ELSEVIER

Thermochimica Acta 360 (2000) 47–56

thermochimica acta

www.elsevier.com/locate/tca

The monomer of the yeast transcriptional activator GCN4 recognizes its dimer binding DNA target sites without dimerization

Wei Cao, Liang Liu, Daryl E. Klein, Linyi Wei, Luhua Lai*

Institute of Physical Chemistry, College of Chemistry and Molecular Engineering, Peking University, Beijing 100871, China

Received 10 January 2000; received in revised form 6 April 2000; accepted 20 April 2000

Abstract

Dimerization is widely believed to be a requirement for the yeast transcriptional activator GCN4 to recognize its specific DNA target sites. We used the basic region (226–252) of the yeast transcriptional activator GCN4, both as a monomeric peptide and a disulfide-linked dimer to investigate the interaction of GCN4 peptides with the DNA target sites AP-1 and CRE. CD and ITC experiments suggest that the monomeric peptide GCN4-M recognizes the AP-1 and CRE target sites, but it has a weaker affinity with the DNA relative to the disulfide-linked dimer peptide GCN4-D. These results indicate that the basic region of GCN4 alone is sufficient for sequence-specific DNA binding, and that dimerization can stabilize the protein-DNA complex.

Keywords: Recognition; GCN4; DNA; Monomer; CD; ITC

1. Introduction

Sequence-specific DNA binding of gene-regulatory proteins is often mediated by dimeric species, which may recognize DNA binding sites as either homodimers or heterodimers [1–3]. Basic leucine zipper (bZIP) proteins are important transcriptional regulatory proteins, which are characterized by two functional segments: the basic region that directly contacts the DNA [4,5], and an immediately C-terminal α-helical dimerization region 'leucine zipper' [6,7]. One prominent representative among the large family of bZIP proteins is the transcriptional activator GCN4, which regulates many genes by binding to specific DNA target sites [8]. The X-ray crystal structures of the complexes of GCN4 with its DNA target sites AP-1 (5′-ATGACTCAT-3′) and CRE (5′-ATGACGTCAT-3′) have confirmed that [4,5] GCN4 dimerizes via the leucine zipper regions to form a Y-shaped dimer. Each arm of the dimer is a basic region that recognizes half of the DNA dimer binding site. The basic region is unstructured in solution, but changes into an α-helix when bound to the specific DNA site [9–11]. GCN4 forms a relatively stable dimer in the absence of specific DNA binding sites and binds to DNA as a dimer. Most mutant leucine zipper proteins, unable to undergo dimerization, fail to recognize the DNA binding sites [3,12]. Therefore, it is widely believed that dimerization is a prerequisite for GCN4 to specifically recognize its DNA target sites [13]. Talanian et al. [9] have also reported that a disulfide-linked dimer of the basic region binds

* Corresponding author. Tel.: +86-10-62756833; fax: +86-10-62751725.
E-mail address: lai@mdl.ipc.pku.edu.cn (L. Lai)

0040-6031/00/$ – see front matter
PII: S0040-6031(00)00538-4

specifically to the DNA sequences as observed for the native bZIP proteins, but the monomeric basic region without the leucine zipper fails to bind. However, footprinting assays have recently shown that the basic region of other bZIP proteins, such as v-Jun, can bind as monomers to the dimer binding site [14]. Indeed, the Skn-1 protein, which contains a basic region similar to that of bZIP proteins, but lacks a leucine zipper dimerization region, also binds to specific DNA sequences as a monomer [15]. Furthermore, recent kinetic evidence has also shown that GCN4 need not dimerize in order to bind to its specific DNA sites [16].

In order to confirm whether the monomer of protein GCN4 can recognize the dimer DNA binding sites without dimerization, we investigated the recognition of the monomer and the dimer of the basic region of GCN4 with the DNA target sites AP-1 and CRE using CD spectroscopy and isothermal titration calorimetry (ITC) techniques. We report evidence that the basic region of GCN4 can bind as a monomer to its dimer DNA binding sites. These results may have implications for the mechanism by which GCN4 proteins recognize specific DNA target sites for transcriptional regulation.

2. Experimental

2.1. Peptide synthesis

The basic region peptide GCN4-M consisting of residues 226–252 of GCN4 was synthesized on MBHA resin (substitution of 0.97 mmol/g, Advanced ChemTech, USA) using Fmoc/Bu^t strategy and carboxyl group activation by HBTU/HOBT [17]. The peptide was cleaved from the resin and the protecting groups of side chains were removed using the TFMSA/TFA method [18], and purified by reverse-phase HPLC (Gilson Medical electronics, 95 400 Villiers le Bel, France) using preparative Zorbax C_{18} Column. The purity was confirmed by analytical reverse-phase HPLC and MALDI-TOF mass spectra (BIFLEX Model III, Bruker, Germany), giving molecular masses within ± 1 Da of calculated values. The disulfide-linked peptide dimer GCN4-D was prepared by air oxidization of the C-terminal cysteins of two monomeric peptides in 0.2 M phosphate buffer, pH 7.4. Analytical RP-HPLC was used to monitor the oxidization. The oxidized product was isolated by RP-HPLC and the purity was confirmed by analytical RP-HPLC and MALDI-TOF mass spectra. The peptides were >95% pure.

2.2. Oligonucleotide synthesis

The oligonucleotides AP-1 and CRE were obtained from Sheng Gong Bioengineer, Shanghai, China. AP-1 and CRE contain the DNA binding site 5′-ATGACTCAT-3′ and 5′-ATGACGTCAT-3′, respectively. These two sequences are specifically recognized by native GCN4 [4,5]. CONT is a nonspecific control oligonucleotide. The dried individual component strands were synthesized on an automatic DNA synthesizer Model 391 (PE, USA) using standard phosphoramidite chemistry. Each strand synthesized was purified by C_{18} RP-HPLC. Purity of the strands was checked by polyacrylamide gel electrophoresis and analytical RP-HPLC. The DNA duplex was formed by mixing equal amounts of complementary strands and temperature annealing by heating at 80°C for 10 min, followed by slow cooling. The annealed samples were allowed to equilibrate at 4°C for 24 h before analysis. Concentrations were determined spectrophotometrically by measuring the absorbance at 260 nm, the extinction coefficients (ε) were determined directly by using a nearest-neighbor analysis [19].

2.3. Circular dichroism (CD) spectroscopy

CD spectra were measured using CD6 Spectropolarimeter, Jobin–Yvon (38640 Claix, France). This instrument is computerized and equipped with a programmable thermoelectrically controlled cell holder. Circular cells with 0.1-mm path length were used. All solutions contained 10 mM sodium phosphate buffer, 0.1 mM EDTA, 100 mM NaCl, pH 7.4. The buffer for the monomeric peptide GCN4-M, in addition, contains 10 mM DTT to prevent the cysteins oxidation; analytical RP-HPLC was used to confirm that the GCN4-M is in the monomeric form during experiments under this condition. Thermal stability was determined at peptide concentrations of 144 and 72 μM for monomeric GCN4-M and dimeric GCN4-D, respectively, by monitoring the changes in $[\theta]_{222}$ as a function of temperature. The temperature was increased in steps of 0.2°C at a scan rate of 30°C/h from 5 to 80°C. All thermal melts were reversible.

2.4. Isothermal titration calorimetry

The measurements of the heat of mixing peptides GCN4-M and GCN4-D with synthetic DNA target sites AP-1 (5′-GAGATGACTCATCTC-3′) and CRE (5′-GAGATGACGTCATCTC-3′) were carried out with the isothermal titration calorimeter (ITC) from CSC (Provo, UT). The instrument was electrically calibrated by means of a standard electric pulse as recommended by the manufacturer. For peptides binding to DNA, solutions of peptides were used to titrate DNA. A 250-μl syringe was used for the titrant, mixing was affected by stirring this syringe at 200 rpm during equilibration and experimentation. Typically, 25 injections of 10 μl each were performed with a 400-s interval between injections in a single titration. The reference cell of the calorimeter, filled with buffer, acts as a thermal reference to the sample cell. To correct for GCN4 peptide heats of dilution, the control experiments were also performed using similar conditions with buffer solution only. All solutions were degassed by evacuation to reduce noise. The buffer for GCN4-D contains 10 mM sodium phosphate buffer, 0.1 mM EDTA, 100 mM NaCl, pH 7.4. The GCN4-M and DNA samples for the titration of GCN4-M into DNA experiments were dissolved in the same buffer, but containing 10 mM DTT in addition to prevent cysteins oxidation. The heats of each reaction were determined by integration of the peaks observed. After the contribution from the heat of dilution of each injection was subtracted, the heat was plotted against the molar ratio of the peptide to DNA. The binding constants (K_b), enthalpy of binding (ΔH^0), and stoichiometry (N) of the formation of complexes were determined by fitting the binding isotherm against the binding equation described by Freire et al. [20] using an independent binding model. Data analysis was carried out with the software provided with the instrument.

3. Results

3.1. Evidence for GCN4 monomer recognition of its dimer DNA target sites

The yeast transcriptional activator GCN4 is an interesting representative of the large family of bZIP proteins, recognizing the AP-1 and CRE DNA target sites as a dimer. It is a widely studied system to investigate the specific recognition of the DNA binding proteins with its DNA target sites and the mechanism followed by bZIP transcription factors to find its target sites. In order to elucidate the nature of the GCN4–DNA interaction, many artificial sequence-specific DNA binding peptides have been designed and synthesized [9,21–23]. Kim et al. [9] have reported that the disulfide-linked dimer of the basic region specifically binds the DNA sequences as observed for the native bZIP proteins. Here, we used this model to distinguish between the monomer and dimer recognition of DNA target sites, we synthesized a 30-amino acid peptide GCN4-M, corresponding to residues 226–252 of native GCN4 containing a three-residue linker Gly–Gly–Cys. In this short peptide, residue 250 Met is replaced with Leu to inhibit oxidation without affecting DNA-binding affinity and specificity, and the N-terminus was acetylated to avoid introduction of additional charge [9]. This peptide contains the basic region, responsible for the DNA binding recognition of the native protein. By air oxidization of the C-terminal cysteins of two monomeric peptides, the disulfide-linked peptide dimer GCN4-D was obtained. The sequences of these peptides are given in Fig. 1A.

We have investigated the recognition of peptides GCN4-M and GCN4-D with the DNA target sites AP-1 and CRE; the CD spectra of the two free peptides and as complexes with DNA at 20°C are shown in Fig. 2. In the absence of DNA targets, the basic region of the bZIP domain of GCN4 is largely disordered [9]. Similar results were obtained for the GCN4-M and GCN4-D peptides. The characterization of an α-helix was detected by two minima at about 222 and 208 nm, which suggests that the conformation of the two peptides in the absence of specific DNA sequences are similar and exhibit a partial α-helix, nearly disordered in solution. But a significant increase in the intensity of the CD signal at 222 and 208 nm is observed upon introduction of the AP-1 and CRE sequences (Fig. 2A and B). The large changes of intensity at 222 and 208 nm result from changes in the peptide conformation rather than from the introduction of the DNA structure. DNA contributes only a small signal in this region of the CD spectra. Spectra of the peptides in the presence of DNA were calculated

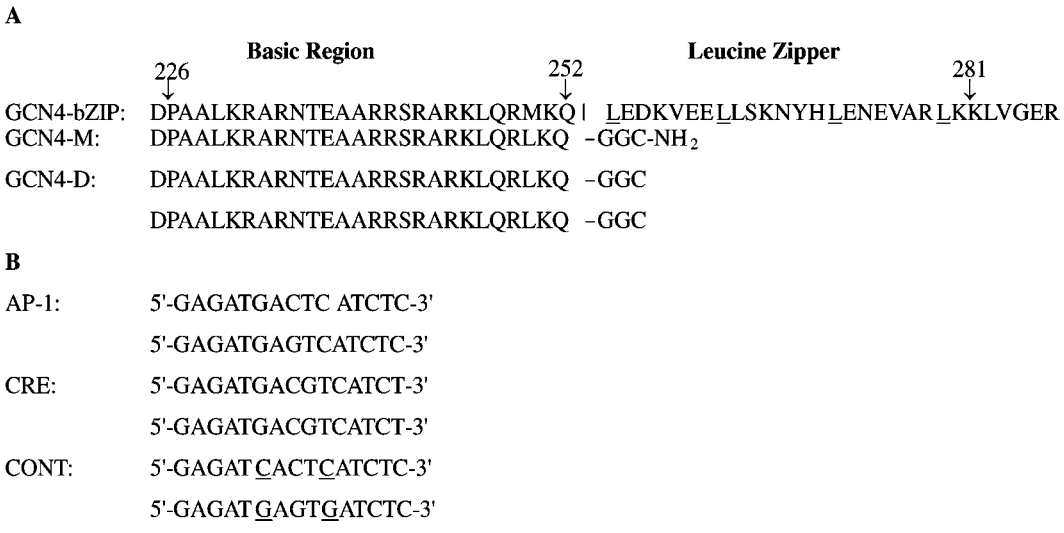

Fig. 1. Sequences of the peptides and oligonucleotides studied. (A) Sequences of the peptides GCN4-M and GCN4-D. (B) Sequences of the oligonucleotides: AP-1, CRE and control DNA CONT.

as the difference between the bound spectrum and the free DNA spectrum. The difference spectra indicate that the basic region of both peptides exhibits conformational transitions from coil to helix when bound to the AP-1 or CRE binding site.

Control CD spectra were obtained with the oligonucleotide CONT (Fig. 2). CONT has the base composition of the AP-1 sequence, but with one base C replaced by G (Fig. 1B). The result shows that CONT also induces the conformational changes of GCN4-M and GCN4-D, but the signal at 222 nm is much smaller than that of the AP-1 or CRE induced signal. This indicates that CONT also has some interaction with the two peptides GCN4-M and GCN4-D. The CD spectra of the disulfide-linked peptide with AP-1 are consistent with the results reported previously [9], which has shown that the basic region of GCN4 has a flexible conformation in solution, but changes into an α-helix when bound to a specific DNA site. Comparison of panel A and B (Fig. 2) indicates that the magnitude of the helicity increase of GCN4-M is similar to that of GCN4-D when adding equimolar amounts of synthetic AP-1 or CRE target DNA. The conformational changes for both peptides are identical, which may suggest that the recognition property of the free basic region of GCN4 to AP-1 and CRE sites is similar to the linked dimer of basic region by a C-terminal disulfide bond.

3.2. Thermal unfolding experiments

In order to further test whether the basic region of GCN4 can specifically bind to both the AP-1 and CRE binding sites in the monomeric form, we have measured the thermal stability of the two peptides and the complexes of the peptides with the two DNA target sites and the control DNA. The peptides were incubated with AP-1, CRE and CONT in 2:1 and 1:1 molar ratios (peptide: DNA) for GCN4-M and GCN4-D, respectively, to make sure the peptides completely bind to DNA. The CD signal associated with helicity at 222 nm was monitored as a function of temperature, as shown in Fig. 3. The thermal unfolding curves of the free peptides of GCN4-M (curve a in Fig. 3A) and GCN4-D (curve a in Fig. 3B) suggest that the structures of the two free peptides are unstable. However, the thermal stability of the complexes with AP-1 and CRE is much greater. The increased thermal stability of the basic region of GCN4-D is consistent with the results reported by Talanian et al. [24], which has demonstrated that it binds to the AP-1 target site in a sequence-specific manner. Thus, our CD spectra studies indicate that the basic region of GCN4-M can also bind to the AP-1 and CRE sites specifically, but with weaker affinity than the dimer. The binding affinity can be inferred from the shape of unfolding curves and the unfolding transition midpoints.

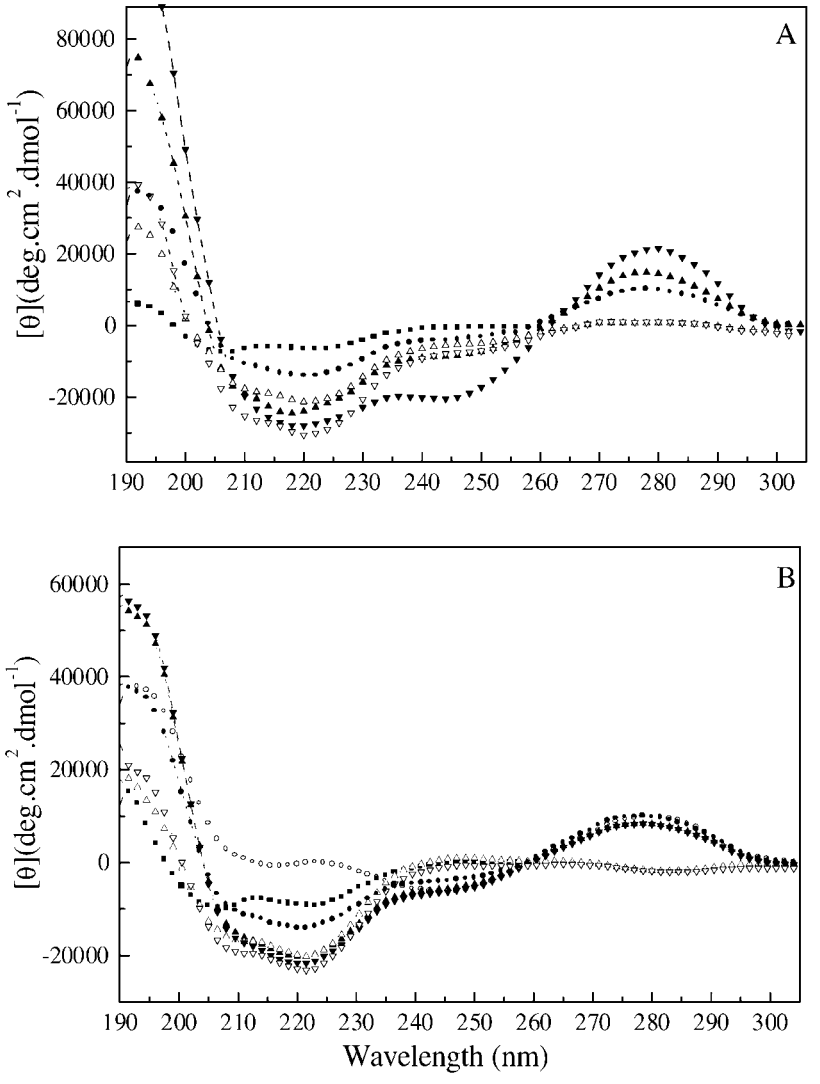

Fig. 2. CD difference spectra indicate that GCN4-M and GCN4-D change into α-helical conformation when bound to AP-1 and CRE target sites at 20°C. (A) GCN4-M. (B) GCN4-D. (■) GCN4 alone; (○) AP-1 alone; (●) Spectra of GCN4 bound to control DNA CONT; (▲) Spectra of GCN4 bound to AP-1 site; (△) Difference spectra of GCN4 bound to AP-1; (▼) Spectra of GCN4 bound to CRE; and (▽) Difference spectra of GCN4 bound to CRE. The concentration is 144 and 72 μM for GCN4-M and GCN4-D, respectively. The buffer for GCN4-D contains 10 mM sodium phosphate, 100 mM NaCl, and 0.1 mM EDTA, at pH 7.4; The buffer for GCN4-M contains 10 mM DTT in addition.

The thermal stability of the complexes of the nonspecific DNA CONT with GCN4-M and GCN4-D were tested under the same conditions. Curve b in Fig. 3A and B show the results. CONT induces a CD signal increase at 222 nm in GCN4-M and GCN4-D at 20°C (Fig. 2), but unlike the results obtained with the AP-1 and CRE sites, the shape of the thermal transition suggests non-cooperative binding. Comparison of the thermal unfolding curves of the complex of GCN4-M with AP-1, CRE and CONT further confirms that the free basic region of GCN4 can recognize the dimer target sites AP-1 and CRE in sequence-specific fashion.

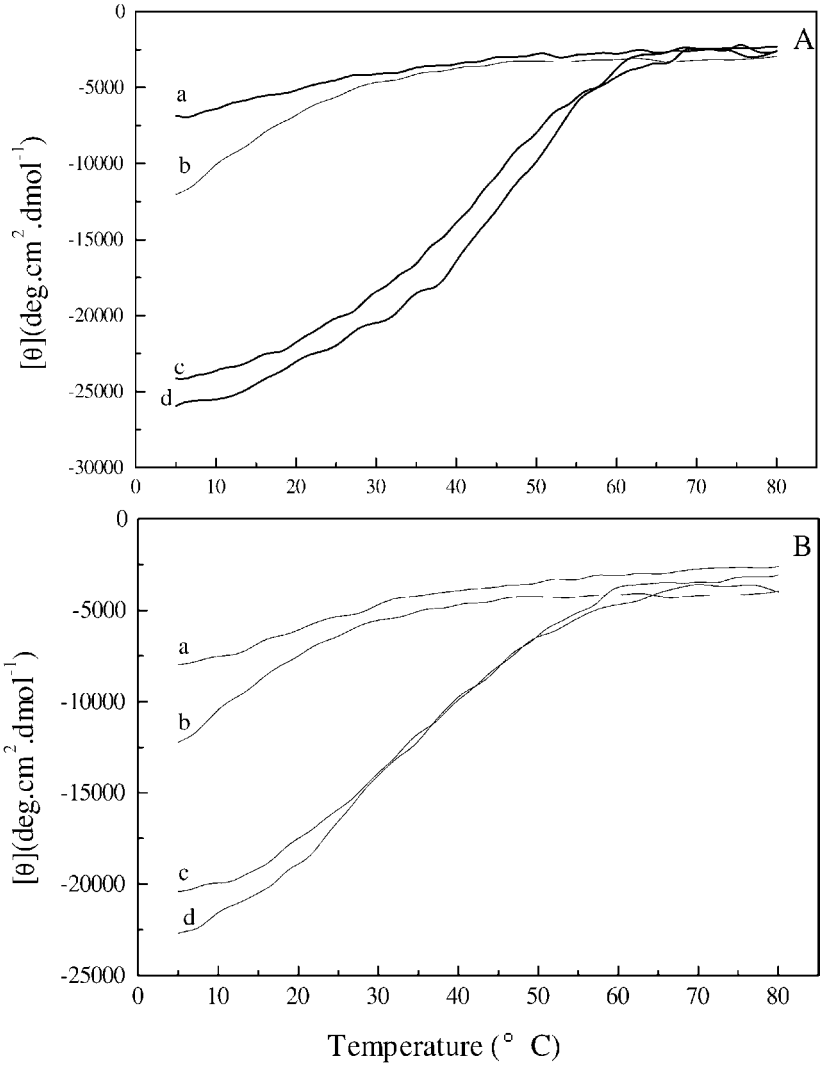

Fig. 3. Thermal unfolding curves of GCN4-M, GCN4-D and the complexes with AP-1 and CRE at 222 nm. (A) GCN4-M. (B). GCN4-D. (a) GCN4 alone; (b) GCN4-CONT; (c) GCN-AP-1; and (d) GCN-4-CRE. The buffer is same as in Fig. 2.

3.3. Thermodynamic properties of GCN4-M and GCN4-D binding to DNA

ITC was used to determine the binding thermodynamic parameters: the binding enthalpy ΔH^0, the association constant K_b, and the stoichiometry N. The thermodynamic parameters for the peptide GCN4-D binding to DNA target sites AP-1 and CRE were obtained in 10 mM sodium phosphate, 0.1 mM EDTA, 100 mM NaCl, pH 7.4; for the peptide GCN4-M, these were obtained in the buffer containing 10-mM DTT in addition. The foregoing analysis indicates that the GCN4-DNA complexes begin to disrupt below 25°C; thus, ITC experiments were performed at 18°C. It is necessary to ensure that the DNA sequences are present in double-stranded form. This is particularly important for the CRE site, which is completely palindromic and has the potential to form hairpins (Fig. 1B). The duplex of AP-1 and CRE was achieved by very slow annealing following heating of the complementary oligonucleotides to 80°C (see Section 2). CD thermal disruptions were carried

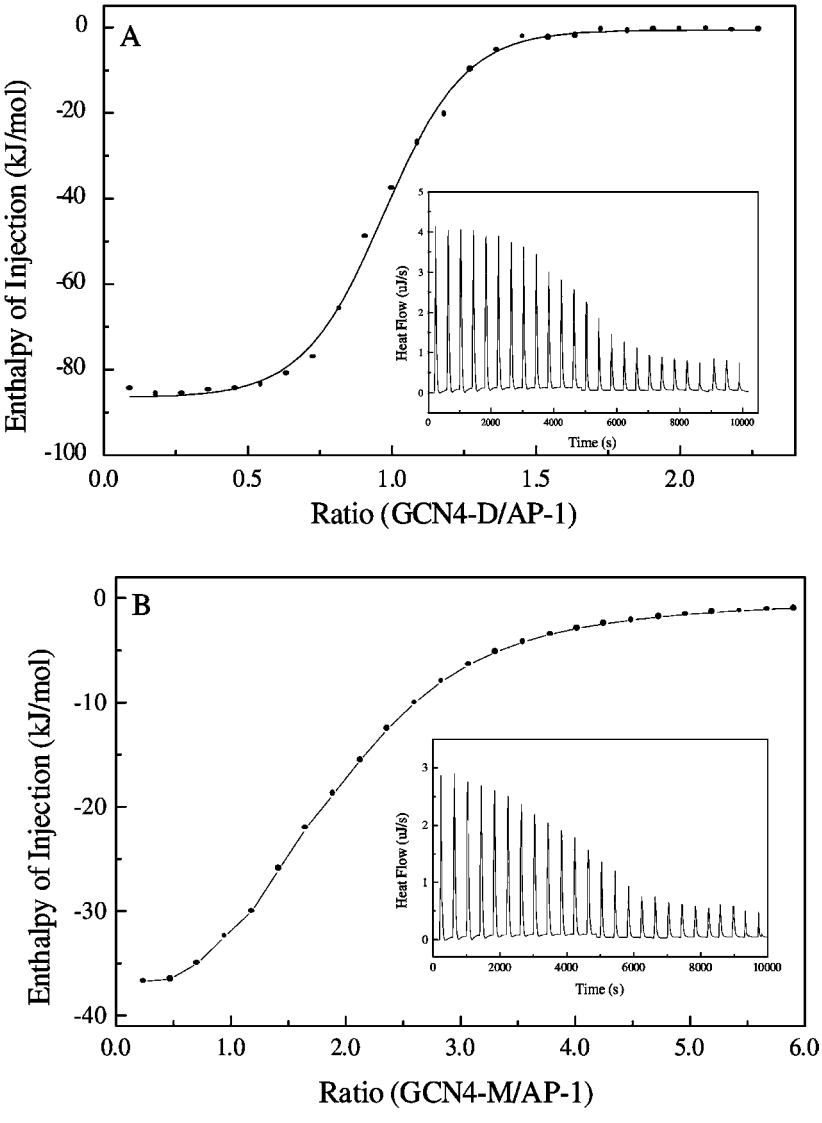

Fig. 4. Typical ITC profile of the binding process between the GCN4 and CRE site at 18°C. (A) GCN4-D: 10 μl, 217 μM GCN4-D was titrated into the cell containing 22 μM AP-1. (B) GCN4-M: 10 μl, 426 μM GCN4-M was titrated into the cell containing 22 μM AP-1. The buffer is same as in Fig. 2.

out on the AP-1 and CRE sites used in ITC experiments (data not shown), to ensure both DNA target sites are in duplex form. Furthermore, we have studied the formation of the AP-1 duplex from its two complementary single strands using ITC [25]. In conclusion, both AP-1 and CRE were in duplex form under the conditions of ITC experiments carried out herein.

Two typical ITC titrations for GCN4-D and GCN4-M binding to the AP-1 site at 18°C are shown in Fig. 4. The inset panel shows the trace recorded for each of the twenty-five 10-μl injections made at 400-s intervals. After each titration, an exothermic heat effect is observed. The area of each peak was integrated and corrected for the peptide heat of dilution, which was estimated by a separate experiment by injecting peptide into the buffer. By fitting the titration curve with a nonlinear least-squares method, the enthalpy change ΔH^0, and the binding constant K_b, of peptide binding

Table 1
Thermodynamic parameters of binding of GCN4-M and GCN4-D to AP-1 and CRE target site at 18°C

Peptide+DNA		N	$K_b \times 10^{-6}$ (M^{-1})	ΔH^0 (kJ/mol)	ΔG^0 (kJ/mol)	$T\Delta S^0$ (kJ/k·mol)
AP-1 site	GCN4-M	1.98±0.03	0.22±0.01	−36.8±0.3	−29.8±0.1	−7.0±0.4
	GCN4-D	1.04±0.05	2.25±0.25	−84.2±0.7	−35.4±0.3	−48.8±1.0
CRE site	GCN4-M	2.04±0.03	0.38±0.02	−35.9±0.4	−31.0±0.1	−4.9±0.5
	GCN4-D	1.03±0.04	2.64±0.40	−74.5±1.4	−35.8±0.3	−38.7±1.7

to DNA can be estimated with the assumption of an independent binding site model. Panels A and B show the fit of each integrated heat to a titration curve calculated on the basis of a single binding site model for GCN4-D and GCN4-M, respectively. The standard free energies (ΔG^0) were obtained from the equation $\Delta G^0 = -RT \ln K_b$, in which K_b is the apparent binding constant. The ΔS^0 function was calculated from the standard thermodynamic relation $\Delta G^0 = \Delta H^0 - T\Delta S^0$.

The results are summarized in Table 1. The values provided are the average of duplicate experiments. The CD results reported above (Fig. 2) indicate that GCN4-M and GCN4-D binding to AP-1 and CRE target sites is coupled with basic region folding. Hence, the thermodynamics of this protein-DNA recognition system include the overall properties of the binding and folding reactions. For both peptides binding to DNA, we obtained exothermic enthalpies and unfavorable entropy, the favorable free energies result from partial compensation of favorable enthalpies with unfavorable entropy.

4. Discussion

As bZIP factors themselves form a relatively stable dimer and bind DNA as dimers in the solution, it is widely believed that bZIP factors have to form a dimer before binding to their target.

DNA sites, and that the monomers do not specifically bind to DNA [9,13]. However, this idea has recently been challenged [14,16,26], and the idea that GCN4 need not dimerize in order to recognize its specific DNA site has been supported by kinetic and mechanism studies on the formation of the DNA-bZIP protein complex [16,26]. CD spectroscopy and ITC have been used to characterize the DNA-binding by the monomeric and the dimeric basic region of GCN4. The objective of this work is to test whether the monomeric GCN4 recognizes its DNA targets as the dimer does in sequence-specific fashion.

The CD difference spectra reported here demonstrate the induction of significant conformational changes from coil to α-helix for both peptides GCN4-M and GCN4-D in the presence of the AP-1 or CRE site. The similarity in conformational changes of the two peptides induced by DNA suggests that the binding property of the monomeric peptide to specific DNA is quite similar to that of the dimeric peptide. The added linker does not change the bound conformations of the dimeric peptide GCN4-D on the DNA binding site. The thermal unfolding of the complexes of GCN4-M and GCN4-D with the AP-1 and CRE sites shows that the three-dimensional structure of the complex disrupts cooperatively and further suggests that, similar to the dimeric peptide GCN4-D, the monomeric peptide GCN4-M also specifically binds AP-1 and CRE target sites. However, the monomer has a weaker binding affinity to the DNA compared to the dimeric form.

Table 1 shows the thermodynamics of the GCN4-D binding to AP-1 and CRE sites at 18°C. Berger et al. [27] have reported the thermodynamic studies on GCN4-bZIP corresponding to sequence 220–281 of the native GCN4 binding to the AP-1 and CRE target sites. In low salt Tris buffer, $\Delta H^0 = -137.12$ (±0.85) kJ/mol, $K_b = 5.42$ (±0.92)×10^7 (M^{-1}) for the AP-1 site, and $\Delta H^0 = -101.37$ (±3.01) kJ/mol, $K_b = 9.18(\pm 6.50) \times 10^7$ (M^{-1}) for CRE site. However, compared with their results, we obtained much higher enthalpy values, $\Delta H^0 = -84.2$ (±0.7) kJ/mol, $\Delta H^0 = -74.5$ (±1.4) kJ/mol, and lower values for the binding constants $K_b = 2.25$ (±0.25)×10^6 (M^{-1}), $K_b = 2.64$ (±0.40)×10^6 (M^{-1}) in 10 mM phosphate, 100 mM NaCl buffer at 18°C, for the GCN4-D binding to AP-1 and CRE, respectively. This discrepancy may be due to the fact that the GCN4-D studied herein

is the basic region of GCN4 alone, without the leucine zipper region, but dimerized at the C-terminus by an engineered disulfide-bond. When bound to DNA, the two basic regions bind to the major groove at each recognition half-site and form a diverged angle, which is probably different between the peptide GCN4-D and the peptide dimerized by the leucine zipper. Using the deoxyribonuclease (Dnase) I footprinting, Talanian et al. [9] has reported that, unlike the native GCN4 with a leucine zipper, DNA binding by the disulfide bond-linked GCN4 is temperature dependent between 4 and 24°C. When Dnase I digestion was carried out at 24°C, the disulfide-linked GCN4 failed to bind specifically. Thus, this discrepancy also suggests that the role of the leucine zipper, orienting the basic region to the DNA binding regions, is not completely modeled by the flexible disulfide bond linker. In other words, the results further confirm that, although the basic region is sufficient for specific DNA binding, the leucine zipper region is also essential for DNA binding because it mediates dimerization, and thus orients and stabilizes the adjacent basic region [6,9,10].

The binding constants (K_b), enthalpy of binding (ΔH^0), and stoichiometry (N) of the formation of GCN4-D−DNA complexes were determined by fitting to an independent binding model. Little is known on how the monomeric peptide binds to the dimer DNA target site; herein, we also used the independent binding model to fit the data. The binding numbers of the GCN4-M to AP-1 and CRE are 1.98 ($\pm$0.03) and 2.04$\pm$(0.03), respectively, which indicates that two monomeric peptides of GCN4-M molecules bind to one DNA molecule. In the crystal structure complex of GCN4−DNA [4,5], the leucine zipper orients the basic region parallel to the panel of the DNA site and identically contacts in the major grooves of both half-sites. Whether the two monomers contact both half sites of the DNA with identical orientation as the native GCN4 necessitates further investigation. However, our CD difference results, as discussed above, show similar conformational changes with GCN4-M and GCN4-D when bound to DNA. Thus, we propose a model in which the two monomers recognize each of the half-sites of AP-1 and CRE with each C-terminus becoming positioned near the other while the two monomers bind to DNA. We believe there must exists cooperativity between the two half binding sites when binding to DNA, binding of the first monomer peptide GCN4-M changes the conformation of the second half site whereby its affinity changes. The thermodynamic parameters for GCN4-M binding to DNA reported here do not pertain to individual binding sites, but rather the average of the two monomeric peptides. Compared with the dimer peptide GCN4-D, the enthalpy ΔH^0 of GCN4-M binding to the AP-1 and CRE sites is not simply equal to, but higher than, half of the ΔH^0 value of GCN4-D, corresponding to the binding heat for one basic region bound to DNA. This also suggests that binding of the first monomer of GCN4-M may affect the binding of the second molecule to the other half site because the conformation of the free basic region of the peptide is rather flexible. We obtained a smaller value of the apparent binding constant K_b for GCN4-M binding to DNA than the disulfide-linked dimer GCN4-D, which indicates that although the monomer can bind to the specific DNA target sites, the binding affinity is much weaker. This also can be inferred from the thermal unfolding studies on the complexes. The observation suggests that the dimerization formed by the engineered C-terminal Cys stabilizes the complex of GCN4-DNA.

We have performed similar titrations for the GCN4-D and GCN4-M binding to the control oligonucleotide CONT under the same conditions, but we failed to measure the thermodynamic parameters for its binding by both peptides (data not shown here). This suggests that the binding of GCN4-M to CONT is non-specific. CD difference spectrum has shown that the control DNA CONT induces the conformational changes of the GCN4-M (Fig. 2B), however, our ITC results and the thermal unfolding experiments (Fig. 3B) reported above indicate that the interactions between GCN4-M and CONT are presumably due to the electrostatic attraction, other than the specific interactions. Therefore, we confirm the sequence-specificity for GCN4-M recognizing the dimer DNA targets AP-1 and CRE.

In this article, we report evidence that the basic region of GCN4 can bind as monomers to the dimer binding site AP-1 and CRE. However, previous gel retardation assay studies suggested that the monomer failed to bind the DNA target site [9]. This discrepancy, we think, can be rationalized because the much weaker DNA binding affinity of the monomer as compared to the dimer might prevent detection of

the monomer during gel retardation assays at the concentrations used. The results reported here have implications for the mechanism by which bZIP transcription factors bind their target sites for transcriptional regulation.

Acknowledgements

We thank the Department of Science and Technology of China, the National Natural Science Foundation of China and the Department of Education of China for financial support.

References

[1] T. Curran, B.R. Franza Jr., Cell 55 (1988) 395.
[2] T. Kouzarides, E. Ziff, Nature (London) 340 (1989) 568.
[3] R. Turner, R. Tjian, Science 243 (1989) 1688.
[4] T.E. Ellenberger, C.J. Brandle, K. Struhl, S.C. Harrision, Cell 71 (1992) 1223.
[5] P. König, T.J. Richmond, J. Mol. Biol. 233 (1993) 139.
[6] E.K. O'Shea, R. Rutkowski, P.S. Kim, Science 243 (1989) 538.
[7] W.H. Landschulz, P.F. Johnson, S.L. Mcknight, Science 240 (1988) 1759.
[8] I.A. Hope, K. Struhl, Cell 46 (1986) 885.
[9] R.V. Talanian, C.J. McKnight, P.S. Kim, Science 249 (1990) 769.
[10] M.A. Weiss, T. Ellenberger, C.R. Wobbe, J.P. Lee, S.C. Harrison, K. Struhl, Nature 347 (1990) 575.
[11] K.T. O'Neil, R.H. Hoess, W.F. DeGrado, Science 249 (1990) 774.
[12] M. Neuberg, J. Adamkiewica, J.P. Hunter, R. Muller, Nature (London) 341 (1989) 243.
[13] I.A. Hope, K. Struhl, EMBO J. 6 (1987) 2781.
[14] C. Park, J.L. Campbell, W.H. Goddard, J. Am. Chem. Soc. 118 (1996) 4235.
[15] T.K. Blackwell, B. Boweman, J.R. Priess, H. Weintraub, Science 226 (1994) 621.
[16] C. Berger, L. Piubelli, U. Haditsch, H.R. Bosshard, FEBS Lett. 425 (1998) 14.
[17] R. Knorr, A. Trzeciak, W. Bannwarth, D. Gillessen, Tetrahedron Lett. 30 (1989) 1927.
[18] J.P. Tam, W.F. Heath, R.B. Marifield, J. Am. Chem. Soc. 108 (1986) 5242.
[19] C.R. Canter, M. Warshaw, H. Shapiro, Biopolymers 9 (1970) 1059.
[20] E. Freire, O.L. Mayorga, M. Straume, Analy. Chem. 62 (1990) 950A.
[21] M. Ueno, A. Murakami, K. Makino, T. Morii, J. Am. Chem. Soc. 115 (1993) 12575.
[22] C.R. Palmer, L. Sloan, J. Adrian, B. Cuenoud, D. Paolella, A. Schepartz, J. Am. Chem. Soc. 117 (1995) 8899.
[23] M. Pellegrini, R.H. Ebright, J. Am. Chem. Soc. 118 (1996) 5831.
[24] R.V. Talanian, C.J. McKnight, R. Rutkowski, P.S. Kim, Biochemistry 31 (1992) 6871.
[25] W. Cao, L. Lai, Biophy. Chem. 80 (1999) 217–226.
[26] S.J. Metallo, A. Schepartz, Nature Struct. Biol. 4 (1997) 115.
[27] C. Berger, I. Jelisarov, H.R. Bosshard, Biochemistry 35 (1996) 14984.