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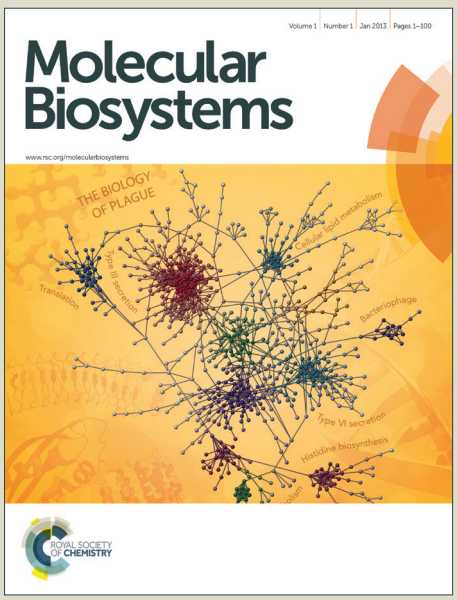

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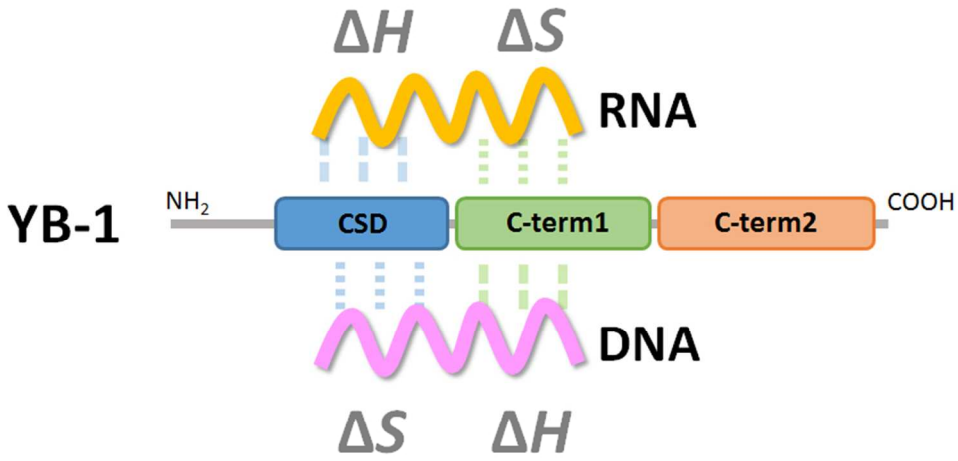

233x127mm (96 x 96 DPI)

Journal Name

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ARTICLE

Thermodynamic characterization of the interaction between human Y-box binding protein YB-1 and nucleic acids

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012,
Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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Y-box binding protein 1 (YB-1) binds to both RNA and DNA to control transcription and translation for the regulation of various cellular systems. YB-1 is overexpressed in some cancer cells and is a potential target for treatment of cancer. Herein, we describe isothermal titration calorimetry analyses of the interaction between a number of recombinant YB-1 domains and nucleic acids to identify the RNA and DNA binding sites and their binding mechanisms. These results demonstrated that the C-terminal domain of the protein interacts with single-stranded DNA and RNA in exothermic and endothermic reactions, respectively. The highly conserved cold-shock domain (CSD) also bound to single-stranded RNA and DNA in exothermic and endothermic reactions, respectively. The specific binding manner for RNA is in the CSD, whereas DNA binds with the most affinity to the C-terminal region (amino acids 130-219). We found further that C-terminal region (amino acids 220-324) regulates the binding stoichiometry of RNA. These quantitative thermodynamic results provide preliminary indication on the molecular mechanism of multifunctional protein YB-1 binding to nucleic acids to regulate it biological function.

Introduction

Y-box binding protein 1 (YB-1) was initially identified as a transcription factor that specifically binds to the Y-box sequence (5′-CTGATTGG-3′) in the promoter regions genes encoding class II major histocompatibility antigens.[1] YB-1 contains an evolutionarily highly conserved cold-shock domain (CSD) .[2] The cold-shock domain containing proteins are found in messenger ribonucleoprotein particles (mRNPs) in the cytoplasm of mammalian cells.[3-5] Previous reports indicate that YB-1 belongs to a family of multifunctional proteins that regulate gene expression on both transcriptional and translational levels, and it binds to both RNA and DNA.[6,7]

YB-1 is ubiquitously expressed in normal cells and tissues, and its overexpression has been reported in a variety of human cancers.[8] High levels of YB-1 are correlated with poor prognosis and cancer recurrence. Depending on the cancer, levels of YB-1 may be high in either the cytoplasm or the nucleus.[8] Recent reports indicate that YB-1 plays a crucial role in many cellular processes, including proliferation, differentiation, drug resistance and stress responses, through control of the stability and localization of mRNAs.[9-12] YB-1 has potential as a diagnostic marker and as a drug target.

The human YB-1 protein has three major functional domains[6,7] as diagrammed in Figure 1. The N-terminal domain (amino acids 1-51) is rich in alanine and proline and is called the A/P domain. The evolutionarily highly conserved cold-shock domain (CSD; amino acids 52-129) acts as a nucleic acid binding domain that has both specific and non-specific interactions with RNA and DNA.[6] The hydrophilic C-terminal region (CTD, amino acids 130-324) has been reported to bind nucleic acids and a number of proteins including YB-1 itself.[13-16] Furthermore, the CTD contains a non-canonical nuclear localization signal (NLS, amino acids 186–205) and a cytoplasmic retention site (CRS, amino acids 267–293).[6] It has been recently reported that YB-1 can be cleaved between Glu-219 and Gly-220 by the 20S proteasome, resulting in the accumulation of a truncated N-terminal YB-1 fragment in the nuclei of cells.[17]

To better understand the molecular mechanism of the interaction between YB-1 and nucleic acids, we carried out isothermal calorimetric (ITC) analyses of the binding of YB-1 to RNA and DNA. The binding of RNA and DNA to full-length and truncated recombinant YB-1 proteins produced in an *Escherichia coli* expression system were analyzed. Our data suggest that YB-1 has distinct RNA- and DNA-binding sites and that it interacts with nucleic acids in an exothermic binding manner.

Results

Design of domain fragments of YB-1 and sequences of nucleic acids

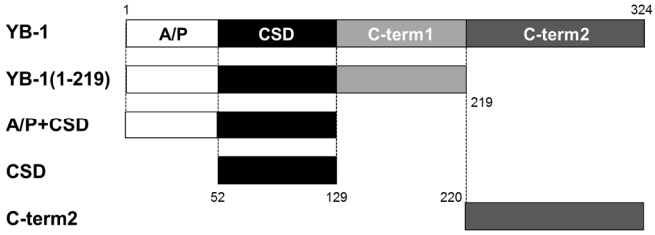

Figure 1. YB-1 constructs used in this study.

The DNA and RNA binding sites of YB-1 were investigated by analysis of binding of nucleic acids to the full-length protein, YB-1(Full), and to fragments of YB-1 including the entire N-terminal domain (amino acids 1-219, YB-1(1-219)), the A/P and CSD containing region (amino acids 1-129, A/P+CSD); the CSD (amino acids 52-129, CSD); and the C-terminal region (amino acids 220-324, C-term2) (Figure 1). A single-stranded RNA (ssRNA) oligonucleotide, 5′-CAUCCAACAAGA-3′, that is the YB-1 binding motif in the *YB-1* mRNA[18] served as the model RNA. The model DNA was a single-stranded DNA (ssDNA) oligonucleotide 5′-TTGGCCAATCAG-3′ that contains the CCAAT box (or Y-box) found in numerous promoters and enhancers.[18]

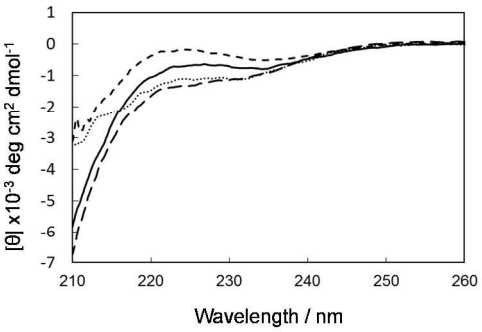

Figure 2. CD spectra of YB-1 and its mutants. YB-1 (Full) (solid line), YB-1(1-219) (short-dashed line), CSD (dotted line) and Cterm-2 (long-dashed line).

Secondary structural analysis of YB-1 and its mutants

Circular dichroism (CD) analysis was carried out to identify the folding of recombinant proteins (YB-1(Full), YB-1(1-219), CSD and C-term2. All proteins had similar spectra with a large negative peak below 220 nm (Figure 2). The noisy spectra below 210 nm were

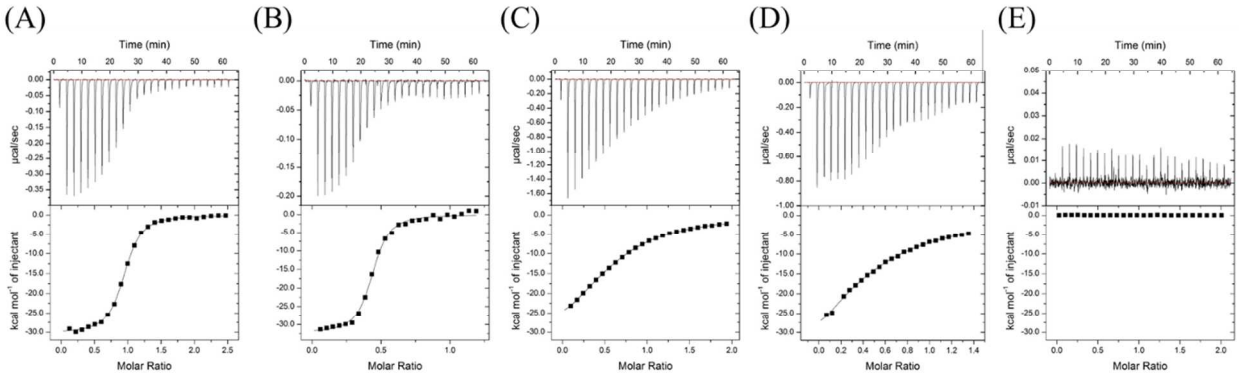

Figure 3. ITC profiles of the binding of YB-1(Full) and fragments to ssRNA. (A) YB-1(Full), (B) YB-1(1-219), (C) CSD, (D) A/P+CSD, (E) C-term2. Conditions were 20 mM HEPES, pH 7.5, 500 mM NaCl; 100-500 µM ssRNA were titrated into 10-50 µM protein solution.

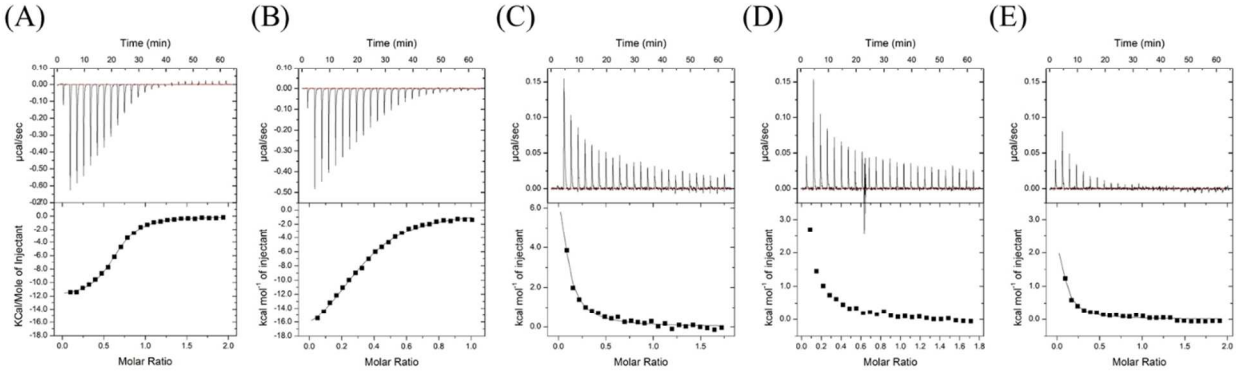

Figure 4. ITC profiles of the binding of YB-1(Full) and fragments to ssDNA. (A) YB-1(Full), (B) YB-1(1-219), (C) CSD, (D) A/P+CSD, (E) C-term2. Conditions were 20 mM HEPES, pH 7.5, 500 mM NaCl; 100-500 µM ssDNA were titrated into 10-50 µM protein solution.

observed due to the buffer solution containing salt. These spectra showed mainly the random coil, suggesting that main structure of these proteins is disordered. The CD spectrum of YB-1(Full) is similar to that of FRGY2 (Y-box protein from *Xenopus laevis*) [19] and YB-1 from Guryanov's group reported previously[20]. Although it has been reported that CSD of YB-1 has an antiparallel β-barrel structure in only 95% H_2O and 5% D_2O solution[21], our CD data showed that the domain had mainly random coil under the buffer condition. The NMR relaxation measurements have demonstrated reportedly that the CSD exhibits an equilibrium between 70% folded and 30% unfolded state [21].

Analysis of the binding of YB-1 and fragments to single-stranded RNA

The ITC analyses of the binding of full-length and truncated YB-1 to ssRNA at 25 °C are shown in Figure 3. ITC data of YB-1(Full) and YB-1(1-219) were indicative of exothermic reactions (Figure 3a, b). The binding of CSD and A/P+CSD fragments to ssRNA was also exothermic (Figure 3c, d). In contrast, C-term2 bound to ssRNA in an endothermic reaction (Figure 3e). The thermodynamic parameters of the binding of YB-1 and fragments to ssRNA are shown in Table 1. The thermodynamic parameters of C-term2 binding to ssRNA could not be determined due to its weak binding affinity. The interaction of CSD with ssRNA had favorable large binding enthalpy that exceeded the unfavorable entropy. The interactions of YB-1(Full) and YB-1(1-219) had also favorable large binding enthalpies. These results suggest that CSD region in YB-1 contains the major binding site for ssRNA.

Analysis of interactions between YB-1 constructs and single-stranded DNA

Calorimetric analyses were also used to analyze the binding of full-length and truncated YB-1 to ssDNA using ITC (Figure 4). ITC profiles of YB-1(Full) and YB-1(1-219) were indicative of exothermic reactions (Figure 4a, b), whereas those of CSD, A/P+CSD, and C-term2 showed endothermic reactions (Figure 4c, d, e). The thermodynamic parameters are shown in Table 2. The interactions of YB-1(Full) and YB-1(1-219) had favorable binding enthalpies that exceeded unfavorable binding entropies, suggesting that the binding of YB-1(Full) and YB-1(1-219) to ssDNA was enthalpy driven. The interactions between CSD, A/P+CSD, and C-term2 with ssDNA were too weak to enable determination of thermodynamic parameters. There was no difference in the ITC profiles between A/P+CSD and CSD, indicating that the A/P site in YB-1 does not contribute to binding to ssDNA. That the C-term1 region is important for binding of YB-1 to ssDNA was supported by the larger binding enthalpy of YB-1(1-219) than that of YB-1(Full). These results suggest that amino acids 130-219 in the C-terminal region in YB-1 contain the major binding site for ssDNA.

Binding stoichiometries of YB-1 to ssRNA and ssDNA

When analyzed by size exclusion chromatography, YB-1(Full) and YB-1(1-219) migrated as dimers (Figure S1a, b, Table S1) at protein concentrations around 20-50 μM, close to concentrations used in ITC analyses. The binding stoichiometries were determined by ITC and are given in Tables 1 and 2. The binding stoichiometry of YB-1(Full) to ssRNA was 0.99±0.10 based on ITC, suggesting that one YB-1(Full) binds to one ssRNA oligonucleotide. In contrast, the binding stoichiometry of YB-1(1-219) to ssRNA was 0.41±0.04,

Table 1. Thermodynamic parameters of the interaction between YB-1(Full) and YB-1 fragments with ssRNA in 500 mM NaCl [1]

	n	K_a (10^5 M^{-1})	ΔH (kcal mol^{-1})	$-T\Delta S$ (kcal mol^{-1})	ΔG (kcal mol^{-1})	ΔC_p (kcal mol^{-1} K^{-1})
YB-1(Full)	0.99±0.10	80.7±12.7	−29.6±1.1	20.1±1.2	−9.4±0.1	n.d.
YB-1(1-219)	0.41±0.04	71.7±18.6	−28.7±1.3	19.3±1.5	−9.4±0.1	−0.98±0.30
CSD	0.49±0.13	1.29±1.17	−34.3±3.7	27.5±4.1	−6.9±0.4	n.d.
A/P+CSD	0.61±0.22	0.52±0.20	−40.8±25.5	34.3±25.6	−6.5±0.1	
C-term2	n.d.	n.d.	n.d.	n.d.	n.d.	

[1] *n* indicates binding stoichiometry; n.d. indicates "not determined".

Table 2. Thermodynamic parameters of the interaction between YB-1(Full) and YB-1 fragments with ssDNA in 500 mM NaCl [1]

	n	K_a (10^5 M^{-1})	ΔH (kcal mol^{-1})	$-T\Delta S$ (kcal mol^{-1})	ΔG (kcal mol^{-1})	ΔC_p (kcal mol^{-1} K^{-1})
YB-1(Full)	0.63±0.01	6.35±1.71	−11.4±1.3	3.5±1.4	−7.9±0.2	n.d.
YB-1(1-219)	0.41±0.07	4.36±1.05	−15.0±1.9	7.3±2.0	−7.7±0.1	−2.20±0.30
CSD	n.d.	n.d.	n.d.	n.d.	n.d.	
A/P+CSD	n.d.	n.d.	n.d.	n.d.	n.d.	
C-term2	n.d.	n.d.	n.d.	n.d.	n.d.	

[1] *n* indicates binding stoichiometry; n.d. indicates "not determined".

Table 3. Thermodynamic parameters of YB-1(Full), YB-1(1-219), and CSD to ssRNA and ssDNA in 150 mM NaCl [1]

	n	K_a (10^5 M^{-1})	ΔH (kcal mol^{-1})	$-T\Delta S$ (kcal mol^{-1})	ΔG (kcal mol^{-1})	ΔC_p (kcal mol^{-1} K^{-1})
ssRNA						
YB-1(1-219)	0.45±0.10	355±54	−33.1±3.7	22.8±3.7	−10.3±0.1	n.d.
CSD	0.55±0.10	1.97±0.61	−37.1±3.6	29.9±3.7	−7.2±0.1	−0.66±0.04
ssDNA						
YB-1(1-219)	0.40±0.02	1.28±0.25	−42.3±3.9	35.4±4.1	−7.0±0.1	−2.11±0.29
CSD	n.d.	n.d.	n.d.	n.d.	n.d.	

[1] YB-1(Full) was not evaluated due to low solubility. n indicates stoichiometry; n.d. indicates "not determined".

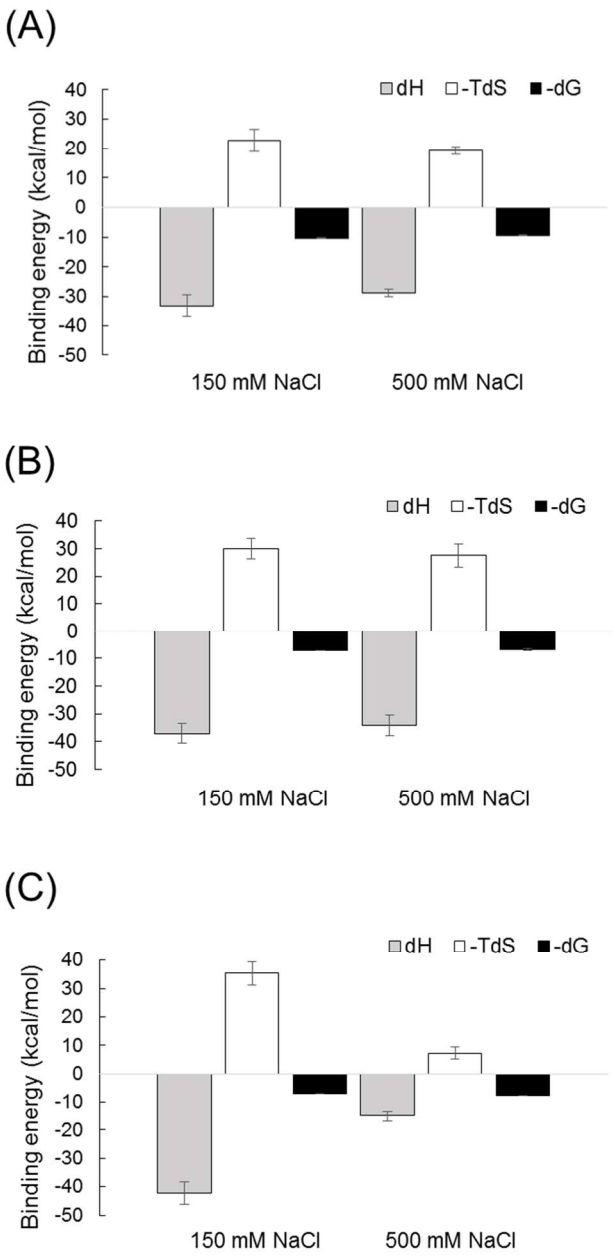

Figure 5. I Salt effect on the thermodynamic parameters of the interactions between YB-1 fragments and nucleic acids. (A) YB-1(1-219) and ssRNA, (B) CSD and ssRNA, (C) YB-1(1-219) and ssDNA. Conditions were 20 mM HEPES, pH 7.5, 150 or 500 mM NaCl.

suggesting that dimer YB-1(1-219) binds a single oligonucleotide of RNA. The CSD contains the major binding site for ssRNA based on our analysis. Therefore, whereas each CSD in dimer YB-1(Full) is capable of binding to ssRNA, the CSD in dimer YB-1(1-219) loses partially the binding activity of ssRNA. The binding stoichiometries of A/P+CSD and CSD were 0.61±0.22 and 0.49±0.13, respectively. The results suggested that CSD has a potential function for alteration of the binding stoichiometry to ssRNA in YB-1.

The binding stoichiometries of YB-1(Full) and YB-1(1-219) to ssDNA were 0.63±0.01 and 0.41±0.07, respectively, suggesting that dimer YB-1 binds to one ssDNA.

Effect of salt on the interactions between YB-1 and nucleic acids

To understand the binding mechanisms of YB-1 to ssRNA and ssDNA, the effects of salt on the binding were investigated using ITC. Binding analyses were performed in low salt (150 mM NaCl) and compared to data collected in buffer containing 500 mM NaCl. We focused on YB-1(1-219) and CSD as these constructs bound with high affinity to the model ssRNA or ssDNA. Due to low solubility, YB-1(Full) could not be studied under these conditions. The thermodynamic parameters are shown in Table 3.

For YB-1(1-219), the binding free energy of ssRNA became less negative with an increase in NaCl concentration (150 mM NaCl, -10.3 ± 0.1 kcal mol^{-1}; 500 mM NaCl, -9.4 ± 0.1 kcal mol^{-1}). The CSD binding to ssRNA showed the same trend but within error (150 mM NaCl; -7.2 ± 0.1 kcal mol^{-1}, 500 mM NaCl; -6.9 ± 0.4 kcal mol^{-1}). These results indicated that salt effect of YB-1(1-219) is more sensitive than that of CSD. The opposite was observed for YB-1(1-219) bound to ssDNA (150 mM NaCl, -7.0 ± 0.1 kcal mol^{-1}; 500 mM NaCl, -7.7 ± 0.1 kcal mol^{-1}). A salt-dependent change in enthalpy was also observed. The extent of change in ΔH of ssDNA binding to YB-1(1-219) was higher than that of ssRNA binding to CSD (Figure 5). The direction of the change was different for ssDNA binding to CSD than for ssDNA binding to YB-1(1-219) (Figure S2).

Temperature dependency of thermodynamic parameters

The temperature dependency of the binding enthalpy and entropy for ssRNA and ssDNA to the YB-1 fragments was also analyzed. The results are shown in Figure 6. The change in heat capacity (ΔC_p) upon binding of YB-1(1-219) to ssRNA was −0.98 ± 0.30 kcal mol^{-1} K^{-1} at 500 mM NaCl; the interaction was unstable at 150 mM NaCl and the change in heat capacity could not be determined. The value of ΔC_p upon binding of YB-1(1-219) to ssDNA was −2.11 ± 0.29 kcal mol^{-1} K^{-1} at 150 mM NaCl and −2.20 ± 0.30 kcal mol^{-1} K^{-1} at 500 mM NaCl. The value of ΔC_p to for binding of CSD to ssRNA was −0.66 ± 0.04 kcal mol^{-1} K^{-1} at 150 mM NaCl; low binding affinity at 500 mM NaCl precluded evaluation. An accurate value of ΔC_p for binding of CSD to ssDNA could not be determined due to low binding affinity, but the value appeared to increase gradually with an increase in temperature between 15 and 37 °C (see Figure S2), suggesting ΔC_p for binding of CSD to ssDNA was positive.

Discussion

Characterization of nucleic acid binding to YB-1 using ITC

(YB-1) binds to both RNA and DNA to control transcription and translation but the molecular mechanism of the interaction between YB-1 and nucleic acids is not well characterized. Here, we carried out isothermal calorimetric (ITC) analyses of the binding of YB-1 to RNA and DNA oligonucleotides. The sequences of the oligonucleotides were chosen as these sequences are present in mRNA or genes known to interact with YB-1. The binding of RNA and DNA to full-length and truncated recombinant YB-1 proteins produced in an *Escherichia coli* expression system were analyzed using ITC.

Characterization of ssRNA binding site in YB-1

The ITC analysis showed that the CSD is the region of YB-1 that binds with highest affinity to ssRNA (Tables 1 and 3). The K_a of the CSD for ssRNA is 10^5 M^{-1} and binding is driven enthalpically. The CSD has low affinity for ssDNA and binding is driven entropically. Heat capacity change of the interaction between CSD and ssRNA was −0.66 kcal mol^{-1} K^{-1}. RNA-specific recognition is accompanied by solvation changes from various noncovalent bonds formed between protein and nucleic acid.[22] The ΔC_p of the interaction between YB-1(1-219) and ssRNA was large and negative (−0.98 kcal mol^{-1} K^{-1} at 500 mM NaCl) and similar to that of the interaction of CSD. The binding affinity of ssRNA to YB-1(1-219) was higher than that of CSD and enthalpy was less favorable.

Analysis of salt effect on the interaction between YB-1(1-219) and RNA demonstrated that binding was less favorable at 500 mM NaCl than at 150 mM NaCl. This suggests that electrostatic interactions are a driving force of its binding. Furthermore, a salt-dependent change in enthalpy change was observed. The enthalpic penalty at higher salt concentration might be due to inhibition of cation and anion release and binding.[23] On the other hand, electrostatic force of CSD is less than that of YB-1(1-219) to ssRNA, resulting from the low salt-dependency of CSD binding rather than that of YB-1(1-219). Therefore, the salt effect indicates that the binding of YB-1(1-219) to ssRNA is specific and C-term1 assists cooperatively the binding of RNA.

Characterization for the unique thermodynamics of ssDNA binding

ssDNA binds enthalpically to YB-1(1-219) with high binding affinity of 10^5 M^{-1} and binds entropically to CSD with low binding affinity (Tables 2 and 3), indicating that the region labeled C-term1 in YB-1 is DNA-specific binding domain. The ΔC_p of the interaction

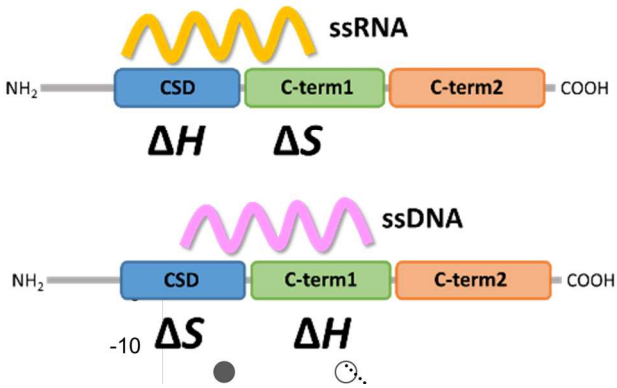

Figure 7. Schematic illustration of sites of interaction in YB-1 for ssRNA (yellow) and ssDNA (pink). The regions that interact enthalpically and entropically are indicated.

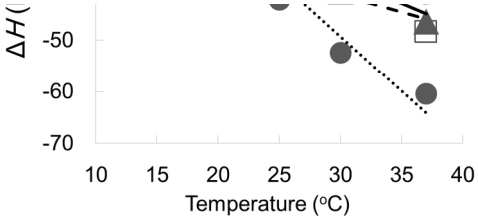

Figure 6. Binding enthalpy as a function of temperature of YB-1(1-219) to ssRNA in 500 mM NaCl (open squares), YB-1(1-219) to ssDNA in 150 mM NaCl (filled circles), YB-1(1-219) to ssDNA in 500 mM NaCl (open circles), and CSD to ssRNA in 150 mM (filled triangles) Conditions were 20 mM HEPES, pH 7.5.

between ssDNA and YB-1(1-219) was large and negative. Although the value of ΔC_p of the interaction between ssDNA and CSD was not determined due to its low binding affinities, the endothermic heat of ssDNA increased gradually with increase in temperature, suggesting that the ΔC_p of CSD to ssDNA was positive. In general for the heat capacity changes in the interactions between proteins and nucleic acids range from −0.25 to −0.55 kcal mol^{-1}K^{-1}.[23] Therefore, our calorimetric analysis suggests that the interactions between the C-terminus of YB-1 and DNA are specific and involves conformational changes such as unstacking of nucleobases.[23]

Since the binding affinity of the interaction between C-term1 and ssDNA increased with increase in salt concentration (150 mM NaCl; −7.0±0.1 kcal mol^{-1}, 500 mM NaCl; −7.7±0.1 kcal mol^{-1}), the force driving the interaction is not electrostatic. We hypothesize that hydrophobic interactions are not a major force in the interaction between C-term1 and DNA, because the thermodynamic analysis of hydrophobic interaction in a protein-DNA complex indicated that the reaction is entropy-driven, and entropy, but not enthalpy, changed favorably with change in salt concentration as shown in the interaction between TATA-box binding protein and DNA[24]. Our data of salt-dependent thermodynamics and the large negative heat capacity changes implies that the interaction between C-term1 and ssDNA requires unstacking of nucleobases as shown for the interaction between single-stranded DNA binding protein and DNA[25]. These findings support that the major interaction of C-term1 with ssDNA contains basic residues and nucleobases. One of the candidate mechanisms is cation-π interaction that reportedly

maintains its strength across a range of solvents, unlike salt bridges.[26] In protein-nucleic acid complexes, cation-π interactions occur between basic residues (lysine and arginine) and purines (adenine and guanine). The arginine-guanine pair has the strongest interaction energy.[27] A sequence motif in DNA that correlates with YB-1 binding has guanine nucleobases[6,7] and the C-term1 region has several arginine residues.

The CSD also interacted with ssDNA, but the reaction was endothermic reaction and resulted in a positive heat capacity change. The interaction was less stable in 500 mM NaCl than in 150 mM NaCl. These results suggest that the interaction of the CSD with ssDNA is stabilized by electrostatic interactions and is accompanied by dehydration from the charge-charge interface. Similar data was obtained for other DNA protein systems.[28-30]

Binding features of YB-1 to nucleic acids

The present study revealed YB-1 is a multifunctional nucleic acid-binding protein that has independent RNA-specific and DNA-specific binding manner. The CSD and C-term1 region has the binding specificity to RNA and DNA, respectively. The CSD also bound weakly to ssDNA in an endothermic reaction. In sequence-specific DNA recognition, hydrogen bonds result in enthalpic, high affinity interactions between amino acid side chains and DNA nucleobases[31]. In non-specific interactions of protein and DNA electrostatic salt bridges between the phosphate oxygens and basic amino acids of DNA provide an entropic driving force.[28-30] In addition, most reports for protein-DNA interactions have suggested that sequence-nonspecific interactions have zero heat capacity.[30] Thus, it is expected that YB-1 interacts specifically with RNA and DNA through exothermic reactions and non-specifically with endothermic reaction (Figure 7). Further binding analysis for other sequences of RNA/DNA would reveal clearly the sequence-specificity.

From the binding stoichiometry obtained using ITC, we propose a model for the interactions of YB-1 with nucleic acids. Our data of full-length YB-1 and YB-1 (1-219) in size exclusion chromatography showed that these proteins have homodimetic structures, which has been previously reported both in vitro and in vivo analysis[32]. Our data showed these homodimer binds one ssRNA. The binding of ssRNA occurs primarily in the CSD and that to ssDNA in the C-term1 regions. The lacking the C-term2 region decreases the binding activity of CSD to ssRNA. It has been reported that YB-1 is cleaved by 20S proteasome between E219 and G220, resulting in the accumulation of truncated an N-terminal YB-1 fragment in the nuclei of cells.[17] Based on our data, we predict that this cleavage results in inactivation of CSD binding to RNA and that DNA binding is enhanced simultaneously with localization in nucleus. A previous report demonstrated that YB-1 promotes strand separation of duplex DNA.[33] Our results suggest that this activity is due to interaction between cationic residues of YB-1 and the nucleobases of DNA.

Understanding of biological functions of YB-1 is important as overexpression of this protein is correlated with rick for cancer development and it is a potential target for development of anticancer agents.[7,8] Herein we performed a calorimetric study of the interaction between YB-1 and nucleic acids. The RNA- and DNA-binding sites in the protein were identified. The specific binding and the assisted affinity enhancement is caused by exothermic interactions and endothermic interactions, respectively. The C-terminal domain, which has the DNA-specific binding manner and regulates the binding stoichiometry of RNA, is reportedly an intrinsically disordered region.[7] This domain might be targeted by a peptide to regulate the binding of YB-1 to nucleic acids.

Conclusions

Herein, we performed the thermodynamic quantitative analysis of the interaction between YB-1 and nucleic acids. The calorimetric results demonstrated that CSD and C-term1 in YB-1 has exothermic binding mechanism to ssRNA and ssDNA, respectively. On the other hand, CSD and C-term1 in YB-1 has endothermic binding mechanism to ssDNA and ssRNA, respectively. Our physicochemical quantitative analysis provides an attractive viewpoint of molecular mechanisms for the binding to nucleic acids in YB-1.

Experimental

Expression and purification of recombinant YB-1 full-length protein and domains

The gene encoding YB-1 amplified by a polymerase chain reaction was inserted into the pET28b vector (Merck, Germany). Using the *Escherichia coli* BL21 (DE3) expression system (Merck, Germany), we obtained recombinant YB-1 as a soluble protein. BL21 (DE3) cells containing the appropriate expression plasmid were pre-cultured in 3 mL LB medium with 50 mg L^{-1} kanamycin overnight at 37 °C. The pre-cultured cells were then inoculated into 1000 mL LB medium containing 50 mg L^{-1} kanamycin and shaken at 37 °C until the optical density at 600 nm reached 0.4. Isopropyl β-D-thiogalactopyranoside was added to a final concentration of 0.5 mM, and the mixture was shaken at 20 °C for 2 hours. Cells were harvested by centrifugation at 7000 *g* for 10 min at 4 °C, and the pellet thus obtained was resuspended in 50 mL of a solution containing 50 mM Tris-HCl, pH 8.0, and 500 mM NaCl (buffer A). Phenylmethylsulfonyl fluoride (100 mM in 100% ethanol) was added to a final concentration of 1 mM, and the cells were sonicated with an ultrasonic cell-disruptor instrument (Tommy, Japan) for 15 min (Output 7, Duty 50) and then centrifuged at 40,000 *g* for 30 min. To the soluble fraction was added $MgCl_2$ (final concentration, 2 mM) and Turbo Nuclease (750-1500 U, Nacalai Tesque, Japan), and the solution was incubated at 37 °C for 6-12 hours. The solution was applied onto a Ni-NTA column (Novagen, Japan) equilibrated with buffer A containing of 5 mM imidazole. Protein was eluted with stepwise increase of imidazole (10, 20, 50, 100, 200, and 500 mM) in buffer A. The eluate was purified over a Hitrap SP HP column. The full-length protein, YB-1(Full), was further purified over a Resource S column (GE Healthcare, USA) and then through a CHT™ Ceramic Hydroxyapatite Type II column (Bio-Rad, USA). The purity of the YB-1(Full) and the fragments were confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis and by the ratio of UV absorbance at 260 nm to 280 nm (below 0.65). The concentrations of proteins were determined using the molecular absorption coefficient at 280 nm.

Preparation of oligonucleotides

As the target nucleic acids for the YB-1 binding experiments, we used the DNA oligonucleotide 5′-TTGGCCAATCAG-3′ [2, 18] and the RNA oligonucleotide 5′-CAUCCAACAAGA-3′.[18,34] The purified oligonucleotides were purchased from JBioS (Japan), and their concentrations were determined from their molecular absorption coefficients at 260 nm.

Isothermal titration calorimetry

Binding energies for the interactions between proteins and nucleic acids were determined in a buffer of 20 mM HEPES, pH 7.5, 150 mM or 500 mM NaCl using isothermal titration calorimetry on a MicroCal iTC200 (GE Healthcare, Japan). Nucleic acids solutions (100-500 μM) were titrated into protein solutions (10-50 μM). Control experiments were carried out to calculate the heat of dilution for nucleic acids. The thermograms were determined by subtracting the heats of sample experiments from those of the control experiments. Each experiment was repeated three times. The thermograms were fitted to obtain K_A using Origin 7 software (MicroCal Inc., USA) assuming a single set of identical binding sites. Thermodynamic parameters governing protein-nucleic acid interactions were estimated by the following standard relationships [Eqs. (i) and (ii)]:

$$\Delta G = -RT \ln K_A \quad \text{(i)}$$

$$\Delta G = \Delta H - T\Delta S \quad \text{(ii)}$$

where ΔG is the binding free energy change, ΔH is the binding enthalpy change, ΔS is the binding entropy change, R is the gas constant, and T is the temperature in Kelvin. The heat capacity change, ΔC_p, was estimated from the temperature dependence of ΔH.

Analytical size exclusion chromatography

Analytical size exclusion chromatography was performed on a Superdex 200 10/300 GL column (GE Healthcare, USA) at a flow rate of 0.5 mL min^{-1} at 25 °C. The equilibration buffer contained 10 mM HEPES, pH 7.5, 500 mM NaCl. L-arginine hydrochloride was added to the equilibration buffer at 200 mM final concentration to limit adsorption of proteins to the resin. In a typical experiment, the protein was loaded at 20-50 μM. Elution was monitored at 260 nm and 280 nm.

Acknowledgements

This work was supported in part through funding from Grant-in-Aid for Young Scientists B (Grant Number 24750166 to SN) from the Japan Society for the Promotion of Science (JSPS). The authors thank Ms. Yumiko Yamagami for collecting SEC data. We thank Prof. N. Sugimoto (FIBER, Konan University) and Prof. T. Nishikata (FIRST, Konan University) for many start-up discussions.

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Electronic Supplementary Information (ESI) available: See DOI: 10.1039/b000000x/

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