGTGTA
ATTAAAAGCCTGTTTTTATGTGTA
ATTTTAAAGGCTTTTAAATACTCG
ATTTTAAAGGCTTTTAAATACTCG
ATTTTAAAGGCTTTTAAATACTCG
ATTTTAAAGGCTTTTAAATACTCG (ODN50H)
TAACGCTTTTTAAGTTTGTCATGT
CCGTCTAATAAGTCTGCTTTTTAA
CCGTCTAATAAGTCTGCTTTTTAA
GGATATAAGACGGCTTTATATTGT
CACTGTCATTCTTTTAAGTCTTAT
CACTGTCATTCTTTTAAGTCTTAT
GTTAATAGTTATTTTAAAAGAGTA
TGTATTTTAAAAGAGGTCCTTTAG

Figure S1. DNA sequences of the centrally positioned 24-nt random region, selected from seven cycles of the in vitro selection experiment in 1 M NaCl at 50 °C.

CATTATAATGACTTTGTATATGGA
TTTTATAATTTACATTATAATGGT
CTTTATAATGTGAGCTTATAAGTT
TTATATTGCGCGGCATTTATAATG
CATTATAAACTTTATTTTAAGGCT
AATAATAGCTACTGGGTTATATTG
ATATAAAGCTACACTTATATTGTA
CTTTATAAGCTACTCTTATATTGA (ODN25H)
CATTATAAGGAACGTAAATAAAGG
CTTATAATTGCATTATATAAGAAT
CAGTTATTACTACAGTATATAATG
TATATAATGGAGACAGTTATATGA
ACAAATAATCCGCTGATATATTGT
TCTGGTATATAATTACTTTATATC
GCTTTATATCTGACGATTAATATC
ACTATATATAGTCTATTATATGTA

Figure S2. DNA sequences of the centrally positioned 24-nt random region, selected from seven cycles of the in vitro selection experiment in 1 M NaCl at 25 °C.

CCCCTTATAAAGCATTTATAAAGG
CCCCTTATAAAGCATTTATAAAGG
CCCCTTATAAAGCATTTATAAAGG
CCCCTTATAAAGCATTTATAAAGG (ODN50L)
AGGTTTATAAAGTGCTTATAAACT
AGGTTTATAAAGTGCTTATAAACT
ACTTTATAAAGTCTTTATAAAGTA
ACTTTATAAAGTCTTTATAAAGTA
CGTTTATAAGCAGTTTATAAAGCG
ACTTATAAACGGTTTATAAAGTCA
CTTTTTATAAAGTGTTTATAATGG
CTTTATAAGCACACTTTATAAAGG
CTTTATAAGCACACTTTATAAAGG
ACTTATAAGTCCCTTTATAAAGTT
ACTTATAAGTCCCTTTATAAAGTT
ATTTATAAAGGCGCACTTTATAAA
TTTATAAGCTTTATAAAGGCGACA

Figure S3. DNA sequences of the centrally positioned 24-nt random region, selected from seven cycles of the in vitro selection experiment in 150 mM NaCl at 50 °C.

GTAATGGCGGTATCGTCTTAACGA
CATGATTGTCCGGCTGCAAGACAT
ATTTCGCCGTTACGCCAAAGACTT
GAAGTTAAACGAGCCGCTAATCTT
AATATTGCCATTCGTCAACTTGTA
TCTCCTCAGCACTTGTACGTGGGT
GGACACATCTCAACGGGAATGGTC
TTAAGACACGTGCCGCGGTAATGA
GACTTATGCCTATGCATTAAGTGC
TATAGCGCCGGCCTTAGCTTATAA
CATGATATGCTTTTGGCCAGCTAT
TCTCGATAGCACTTGGGATTCGCG
ATTAACCTGTGCACATGTTATGTA
TGAAGGTCCATTACGAGCATTCGG
ATGGGCCCAACCGGCGCATTGGCT
CCGTGTCAATAACTTTGTTTTGAT
GCCAATACTGCTTGGCAGTTTGTG
GAAGATTGACGCAGTTCAACTCGA
ATGATTAAGCCAGCGTGGTCTTAT
GCCATTAGCTAGGGCATAAGGCGG
TCAAAGCGAAGTCGCATTTGGACG
ATTCCCCAGTACCAGGGGATGTAC
ATTATCTCTGCGGAGGTTAAGACG
GCTTATAACAGCGTTTATATGTGG
ATTAACCCGTGCACATGTTATGTA
ATTCACCGGTTCCCGCTGATGCAC
AACTAGCGCTAGCTTGATTGCTTA
TGGGTTTTACTCGCAGAGATAAAC
AAATGCGCGCATTGGCGCTTTGTA
TATAAGCGCGGGGTCGCTATATAA
CAATGTGGCAGGCACTTGCGACCT
TCAAAGCGAAGTCGCATTTGGACG
ATATATGACGGCGGTCATTATAGA
CTTTATAAGCTGGTGCTATATAAG
ACGCATGTAGAAGCCACAATGGTG
GACTAATACGCGGGCCGTTTAAGA
TCATAGTGCAAGGCCTCATATGGA
ATATCGCCTATGGTCGCTGATAGA

Figure S4. DNA sequences of the centrally positioned 24-nt random region, selected from seven cycles of the in vitro selection experiment in 150 mM NaCl at 25 °C.

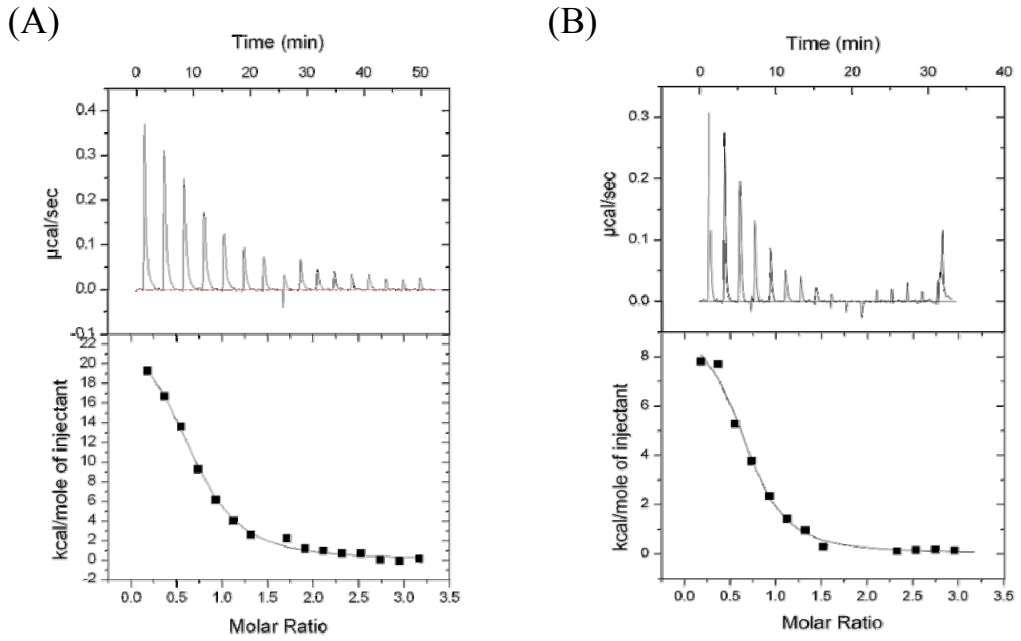

Figure S5. Top panel: ITC profiles of the interaction between 240 µM *Pho*TBP and 15 µM ODN25H at 25 °C, 1 M NaCl (A), 225 µM *Pho*TBP and 15 µM ODN50L at 50 °C , 150 mM NaCl (B), in 20 mM Tris-HCl (pH 7.5) and 1 mM EDTA. Bottom panel: Integration plot of data calculated from the ITC profile after correction for the heat of *Pho*TBP dilution. Solid line corresponds to the best fit curve obtained by least-squares deconvolution.

Table S1. Thermodynamic parameters for the interaction between *Pho*TBP and selected DNAs (ODN25H, and ODN50L), obtained by ITC under the conditions used in each SELEX experiment. Each value is the average of at least three independent measurements.

Sample	Conditions	K_a	ΔH^o	ΔS^o
		10^6 M^{-1}	kcal mol^{-1}	cal $mol^{-1}K^{-1}$
ODN25H	1 M NaCl, 25 °C	0.53 ± 0.062	24.2 ± 0.90	107
ODN50L	150 mM NaCl, 50 °C	0.83 ± 0.19	9.5 ± 0.52	56.3