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The Effects of Salt on the TATA Binding Protein-DNA Interaction from a Hyperthermophilic Archaeon

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This study investigates the thermodynamics of the interaction of the TATA box binding protein (TBP) from *Pyrococcus woesei* (*Pw*) with an oligonucleotide containing a specific binding site. *Pw* is a hyperthermophilic archeal organism which exists under conditions of high salt and high temperature. A measurable protein-DNA interaction only occurs at high salt concentrations. Isothermal titration calorimetric binding studies were performed under a range of salts (potassium chloride, potassium phosphate, potassium acetate and sodium acetate) at varying concentrations (0.8 to 1.6 M). At the high salt concentrations used the observed equilibrium binding constant increases with increasing salt concentration. This is very different to the effect reported for all other protein-DNA interactions which have been studied at lower salt concentrations. Thermodynamic data suggest that the protein-DNA interaction at high salt concentration is accompanied by the removal of large numbers of water molecules from the buried hydrophobic surface area. In addition, the involvement of ions appears to influence the binding which can be explained by binding of cations in the interface between the electrostatically negative lateral lobes on the protein and the negatively charged DNA.

Keywords: protein-DNA interactions; isothermal titration calorimetry; hyperthermophile; cations; salt dependence

Introduction

Some proteins with a high level of sequence, structural and mechanistic similarity are able to exist in dramatically different environments. This is particularly apparent when comparing proteins from hyperthermophiles with those from mesophilic organisms. A knowledge of how proteins adapt to their environments is of fundamental importance in understanding the determinants of proteins stability and protein-ligand interactions. Here we explore the thermodynamic-structural relationship of the TATA box-binding protein (TBP) from the hyperthermophilic *Pyrococcus woesei* (*Pw*) archea interacting with an oligonucleotide and compare this to the equivalent interaction in mesophilic counterparts.

The *Pw*TBP has approximately 35% sequence identity with its counterparts from the mesophilic species *Arabidopsis thaliana* (*At*) and *Saccharomyces cerevisiae* (*Sc*) (Figure 1). Furthermore, crystal structures of the C terminus of *Pw*TBP, *At*TBP and *Sc*TBP determined in the free state (DeDecker *et al.*, 1996; Nikolov *et al.*, 1992; Chasman *et al.*, 1993; Geiger *et al.*, 1996), and bound to a TATA-box oligonucleotide sequence (Kosa *et al.*, 1997; J. Kim *et al.*, 1993; Y. Kim *et al.*, 1993) show a high degree of structural homology. It is interesting that despite the hyperthermophilic TBP being active at much higher salt conditions and at temperatures elevated by approximately 75°C, it maintains a clear structural resemblance to proteins with similar function in other organisms.

The archeal *Pw* lives in the highly saline conditions found near deep sea vents (Zillig *et al.*, 1993) and has an optimal growth temperature of 105°C. The intracellular salt concentration in the organism is in the region of 0.8 M (Scholz *et al.*,

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Abbreviations used: TBP, TATA box binding protein; *Pw*, *Pyrococcus woesei*; AUC, analytical ultracentrifugation; ITC, isothermal titration calorimetry.

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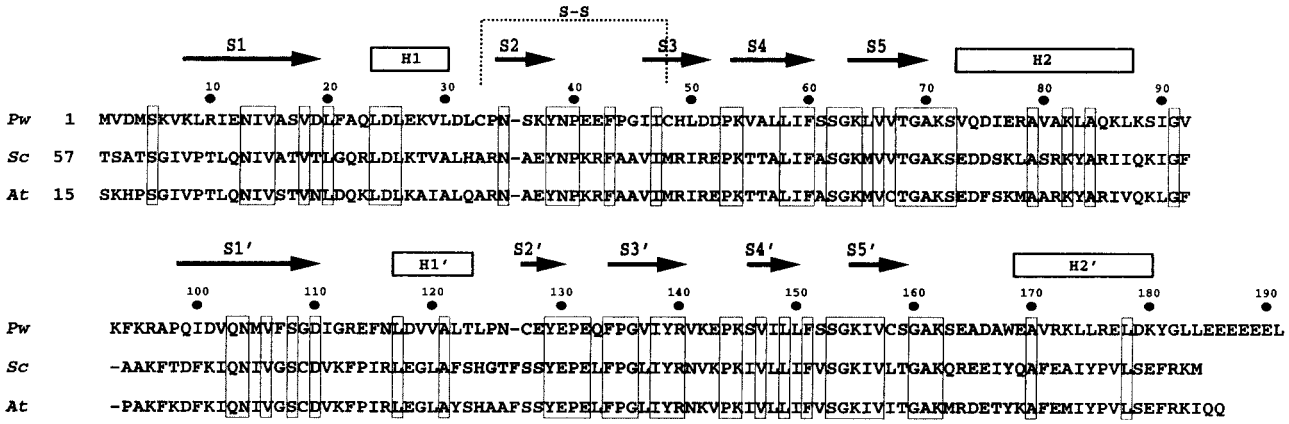

Figure 1. Primary sequence alignment of the conserved C termini of *Pw*, *Sc*, and *At* TBP. Numbering and secondary structure (S, beta sheet; H, alpha helix) are labeled according to the *Pw*TBP structure. Identical residues are boxed.

1992). TBP is the primary factor of the basal transcription machinery in both archaeal and eukaryotic species (Qureshi *et al*., 1995a,b; Rowlands *et al*., 1994). The archaeal TBP binds upstream of transcription sites at promoters containing A + T-rich sequences called ''box A motifs'' which have the consensus sequence of TTTA(T or A)ANN (where N is any nucleotide; Zillig *et al*., 1993) which is analogous to that of the eukaryotic TATA box of TATA(T or A)ANN (Hahn *et al*., 1989).

Archeal and eukaryotic TBP bind to the minor groove of DNA, incurring negligible structural changes in the protein but inflicting a severe bend on the DNA which results in an untwisting of the duplex by a third of a helical turn over the seven inter-base-pair steps of the contact surface. This deformation is required for, and is incorporated into, higher order complexes that involve the transcription factor TFIIB and presumably the complete pre-initiation assembly. In all cases the surface of the protein binding site is largely hydrophobic. The major difference in the structures is found in the distribution of electrostatic charge potential (DeDecker *et al*., 1996). Whereas the potential surrounding the *At*TBP and *Sc*TBP is almost entirely positive, on *Pw*TBP the substitution of neutral by acidic residues leads to a nearly net neutral charge. However, a distinctly negative potential is localized to the lateral extremes (stirrups) of the *Pw*TBP which form part of the DNA binding site (DeDecker *et al*., 1996).

Here we use the thermodynamic data in relation to structural detail to analyze the binding of TBP from an archeal organism with an oligonucleotide corresponding to its physiological binding site. By investigating this interaction in a range of salts at different concentrations we are able to discuss the physico-chemical mechanism of binding.

Results

ITC has been used to characterize the binding of the *Pw*TBP to a 16 bp stem of a hairpin oligonucleotide containing a box A motif sequence at a range of temperatures and salt concentrations. The box A motif sequence is a specific binding site for the protein and we observe no effects associated with non-specific binding. Since it is generally considered to be of an electrostatic nature, non-specific binding would be unlikely to prevail at the high salt concentrations used in these binding studies. The thermodynamic data derived from the titrations are given in Tables 1 and 2. Binding is only detectable in elevated salt concentrations($\geqslant$200 mM). In all cases the stoichiometry of the interaction was unity (mean for all experiments is 0.96 ± 0.10).

Analytical ultracentrifugation (AUC) studies

Since TBP is known to show a tendency to dimerize it was important to define the oligomeric state of the protein under the conditions adopted in the binding experiments. Table 3 shows the AUC data obtained at two representative salt concentrations and three different temperatures. The equilibrium association constants for homodimer formation at 0.8 to 1.6 M NaOAc are very low and appear to be temperature invariant in the range 4 to 35°C. From these data we can calculate the amount of dimer apparent at various protein concentrations. In all the binding studies we used concentrations of the protein such that the presence of dimer had a negligible effect on the thermodynamic data.

The effect of salt concentration

The *Pw*TBP-DNA interaction at any given salt concentration (in the range 0.8 to 1.6 M) has a favorable entropic and unfavorable enthalpic contribution to the observed free energy. Titrations were performed in a range of concentrations of the potassium salts of acetate, chloride and phosphate as well as in sodium acetate (the thermodynamic data at 35°C are shown in Table 1). The observed binding constants (K_{obs}) are highly sensitive to salt concentration. For example, in potassium acetate at

Table 1. Thermodynamic data for the interaction of *Pw*TBP with DNA at 35°C in different salts

[Salt] (M)	[DNA] (mM)	[Protein] (μM)	N	K_{obs} (10^6 M^{-1})	ΔG_{obs} (kcal mol^{-1})	ΔH_{obs} (kcal mol^{-1})	$T\Delta S_{obs}$ (kcal mol^{-1})
Sodium acetate							
0.80	0.10	10	0.88	4.79 ± 0.72	−9.4	17.7 ± 0.4	27.1
1.30	0.10	10	0.97	20.3 ± 3.30	−10.3	17.9 ± 0.2	28.2
1.60	0.09	9.5	0.74	50.6 ± 7.40	−10.9	176 ± 0.1	28.5
Potassium acetate							
0.80	0.11	12	1.02	0.77 ± 0.05	−8.3	19.6 ± 0.3	27.9
1.00	0.31	25	0.88	1.36 ± 0.06	−8.7	21.3 ± 0.2	29.9
1.30	0.13	13	0.83	3.50 ± 0.26	−9.2	21.8 ± 0.2	31.0
1.60	0.12	12	0.90	12.4 ± 1.30	−10.0	21.0 ± 0.2	31.0
Potassium phosphate							
1.00	0.42	35	1.02	1.49 ± 0.08	−8.7	21.6 ± 0.1	30.3
1.15	0.38	31	0.97	3.64 ± 0.06	−9.3	21.5 ± 0.1	30.8
1.30	0.53	36	1.04	5.54 ± 0.53	−9.5	21.6 ± 0.4	31.1
Potassium chloride							
1.00	0.31	25	1.17	0.29 ± 0.01	−7.7	26.1 ± 0.3	33.8
1.30	0.31	25	1.04	0.75 ± 0.03	−8.3	29.5 ± 0.2	37.8
1.60	0.16	13	0.91	2.74 ± 0.13	−9.1	23.1 ± 0.1	32.2

The errors shown correspond to standard deviation of the non-linear least squares fit of the data points on the titration curve.
35°C was chosen as a suitable temperature, since it is comparable to mesophilic physiological temperatures and it is in the middle of the temperature range at which we were able to obtain binding data.
N is the stoichiometry of the interaction as determined by the fitting of the ITC binding isotherm (Wiseman *et al.*, 1989).

0.8 M, $K_{obs} = 7.7 \times 10^5$ M^{-1} while at 1.6 M, $K_{obs} = 10^7$ M^{-1}. The salt dependence of K_{obs} for the binding of TBP and DNA in a range of salts at 35°C are shown in Figure 2. For each type of salt the log K_{obs} increases with a similar, but not identical, dependency, as the log [salt] is increased. This is the reverse of what has been experimentally observed for protein-DNA interactions at lower salt concentrations (Ha *et al.*, 1992).

The entropic and enthalpic contributions to the free energy over a range of salt concentrations for each of the salts investigated are given in Table 1. The free energy becomes more favorable with increasing salt, whilst the enthalpic contribution remains relatively constant, showing that the increase in binding affinity with increased salt is an entropically driven phenomenon.

The effect of salt type

The dependence of K_{obs} on salt concentration at 35°C changes as a function of salt type (Figure 2). Most significantly the magnitude of log K_{obs} appears to be dictated by the position of the ions in the Hofmeister series (discussed below), suggesting the importance of the role of water in the binding event.

The effect of temperature

The enthalpy of binding is highly temperature dependent as shown in Table 2. At a representative salt concentration (1.3 M Kphos) the change in heat capacity, ΔC_{Pobs}, is −791 cal mol^{-1} K^{-1} (see Figure 3). The large negative ΔC_{Pobs} obtained is consistent with other highly complementary specific protein/DNA interactions (Ha *et al.*, 1989; Spolar & Record, 1994; Ladbury *et al.*, 1994; Hyre & Spicer, 1995; Lundbäck, 1996).

It is noticeable that at low temperatures there is a significant deviation from linearity of the graph ΔH *versus* temperature at 1.3 M. This results from another equilibrium which becomes more significant at lower temperatures and lower salt conditions. This is most probably due to structural changes that have been observed at low temperatures for some proteins in other thermophiles. The linearity of the plot observed at the higher temperatures indicates that any non-binding event has a negligible effect on the calculated ΔC_{Pobs}. The data reported herein on the effects of salt concen-

Table 2. Thermodynamic data for the interaction of *Pw*TBP with DNA at different temperatures

Temp. (°C)	[Protein] (μM)	N	K_{obs} (10^6 M^{-1})	ΔG_{obs} (kcal mol^{-1})	ΔH_{obs} (kcal mol^{-1})	$T\Delta S_{obs}$ (kcal mol^{-1})
1.3 M potassium phosphate						
15	65	0.91	0.18 ± 0.07	−6.9	29.5 ± 0.7	36.4
25	46	0.89	1.86 ± 0.53	−8.6	29.8 ± 0.5	38.4
30	35	0.97	2.59 ± 0.36	−8.9	25.3 ± 0.3	34.2
35	36	1.04	5.54 ± 0.50	−9.5	21.6 ± 0.4	31.1
40	14	1.01	19.8 ± 2.80	−10.5	17.1 ± 0.3	27.6
45	14	1.07	28.7 ± 1.50	−10.9	14.2 ± 0.1	25.1

The errors shown correspond to standard deviation of the non-linear least squares fit of the data points on the titration curve. N is the stoichiometry of the interaction as determined by the fitting of the ITC binding isotherm (Wiseman *et al.*, 1989).

Table 3. The calculated association constants, K_A, at different temperatures from analytical ultracentrifugation studies on *Pw*TBP

Temperature (°C)	$K_A(\times 10^3\ M^{-1})$
0.8 M NaOAc	
4	9.1
20	8.3
35	5.6
1.6 M NaOAc	
4	3.6
20	3.7
35	3.7

tration and type were at 35°C, which is not affected by structural changes, as shown by the linearity of the plot in Figure 3 in this region.

The magnitude of the ΔC_P values for *Pw*TBP is significantly larger (i.e. less negative) than that measured using quantitative footprinting titrations and van't Hoff analysis for the non-specific interaction of *Sc*TBP with the adenovirus E4 promoter (−4 kcal mol^{-1} K^{-1}; Petri *et al.*, 1995).

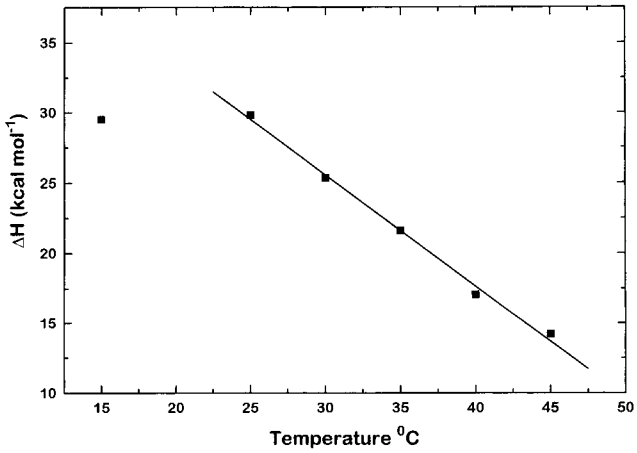

Figure 3. The effect of temperature on ΔH for binding of the *Pw*TBP to the DNA. The lines are linear least-squares regressions to the data at and above 25°C for the titrations performed in 1.3 M Kphos. Data taken from Table 2.

Discussion

Here we address the question: how do high salt and change in temperature affect the binding of *Pw*TBP to a specific oligonucleotide? We have determined the thermodynamics of binding in different salts and concentrations thereof, and at a range of temperatures. To discuss our findings we deal with the individual components of the protein-DNA interaction separately and where possible compare hyperthermophilic and mesophilic interactions.

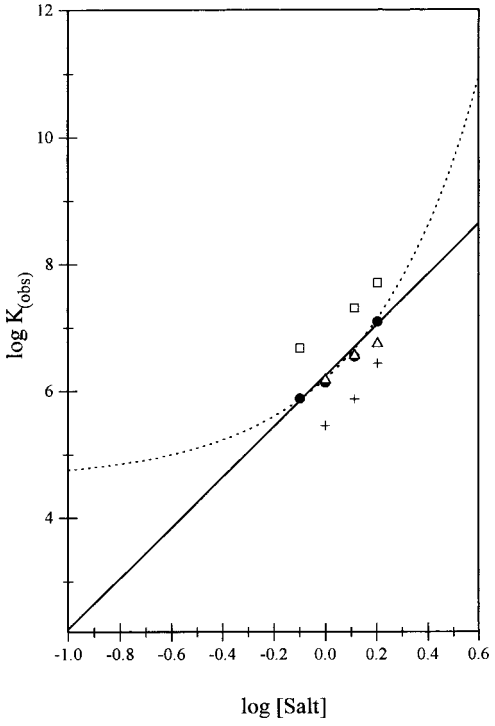

Figure 2. Plot of log K_{obs} against log [MX]. The data are shown for the *Pw*TBP interaction in different salts; □, NaOAC; ●, Kphos; +, KCl and △, KOAC. Simulations using equation (2) show approximate fits for line ----- when $K_{REF} = 4.6$, $A = 0$ and $B = 100$ water molecules released; and for line —— when $K_{REF} = 6.25$, $A = 4$ ions binding and $B = 0$ (see equations (1) and (2).

The oligonucleotide

The DNA is a highly negatively charged polyelectrolyte and clearly favors interaction with molecules with net positive charge in both the hyperthermophilic and mesophilic interactions. There are some differences in the recognition nucleotide sequences for the hyperthermophilic and mesophilic interactions. These are unlikely to be determinants for selecting high salt over low salt binding, since the net negative charge remains unaltered. In addition, the available structural data show that, although the DNA undergoes a significant conformational change upon binding, this effect is similar for both hyperthermophilic and mesophilic interactions and hence there is little net difference in the free energy associated with deformation of the DNA between the two forms.

The protein

The surfaces of the DNA binding sites of both the hyperthermophilic and mesophilic TBP are dominated by a large number of hydrophobic residues. This apolar surface interacts intimately with the oligonucleotide bases that are exposed in the severe distortion of the DNA on forming the complex. The charge potential surrounding most of *Pw*TBP is neutral at pH 7. Distinct regions of negative potential are, however, localized to the lobes (which are likened to stirrups of TBP whose overall

structure resembles a saddle) at the lateral extremes of the protein and these are in close proximity to the boundary of the eight-base TATA-like element despite the DNA being polyanionic.

The salt concentration

Here we report the first experimental demonstration of an increase in K_{obs} with increasing salt concentration for a protein-DNA interaction. Previously reported experimental data on the effects of salt concentration on protein-DNA interactions show that at lower salt concentrations than used in this study, and where the removal of large numbers of water molecules is not involved, the affinity decreases with increased salt (for example, see Ha *et al*., 1992). In these studies the reduction in the observed binding constant is ascribed to the decrease in favorable entropy of ion release from the polyelectrolyte DNA as the concentration of salt in the bulk solvent is increased (Ha *et al*., 1992; Lohman *et al*., 1996).

The relationship between K_{obs} and salt concentration has previously been successfully modelled using an equation based on the relative stoichiometries of cations, anions and water molecules involved in coupled equilibria which occur on formation of the complex (Record *et al*., 1978; Ha *et al*., 1992). The following equation derived from Ha *et al*. (1992) allows prediction of the net uptake or release of ions and water molecules from the dependence of K_{obs} on salt concentration (SK_{obs}):

$$-SK_{obs} = -d\log K_{obs}/d\log[MX] = A - 2(B)[MX]/[H_2O] \quad (1)$$

where [MX] is the univalent salt concentration, A is the sum of the net stoichiometries of cation and anion release upon binding, respectively and B is the total number of water molecules that are displaced from both protein and DNA. An approximate integrated form of the above equation demonstrates how ion and water stoichiometries can affect K_{obs}:

$$\log K_{obs,MX} = \log K_{REF,MX} - A\log[MX] + 0.016(B)[MX] \quad (2)$$

where A is the total ion stoichiometry released (a positive number) or taken up (a negative number); [MX] and B are defined as above, and K_{REF} is the binding constant in a hypothetical reference state ([MX] = 1 M) where the effects of ion and water displacement are non-existent (the subscript MX denotes that the term is based on an equilibrium in a given salt). We have simplified this equation from that described by Ha *et al*. (1992) by assuming that the water, cation and anion stoichiometries are independent of salt concentration and the nature of the salt. This allows us to incorporate the cumulative effects of cations and anions in one term of the equation.

The relationship described by equation (2) can be used to fit the data obtained here. However, to produce the log K_{obs}−log[MX] dependence at high salt concentrations we have to invoke two effects. (1) A large differential hydration between free and bound molecules. (2) Incorporation of ions into the complex interface. Figure 2 shows simulations of data obtained using equation (2) to demonstrate the effect of both large differential hydration and the incorporation of ions in the binding site. By changing the value of K_{REF} we are able to get reasonable fits to our limited data set (only that for Kphos is shown in Figure 2). The two effects described above are not mutually exclusive and could occur as coupled equilibria in the formation of the *Pw*TBP-DNA complex in high salt concentrations. We have no direct evidence for either of the two effects described above; however, from the ITC data we are able to hypothesize that at 35°C it is both dehydration, and the involvement of cations in the binding site that contribute to the ΔG_{obs} of the interaction, although the former is likely to dominate as described below.

The effect of a large differential dehydration on forming the protein-DNA interface

From the calorimetric studies at 35°C it is apparent that the more favorable free energy of the *Pw*TBP-DNA interaction with increasing salt concentration is dominated by a large favorable entropy accompanied by an unfavorable enthalpy. This entropic effect was observed in previously reported protein-DNA interactions at lower salt concentrations and attributed to the release of ions from the interacting macromolecules. However, since this source of an entropic effect is likely to be reduced significantly in high salt conditions (Ha *et al*., 1992), we propose that it is largely based on the release of water. Increasing the salt concentration serves to decrease effective concentration of water in the bulk phase and hence decrease the water activity (Record *et al*., 1978). Water in contact with hydrophobic residues will adopt an ordered conformation. Thus, in the largely hydrophobic binding site of the free protein or DNA a large number of water molecules will be restricted. On forming the complex the release of this water into bulk solvent with a reduced water activity results in an increase in entropy contributing favorably to the free energy of the system.

As the salt concentration of any of the salts is increased the enthalpic contribution remains approximately constant (Table 1). This is also consistent with the idea that the release of water on the burial of hydrophobic surface is a major contribution to the observed free energy of binding, since, for a given salt at different concentrations, the actual amount of water liberated and hence the number of hydrogen bonds between ordered water molecules that are broken on forming the complex is likely to be constant. This would result in little

change in enthalpies of binding measured at the different salt concentrations.

The effect of incorporation of ions into the protein-DNA interface

As described above the direct interaction of the electrostatically negative protein stirrups with the DNA is unfavorable due to charge repulsion. Thus, the presence of cations in these regions of the *Pw*TBP-DNA interface would reduce the expected repulsive effect. Although the crystal structure of the complex (Kosa *et al.*, 1997) shows electron density between the protein-DNA interface in these regions, it is not possible to unequivocally assign these as cations. The increasingly favorable entropy of binding with higher salt concentration would argue against cations being drawn from bulk solvent to be incorporated in the binding site (an entropically unfavorable effect). Thus a compelling argument can be made for the cations that are condensed on the DNA being incorporated in the interface.

The type of salt

Salts have been shown to play an important role in stabilization of proteins *via* their effect on water structure and thus the hydrophobic effect (Collins & Washabaugh, 1985). The relative effects of the salts are predicted by their relative position in the Hofmeister series. In this series anions and cations are ordered based on their respective kosmotropic or chaotropic nature. Kosmotropes have the effect of increasing the ordered nature of water on hydrophobic surfaces of a solute molecule. As mentioned above the formation of this ''water structure'' is an energetically unfavorable process. Therefore, the burial of hydrophobic surface is favored in kosmotropic salts. The salts used in this study form the following series with respect to kosmotropic nature: anions, phosphate > acetate > chloride; cations, sodium > potassium.

A series of ITC experiments were performed to explore the effects of using salt with different kosmotropic nature. At the high salt concentrations used in these studies the K_{obs} increases with the kosmotropic character of the anion (Table 1). Comparison of the ITC data at 35°C shows that on progressing along the series towards the more kosmotropic salts small decreases in ΔG_{obs} result from an increasingly less favorable ΔS_{obs} and more favorable ΔH_{obs}. These changes in thermodynamic property from one salt to another are very small compared to the overall values for the binding interaction. For example, on going from Kphos to NaOAc at 1.3 M $\Delta\Delta G_{obs} = \rightarrow 0.8$ kcal mol^{-1}; $\Delta\Delta H_{obs} = -3.7$ kcal mol^{-1} and $T\Delta\Delta S_{obs} = -2.9$ kcal mol^{-1}. This is important to note, since whatever effect is imposed by the change in salt type has a small contribution to the overall binding. The reduction of the entropic term with the increasing kosmotropic nature of the solvent suggests that the lowering of ΔG_{obs} is not the result of differential dehydration, since the more ordered the water on the binding site the greater should be the entropy change on releasing it into bulk solvent. We, thus, conclude that the differential dehydration does not significantly vary between the different salts. This suggests that ions play a role in the binding of the protein to DNA.

As described above, the postulate of the presence of cations in the interface between the regions of negative electrostatic potential on the protein and the DNA is appealing, since they would reduce the repulsion between the interacting macromolecules. This incorporation of ions (and most likely cations) can be used to explain the less favorable entropy and the more favorable enthalpy observed with increasing kosmotropic nature of the solvent. This explanation could result from one of two distinct, but related effects in the binding site. (1) Ions that are condensed on the surface of the unbound components of the interaction become bound in the interface on forming the complex; (2) ions are brought from the bulk solvent to bind in the interface.

In the first case DNA and electrostatically negative regions of the protein would interact favorably with cations in the unbound state. Since there are ions interacting in the binding site, on forming the complex ions would be released if the cumulative concentration on both of the macromolecular electrolytes is too large to accommodate. This would give rise to a favorable entropic and unfavorable enthalpic contributions to the ΔG_{obs}. Since kosmotropic salts tend to preferentially hydrate macromolecules resulting in an exclusion of ions from their surfaces, the entropic benefit and enthalpic loss become less as the kosmotropic nature of the salt increases.

In the second case the increase in kosmotropic nature of the salt reduces the number of cations bound to the macromolecules. If this resulted in cations having to be taken from the bulk solvent to bind in the TBP-DNA interface increasingly unfavorable entropy and favorable enthalpy changes would be observed. It is impossible to unequivocally state which of the above effects is observed in our ITC experiments. Nonetheless, the involvement of cations in the interaction is strongly suggested by the fact that when the anion is constant in the case of the binding data in NaOAc and KOAc, the more kosmotropic the cation, the less favorable the entropy and the more favorable the enthalpy. Furthermore, the effect of binding of cations as shown in Figure 2 could explain why the *Pw*TBP-DNA interaction does not occur at low salt concentrations, since at concentrations below 0.8 M the binding gets progressively weaker.

The dependence on temperature

Figure 3 shows the strong temperature dependence of ΔH_{obs}. This is typical of interactions that involve the burial of large hydrophobic surface

areas. A discrepancy of around 30% is apparent between the ΔC_{Pobs} determined empirically and that calculated from the correlation with buried surface area (Spolar & Record, 1994), i.e. the calculated value is -511 cal mol^{-1} K^{-1}. In interactions dominated by the burial of hydrophobic surface good agreement between the calculated and experimental ΔC_P values would be expected (Haq *et al.*, 1997). Discrepancies have previously been observed in protein/DNA complexes where water is found in the interface (Ladbury *et al.*, 1994; Morton & Ladbury, 1996). These discrepancies were attributed to the reduction of soft vibrational modes of water molecules through their restriction in the complex interface. In the case of the TBP-DNA interaction we hypothesize that the restriction of ions in the interface may be at least partially responsible for the apparent discrepancy.

Summary

We have investigated the interaction of the *Pw*TBP with its specific target oligonucleotide at a range of different concentrations and types of salt and at different temperatures. At 35°C, we hypothesize that the binding is dictated by the release of ordered water from the large hydrophobic surface area but there is a contribution from the incorporation of ions mediating the interaction of electrostatically negative surfaces in the protein and DNA. The experiments at different salt concentrations enable us to demonstrate that the entropic effect from the release of water on the burial of hydrophobic surface on forming the complex is a major contribution to the ΔG_{obs}. However, the experiments in salts with different kosmotropic natures allow us to demonstrate that the contribution to the thermodynamics of complex formation from cations cannot be ignored.

At 35°C the binding of *Pw*TBP is dominated by the release of ordered water from hydrophobic surface (the hydrophobic effect) on forming the complex. However, at the physiological temperature at which the hyperthermophilic organism exists the hydrophobic effect is reduced (Privalov & Gill, 1989). It has been shown from studies on the heats of transfer of small hydrocarbon molecules into aqueous solvents and protein folding/unfolding equilibria that at temperatures of approximately 110°C the entropic contribution from the hydrophobic effect reduces to zero (Privalov & Gill, 2988, 1989; Baldwin & Muller, 1992). Under kosmotropic conditions the hydrophobic effect is likely to prevail to higher temperatures. Furthermore, under conditions of highly kosmotropic salts the binding of cations could be important. If the enthalpic gain from binding the cation is greater than the favorable entropy of removing it from bulk solvent then there will be a net favorable contribution to ΔG_{obs}.

The structures of the TBP molecules from hyperthermophilic and mesophilic organisms are highly conserved even though they exist under very different conditions. It appears that by subtle substitutions of amino acids (particularly in the binding site) the proteins are able to exploit the solvent conditions. The predominance of hydrophobic surface area in the binding site suggests that liberation of water into the bulk phase is crucial to binding for both forms of TBP.

The clearest structural difference between the archea and mesophilic TBP is the overall neutrality of the charge on the potential distribution and greater electronegativity of the lobes in the DNA binding site in the former. It is likely that these lobes sequester cations on binding to DNA. This offsets the repulsive effect of interaction with the negatively charged DNA but could also result in the cationic mediation of definite interactions in the interface adding a higher level of specificity to the interaction.

Materials and Methods

TBP expression and purification

Full length *Pw*TBP (see Figure 1 for amino acid sequence) was expressed and purified as previously described (DeDecker *et al.*, 1996). Protein concentrations were determined using an extinction coefficient of 11.05 mM^{-1} cm^{-1} at 280 nm, calculated from the individual extinction coefficients of the tryptophan and tyrosine residues and disulfides in the protein (Pace *et al.*, 1995).

DNA preparation

The DNA (sequence with binding site underlined; AAGCTTTAAAAA-GTAACCTCCTTACTTTTTAAAGC-TT) was designed to form a hairpin loop. This was synthesized at the Keck facility. DNA concentrations were determined using an extinction coefficient (of 416.3 mM^{-1} cm^{-1}) calculated from the sum of those of the individual bases. The DNA was hydrolyzed with an endonuclease prior to concentration determination. Prior to use the oligonucleotide was heated to approximately 100°C and rapidly cooled in ice to ensure hairpin formation. Thermal unfolding studies by differential scanning calorimetry of the DNA in 1.3 M potassium phosphate at pH 7.0 did not show any concentration dependence of the t_m (between 0.02 mM and 0.12 mM DNA), suggesting that only one species was present.

Analytical ultracentrifugation (AUC)

Sedimentation equilibrium AUC was performed using a Beckman Optima XL-I ultracentrifuge, using the absorbance optics system to visualize the protein at 280 nm. Six sector cells were used, and data were acquired every 0.001 cm with ten replicates. Sample volumes were 110 μl. Data were collected at 35°C at speeds of 20,000, 24,5000 and 30,000 revolutions per minute and at 4°C and 20°C at 24,500 revolutions per minute. Data were collected on samples at three initial concentrations ranging from 260 μM to 760 μM. Absorbance *versus* radius scans were collected at 60 minute intervals, and sedimentation equilibrium was established using the MATCH algorithm. The protein partial specific volume was calculated from the amino acid composition using the values

of Cohn & Edsall (1943). The solvent densities were estimated by summing the density increments of the buffer components (Laue *et al*., 1992).

Global analysis of sedimentation equilibrium data for each temperature was accomplished using the Macintosh version of the NONLIN algorithm (Johnson *et al*., 1981). The value of *s*l was fixed to correspond to the calculated buoyant molecular weight of the monomer. The error of the fit was determined by examination of the residues and minimization of the variance. Apparent absorbance equilibrium constants were converted to molar units using the molar extinction coefficient (see above).

Isothermal titration calorimetry (ITC)

All experiments were conducted using an MCS ITC or an OMEGA ITC (MicroCal Inc., Northampton, MA). Titrations were performed as described elsewhere (Wiseman *et al*., 1989; Ladbury & Chowdhry, 1996). In a typical experiment 16, 15 μl injections of DNA (0.42 mM) were made into TBP (35 μM) in the ITC cell at pH 7.0 and 35°C. The heats of dilution of DNA into buffer and buffer into TBP were determined in separate experiments and subtracted from the titration prior to data analysis. The data were analyzed using the ORIGIN software supplied with the calorimeter. Titrations were performed in 10 mM Mops (3-[*N*-morpholino]propane-sulfonic acid) and either potassium phosphate (Kphos), potassium chloride (KCl), potassium acetate (KOAc) or sodium acetate (NaOAc) was added to the designated concentrations (0.8, 1.0, 1.15, 1.3, or 1.6 M). The pH of each solution was checked prior to each ITC experiment.

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References

Baldwin, R. L. & Muller, N. (1992). Relation between the convergence temperatures T_h* and T_s* in protein unfolding. *Proc. Natl Acad. Sci. USA,* **89**, 7110–7113.

Chasman, D. I., Flaherty, K. M., Sharp, P. A. & Kornberg, R. D. (1993). Crystal structure of yeast TATA binding protein and model for interaction with DNA. *Proc. Natl Acad. Sci. USA,* **90**, 8174–8178.

Cohn, E. J. & Edsall, J. T. (1943). Density and apparent specific volume of proteins. In *Proteins, Amino Acids and Peptides* (Cohn, E. J. & Edsall, J. T., eds), pp. 370–381, Reinhold Corporation, New York.

Collins, K. D. & Washabaugh, M. W. (1985). The Hofmeister effect and the behaviour of water at interfaces. *Quart. Rev. Biophys.* **18**, 323–422.

DeDecker, B. S., O'Brien, R., Fleming, P. J., Geiger, J., Jackson, S. P. & Sigler, P. B. (1996). The crystal structure of a hyperthermophilic archael TATA-box binding protein. *J. Mol. Biol.* **264**, 1072–1084.

Geiger, J. H., Han, S., Lees, S. & Sigler, P. B. (1996). Crystal structure of the yeast TFIIA/TBP/DNA complex. *Science,* **272**, 830–836.

Ha, J.-H., Spolar, R. S. & Record, M. T. (1989). The role of the hydrophobic effect in stability of site-specific protein-DNA complexes. *J. Mol. Biol.* **209**, 801–816.

Ha, J.-H., Capp, M. W., Hohenwalter, M. D., Baskerville, M. & Record, M. T., Jr (1992). *J. Mol. Biol.* **228**, 252–264.

Hahn, S., Buratowski, S., Sharp, P. A. & Guarente, L. (1989). Isolation of the gene encoding the yeast TATA binding-protein TFIID. A gene identical to the SPT15 suppressor of TY element insertions. *Cell,* **58**, 1173–1181.

Haq, I., Ladbury, J. E., Chowdhry, B. Z., Jenkins, T. C. & Chairs, J. B. (1997). Specific binding of Hoechst 33258 to the $d(CGCAAATTTGCG)_2$ duplex: calorimetric and spectroscopic studies. *J. Mol. Biol.* **271**, 244–257.

Hyre, D. E. & Spicer, L. D. (1995). Thermodynamic evaluation of binding interactions on the methionine repressor system of *Escherichia coli* using isothermal titration calorimetry. *Biochemistry,* **34**, 3212–3221.

Johnson, M. L., Correia, J. J., Yphantis, D. A. & Halvorson, H. R. (1981). Analysis of data from the analytical ultracentrifuge by nonlinear least-squares techniques. *Biophys. J.* **36**, 575–588.

Kim, J. L., Nikolov, D. B. & Burley, S. K. (1993). Co-crystal structure of TBP recognizing the minor groove of a TATA element. *Nature,* **365**, 520–527.

Kim, Y., Geiger, J. H., Hahn, S. & Sigler, P. B. (1993). Crystal structure of yeast TBP/TATA-box complex. *Nature,* **365**, 512–520.

Kosa, P. F., Ghosh, G., DeDecker, B. S. & Sigler, P. B. (1997). The 2.1-angstrom crystal structure of an archaeal preinitiation complex: TATA-box-binding protein/transcription factor (II)B core/TATA-box. *Proc. Natl Acad. Sci. USA,* **94**, 6042–6047.

Ladbury, J. E. & Chowdhry, B. Z. (1996). Sensing the heat: the application of isothermal titration calorimetry to thermodynamic studies of biomolecular interactions. *Chem. Biol.* **3**, 791–801.

Ladbury, J. E., Wright, J. G., Sturtevant, J. M. & Sigler, P. B. (1994). A thermodynamic study of the *trp* repressor-operator interaction. *J. Mol. Biol.* **238**, 669–681.

Laue, T. M., Shah, B., Ridgeway, T. M. & Pelletier, S. L. (1992). Interpretation of analytical sedimentation data for proteins. In *Analytical Ultracentrifugation in Biochemistry and Polymer Science* (Harding, S. E., Rowe, A. J. & Horton, J. C., eds), pp. 90–125, Royal Society of Chemistry, Cambridge.

Lohman, T. M., Overman, L. B., Ferrari, M. E. & Kozlov, A. G. (1996). A highly salt-dependent enthalpy change for *Escherichia coli* SSB protein-nucleic acid binding due to ion-protein interactions. *Biochemistry,* **35**, 5272–5279.

Lundbäck, T. & Härd, T. (1996). Sequence-specific DNA-binding dominated by dehydration. *Proc. Natl Acad. Sci. USA,* **93**, 4754–4759.

Morton, C. J. & Ladbury, J. E. (1996). Water mediated protein-DNA interactions: the relationship of thermodynamics to structural detail. *Protein Sci.* **5**, 2115–2118.

Nikolov, D. B., Hu, S.-H., Lin, J., Gasch, A., Hoffmann, A., Horikoshi, M., Chua, N.-H., Roeder, R. G. &

Burley, S. K. (1992). Crystal structure of TFIID TATA-box binding protein. *Nature,* **360**, 40–46.
Pace, C. N., Vajdos, F., Fee, L., Grimsley, G. & Gray, T. (1995). How to measure and predict the molar adsorption coefficient of a protein. *Protein Sci.* **4**, 2411–2423.
Petri, V., Hsieh, M. & Brenowitz, M. (1995). Thermodynamic and kinetic characterization of the binding of the TATA binding protein to the adenovirus E4 promoter. *Biochemistry,* **34**, 9977–9984.
Privalov, P. L. & Gill, S. J. (1988). Stability of protein structure and hydrophobic interaction. *Advan. Protein Chem.* **39**, 191–234.
Privalov, P. L. & Gill, S. J. (1989). The hydrophobic effect: a reappraisal. *Pure Appl. Chem.* **61**, 1097–1104.
Qureshi, S. A., Baumann, P., Rowlands, T., Khoo, B. & Jackson, S. P. (1995a). Cloning and functional analysis of the TATA binding protein from *Sulfolobus shibatae*. *Nucl. Acids Res.* **23**, 1775–1781.
Qureshi, S. A., Khoo, B., Baumann, P. & Jackson, S. P. (1995b). Molecular cloning of the transcription factor TFIIB homolog from *Sulfolobus shibatae*. *Proc. Natl Acad. Sci. USA,* **92**, 6077–6081.
Record, M. T., Jr, Anderson, C. F. & Lohman, T. M. (1978). Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water. *Quart. Rev. Biophys.* **11**, 103–178.
Rowlands, T., Baumann, P. & Jackson, S. P. (1994). The TATA-binding protein: general transcription factor in eukaryotes and archaebacteria. *Science,* **264**, 1326–1329.
Scholz, S., Sonnenbichler, J., Schäfer, W. & Hansel, R. (1992). Di-myo-inositol-1,1′phosphate: a new inositol phosphate isolated from *Pyrococcus woesei*. *FEBS Letters,* **306(2,3)**, 239–242.
Spolar, R. & Record, M. T. (1994). Coupling of local folding to site-specific binding of proteins to DNA. *Science,* **263**, 777–784.
Wiseman, T., Williston, S., Brandts, J. F. & Lin, L.-N. (1989). Rapid measurement of binding constants and heats of binding using a new titration calorimeter. *Anal. Biochem.* **179**, 131–137.
Zillig, W., Palm, P., Klenk, H.-P., Langer, D., Hüdepohl, U., Hain, J., Lanzendörfer, M. & Holz, I. (1993). Transcription in archea. In *The Biochemistry of Archea (Archaebacteria)* (Kates, M., Kushner, D. J. & Matheson, A. T., eds), vol. 26, pp. 367–386, Elsevier, Amsterdam.

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