Sequence-specific DNA-binding dominated by dehydration

(DNA-protein interactions/thermodynamics/hydrophobic effect/isothermal titration calorimetry/fluorescence spectroscopy)

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ABSTRACT Fluorescence spectroscopy and isothermal titration calorimetry were used to study the thermodynamics of binding of the glucocorticoid receptor DNA-binding domain to four different, but similar, DNA-binding sites. The binding sites are two naturally occurring sites that differ in the composition of one base pair, i.e., an A-T to G-C mutation, and two sites containing chemical intermediates of these base pairs. The calorimetrically determined heat capacity change (ΔC_p(obs)) for glucocorticoid receptor DNA-binding domain binding agrees with that calculated for dehydration of solvent-accessible surface areas. A dominating effect of dehydration or solvent reorganization on the thermodynamics is also consistent with an observed linear relationship between observed enthalpy change (ΔH^θ(obs)) and observed entropy change (ΔS^θ(obs)) with a slope close to the experimental temperature. Comparisons with structural data allow us to rationalize individual differences between ΔH^θ(obs) and ΔS^θ(obs) for the four complexes. For instance, we find that the removal of a methyl group at the DNA–protein interface is enthalpically favorable but entropically unfavorable, which is consistent with a replacement by an ordered water molecule.

The physical basis for stability and specificity in DNA–protein complexes is not fully understood even though several high-resolution structures have been determined. The structures generally reveal a large number of interactions between amino acid side chains and DNA bases and backbone. For instance, hydrogen bonding networks involving amino acid side chains, DNA bases, and bound water molecules are often observed (for a review, see ref. 1; refs. 2–6). Still, great care must be taken when interpreting the stabilizing function of a putative interaction (7). The problem is a general one within the field of molecular recognition and it arises because structures of complexes reveal little about the entropy component (TΔS) of the free energy (ΔG) of complex formation. Thermodynamic studies have shown that sequence-specific DNA-binding is often accomplished by a large and negative change in heat capacity (ΔC_p) (8–11). This is generally taken as an indication of the removal of solvent-accessible surface from bulk water on complexation (8, 9), and/or the formation of a highly complementary interface with an accompanying stiffening of molecular vibrations at the interface (10, 12). The heat capacity change results in temperature-dependent and opposing enthalpy (ΔH) and entropy components of the free energy of binding. A consequence of this is that the binding is entropy driven at low temperatures. Moreover, the discrimination between different specific binding sites (13) as well as between specific and nonspecific binding sites (14) can also be entropy driven. Therefore, it is necessary to combine structural studies with detailed thermodynamic analyses to understand the physical basis for sequence specificity (15). Studies of DNA–protein complexes in which only minor alterations have been made on the interacting surfaces can in this way shed light on the stabilizing effects and thermodynamic nature of specific interactions.

We have chosen to study the sequence-specific binding by the glucocorticoid receptor (GR) DNA-binding domain (DBD). Several structures are available for the GR DBD and the closely related estrogen receptor (ER) DBD, both free in solution and in complex with the glucocorticoid response element (GRE) and the estrogen response element (ERE), respectively (2, 3, 16–18). Structures have also been determined for a mutant GR DBD in complex with the GRE (5) and for ER DBD in complex with an altered ERE (6). The DBDs bind as homodimers to the response elements, which are partially palindromic DNA sequences, with the recognition helix of each protein monomer placed in adjacent major grooves on DNA. We previously studied the binding of native and mutant GR DBDs to DNA using equilibrium titrations based on fluorescence spectroscopy (13, 19, 20). The thermodynamics of complex formation investigated by van’t Hoff analyses revealed unfavorable binding enthalpies at low temperatures (i.e., entropy-driven reactions) and negative changes in heat capacity, suggesting contributions from dehydration of interacting surfaces. Differences in observed enthalpy change (ΔH^θ(obs)) for two specific complexes (pGRE and pGRE2; Fig. 14) were interpreted as differences in the number of bound water molecules in the two complexes. This interpretation was subsequently supported by structural studies (5, 21).

In the present study, we investigate the thermodynamics of GR DBD binding to specific sites in more detail using fluorescence spectroscopy and isothermal titration calorimetry. We compare binding to two idealized response elements (pGRE and pGRE2), which differ by an A-T to G-C mutation in each binding half site. We also include two nonnatural binding sites (pGRU and pGRC) in our study to dissect the effect of the removal of the thymine methyl group in the major groove from the effect of changing the A-T base pair to a G-C base pair.

MATERIALS AND METHODS

Protein Preparation. The wild-type GR DBD (equal to the fragment K438-Q520 of the rat GR and the fragment K419-Q501 of the human GR) was overexpressed in Escherichia coli and purified as described (13). The purified protein was extensively dialyzed at 4°C against buffer A containing 50 mM Hepes, 100 mM KCl, 100 mM NaCl, 25 mM NaOH, 1 mM dithiothreitol (DTT), and 5% glycerol by volume at pH 7.4 (20°C). Protein concentrations were determined spectrophotometrically.

Abbreviations: GR, glucocorticoid receptor; ER, estrogen receptor; DBD, DNA-binding domain; GRE, glucocorticoid response element; ERE, estrogen response element; CyT, 5-methylcytosine; DTT, dithiothreitol; ΔG^θ(obs), observed free energy change; ΔC_p(obs), observed heat capacity change; ΔH^θ(obs), observed enthalpy change; ΔS^θ(obs), observed entropy change.

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different amounts of DNA were added at a constant protein concentration. The titration procedure including experimental set-up, fluorescence excitation and emission wavelengths, corrections for background fluorescence, light scattering and optical filtering effects, and precautions to photochemical degradation has been described previously (13). Buffer A (without DTT) was carefully degassed by stirring the buffer while applying vacuum before DTT (1 mM) and GR DBD (2 μM) were added. The DNA was then added in 20 subsequent 1-μl aliquots. Concentrations of free and bound protein were calculated from the fractional fluorescence quenching and equilibrium parameters were determined by nonlinear least square fits of theoretical binding isotherms to data representing the mean of 2–4 independent titrations (13).

**Isothermal Titration Calorimetry.** Calorimetric titrations were carried out in buffer A using the Micro Calorimeter System Isothermal Titration Calorimeter instrument from Microcal (Amherst, MA). All solutions were carefully degassed before the titrations using the equipment provided with the instrument. DNA oligomer solutions (~200 μM) were titrated into protein solutions (14 or 16 μM) using a 100-μl syringe. Each titration consisted of a preliminary 1-μl injection followed by 25 subsequent 4-μl additions. Heats of dilution were measured by adding DNA to buffer and buffer to protein and found similar for these titrations. Comparable heats were also observed toward the end of the DNA–protein titrations as shown in Fig. 2. ΔH°obs was corrected for measured heats of dilution before data analysis. A control experiment was also performed in which a concentrated protein solution (0.4 mM) was titrated into a solution of pGRE (12.5 μM) with a preliminary 2-μl injection followed by 10 subsequent 12-μl injections. Thermodynamic parameters were extracted using the ORIGIN software provided by Microcal.

**Binding Model.** Thermodynamic quantities reported here refer to the formation of a dimeric complex. The binding of GR DBD to a specific response element consisting of two hexameric half-sites arranged as inverted repeats can be described by a two-site cooperative model (19). The model assumes equal binding affinity (binding constant Kobs) for both half-sites with an additional cooperativity parameter (ωco) that is

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**Fig. 1.** (A) Sequences of DNA-binding sites included in this study. The palindromic pGRE and pGRE2 sequences are (idealized) response elements for the GR, pGRU and pGRC5 were included to dissect the effect of the methyl group of the pyrimidine at position four in the hexameric half-site from remaining effects due to the A-T to G-C mutation. All sequences are incorporated in DNA oligomers as described. (B) Molecular structures of the variable base pair at position 4 of the hexameric half-site. The major (where GR DBD binds) and minor grooves are indicated for the A-T base pair.

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**Fig. 2.** Experimental calorimetric data and integrated heats of binding (●) for an isothermal titration of pGRU titrated into a solution of GR DBD (14 μM) at 10°C are shown, as are experimental data and integrated heats of dilution for the corresponding reference titration where pGRU is injected into buffer solution (●).
a measure of the relative change in affinity for binding to a second site when the first site is already occupied. The evaluation of the overall observed free energy change $\Delta G^o_{\text{obs}} = -RT \ln [\omega_{\text{obs}}K_{\text{obs}}^2]$ is relatively insensitive to the best-fit value of $\omega_{\text{obs}}$; we therefore fixed the cooperativity parameter to $\omega_{\text{obs}} = 10$ as previously determined (13) for GR DBD binding to pGRE and pGRE2 at 20°C, and fitted $K_{\text{obs}}$ to obtain $\Delta G^o_{\text{obs}}$.

**Calculation of Surface Areas.** Solvent accessible surface areas (25) were calculated using an analytical method (26) implemented in the CHARMM program (version 24, Harvard University, Cambridge, MA). Lennard-Jones radii were those of the all-atom CHARMM force field and the probe radius was 1.4 Å. Surfaces of carbons and carbon-bound hydrogens were classified as nonpolar whereas those of other atoms were classified as polar. Surface areas were calculated using the x-ray structure of the dimeric GR DBD–GRE complex determined at 2.6 Å resolution (ref. 2; Protein Data Bank entry 1GLU to which hydrogens were added using CHARMM) and the refined NMR structure of the (uncomplexed) GR DBD (ref. 18; Protein Data Bank entry 1GDC). Only one of the GR DBD monomers in the crystal structure is bound to the specific half site. Changes in accessible surface areas for the formation of a specific dimeric complex were therefore calculated from the two (hypothetical) reactions: (i) specific binding of the first GR DBD and (ii) specific binding of the second GR DBD to a complex where the first GR DBD is nonspecifically bound.

**RESULTS**

Gel mobility-shift assays show that the stoichiometry of GR DBD binding to the four binding sites is identical (two proteins per DNA oligomer) with approximately equal binding affinities and cooperativities (data not shown). This is not unexpected because only minor changes are made in the naturally occurring pGRE and pGRE2 sequences to get the pGRU and pGRC5 sequences (Fig. 1). Thus, all DNA-binding sites including the pGRU and pGRC5 sequences can be considered to be specific. Similar binding affinities are also observed in fluorescence equilibrium titrations at 10°C and 34°C. The $\Delta G^o_{\text{obs}}$ values for binding two GR DBD proteins to the palindromic DNA-binding sites obtained from fluorescence titrations are summarized in Table 1. Complexes with pGRE and pGRE2 are somewhat stronger than those with pGRU and pGRC5, although the difference in affinity is never larger than 0.7 kcal-mol$^{-1}$. The differences in affinity cannot

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<th>Table 1. Thermodynamic data for GR DBD binding to DNA</th>
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Errors are estimated from uncertainties in GR DBD (20%) and DNA (10%) concentrations. A minimum error of 0.5 kcal-mol$^{-1}$ for calorimetric $\Delta H^o_{\text{obs}}$ values was estimated from fluctuations in dilution heat titrations. Different GR DBD batches: a, 14 µM; b, 16 µM; c, 0.4 mM.

*Calorimetric measurements. Reported values are the mean of three (10°C) or two (other temperatures) independent titrations. The entropy of binding was calculated from $\Delta S^o_{\text{obs}} = (\Delta H^o_{\text{obs}} - \Delta G^o_{\text{obs}})/T$.

†Fluorescence experiments. Reported values are estimated from the mean of 2–4 independent titrations.
in which the Thy at position 3 in the hexameric half-site is replaced by an Ade (13, 20, 28). The calorimetric signal in a titration using an oligomer containing this half-site sequence was comparable with dilution heat signals. This indicates that nonspecific binding does not occur under the present experimental conditions or that the enthalpy of nonspecific binding of GR DBD to DNA is close to zero. In either case, the experiment shows that contributions from nonspecific binding to measured enthalpies can be neglected. The second control experiment in which a concentrated GR DBD solution (0.4 mM) was titrated into a solution of pGRE (12.5 μM) gave similar results as experiments in which DNA was titrated into protein solutions (Table 1), again indicating that nonspecific binding does not have to be accounted for at the experimental conditions.

**DISCUSSION**

**Sequence-Specific DNA-Binding Is Dominated by Dehydration.** Thermodynamic experiments have shown that sequence-specific DNA-binding is often accompanied by a negative change in heat capacity (8–11). Several studies have also reported that nonspecific DNA-binding lack this behavior (10, 29). The change in heat capacity has been attributed to removal of solvent-accessible surface from bulk water on formation of a specific interface (8, 9). It was recently shown that heat capacity changes that are larger than those expected from a simple docking of protein and DNA surfaces in some cases can be attributed to protein folding reactions that are coupled to DNA-binding (9). It has also been suggested that part of the heat capacity change can be due to a restriction of dynamic fluctuations at the interface (10, 12).

Here we used isothermal titration calorimetry to measure ΔC_p^0 for sequence-specific binding of two GR DBD mole-

| Table 2. Heat capacity changes and characteristic temperatures of sequence-specific DNA-binding by GR DBD |
|-----------------|-----------------|-----------------|-----------------|
| DNA sequence    | ΔC_p^0 obs,*    | T_h ± °C        | T_s ± °C        |
| pGRE            | -0.26 ± 0.05    | 49 ± 4          | 119 ± 39        |
| pGRE2           | -0.28 ± 0.04    | 26 ± 2          | 97 ± 34         |
| pGRU            | -0.25 ± 0.07    | 39 ± 4          | —               |
| pGRC            | -0.22 ± 0.06    | 33 ± 3          | —               |

Errors were estimated in Monte Carlo simulations (27).

*Calculated from the temperature dependence of AH^0 obs [ΔH^0 obs = ΔC_p^0 obs(T − T_h)].

Calculated from the temperature dependence of AS^0 obs [ΔS^0 obs = ΔC_p^0 obs ln(T/T_s)].

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Fig. 4. Comparison of the thermodynamics for binding of GR DBD to the various binding sites (Fig. 1) that differ in a single base pair of each half-site. Differences in binding enthalpies, ΔH^0 obs, and entropies, Δ(TS)^0 obs, between the various complexes are calculated from the data in Table 1 and are given in kcal-mol⁻¹. The fact that ΔH^0 obs and Δ(TS)^0 obs are comparable for the removal of the methyl from the pyrimidine (reactions following vertical arrows) and for mutating the entire base pair (reactions following horizontal arrows) indicate that these effects are thermodynamically independent, as discussed in the text.

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Fig. 3. Integrated heats of binding from calorimetric titrations corrected for heats of dilution. (A) Titrations of pGRE (•), pGRE2 (○), pGRU (♀), and pGRC (■) into a solution of GR DBD (14 μM) at 10°C. (B) Titrations of pGRE (•), pGRE2 (○), pGRU (♀), and pGRC (■) into a solution of GR DBD (16 μM) at 34°C. (C) Titrations of pGRE into a solution of GR DBD (16 μM) at 10°C (•), 18°C (♀), 23°C (■), 29°C (○), and 34°C (■). The insert shows the temperature dependence of ΔH^0 obs, from which ΔC_p^0 obs was calculated (solid line).

T_h, where T_h is the temperature at which ΔH^0 obs = 0]. The best-fit value of the heat capacity change for binding of a dimer GR DBD to pGRE is ΔC_p^0 obs = -0.26 ± 0.05 kcal-mol⁻¹-K⁻¹. Table 2 also includes the best-fit values of ΔC_p^0 obs for the other complexes. It is evident that ΔC_p^0 obs is negative and similar for all complexes. The temperature (T_h) at which the entropy of binding (ΔS^0 obs) becomes zero was calculated from the temperature dependence of ΔS^0 obs using the equation ΔS^0 obs = ΔC_p^0 obs ln(T/T_s) (Table 2).

Two control experiments were done at 10°C to make certain that any additional nonspecific binding did not affect the interpretation of calorimetric data. GR DBD has been shown to bind with very low affinity to a single half-site (TGATCT)
cules to a palindromic GRE binding site. The measured value can be compared with that expected from changes in solvent-accessible nonpolar and polar surfaces ($\Delta A_{\text{pp}}$ and $\Delta A_{\text{p}}$, respectively). Spolar and coworkers derived the empirical relation $\Delta C_{\text{p}}^\circ_{\text{calc}} = 0.32 \pm 0.04 A_{\text{pp}} + 0.14 \pm 0.04 A_{\text{p}}$ cal mol$^{-1}$K$^{-1}$ and showed that it accurately reproduces specific heat capacity changes for protein folding reactions (9, 30) and formation of protein–protein and protein–ligand complexes (9). We calculate $\Delta A_{\text{pp}} = -2044$ $\AA^2$ and $\Delta A_{\text{p}} = -2980$ $\AA^2$, corresponding to $\Delta C_{\text{p}}^\circ_{\text{calc}} = -0.24 \pm 0.15$ kcal mol$^{-1}$K$^{-1}$ for the formation of a specific dimeric GR DBD–GRE complex. A second calculation, in which the free DNA is assumed to be in a B-form conformation, yields $\Delta A_{\text{pp}} = -1919$ $\AA^2$ and $\Delta A_{\text{p}} = -2842$ $\AA^2$ with $\Delta C_{\text{p}}^\circ_{\text{calc}} = -0.22 \pm 0.13$ kcal mol$^{-1}$K$^{-1}$. Calculations in ref. 9, which are based on the same structural data, report $\Delta A_{\text{pp}} = -1885$ $\AA^2$ and $\Delta A_{\text{p}} = -2213$ $\AA^2$, which corresponds to $\Delta C_{\text{p}}^\circ_{\text{calc}} = -0.29 \pm 0.10$ kcal mol$^{-1}$K$^{-1}$. These values agree within errors with the experimentally determined heat capacity change. Thus, it appears that dehydration of interfacial protein and DNA surfaces plays a dominating role for the thermodynamics. Accessible surface areas that are removed upon binding to the other three sites can be expected to be similar to that removed in the complex with pGRE with corresponding differences in $\Delta C_{\text{p}}^\circ_{\text{calc}}$ on the order of 0.01 kcal mol$^{-1}$K$^{-1}$, also when the 5-methyl on the mutated pyrimidine is replaced by a water molecule in the complex, as discussed below. This is consistent with the small variations in $\Delta C_{\text{p}}^\circ_{\text{obs}}$ measured for the various complexes (Table 2).

The binding of GR DBD is also subject to strong enthalpy–entropy compensation, because the temperature dependence of $\Delta G_{\text{p}}^\circ_{\text{obs}}$ is much weaker than the temperature dependence of $\Delta H_{\text{p}}^\circ_{\text{obs}}$. This effect has been taken as a sign of water reorganization (31,32). A linear relationship between $\Delta H_{\text{p}}^\circ_{\text{obs}}$ and $\Delta S_{\text{p}}^\circ_{\text{obs}}$ with a slope close to the experimental temperature is expected in cases where changes in the macromolecular solvation dominate the temperature dependence of these parameters (32). This behavior is observed here for GR DBD binding to the various binding sites as demonstrated in Fig. 5, again implying that the association processes involve removal of water from protein and DNA surfaces on binding.

Dehydration (hydrophobic) effects might also be reflected in the ratio $\Delta S_{\text{p}}^\circ_{\text{obs}}/\Delta C_{\text{p}}^\circ_{\text{obs}}$ (12) and the temperature, $T_S$, at which $\Delta S_{\text{p}}^\circ_{\text{obs}} = 0$ (33). However, care must be taken in the interpretation of these parameters. Association reactions are intrinsically entropically unfavorable because of loss of translational and rotational degrees of freedom. One must also consider the binding-induced release of counterions, i.e., the polyelectrolyte effect (9), which makes a compensating favorable contribution to $\Delta S_{\text{p}}^\circ_{\text{obs}}$. The entropy change of the polyelectrolyte effect is temperature independent and the intrinsically unfavorable association entropy is predicted to have only a weak temperature dependence (9). Thus, if these effects compensate each other and there is no coupled folding reaction, then one might expect to observe $T_S$ values close to 113°C (33) and $\Delta S_{\text{p}}^\circ_{\text{obs}}/\Delta C_{\text{p}}^\circ_{\text{obs}}$ ratios close to the constant value ($\sim 0.26$ at 25°C) observed for the transfer of nonpolar compounds from water to nonpolar solvents (12). We note that these values in fact fall close to those measured here for the GR DBD–DNA interaction, with $T_S$ in the range 97–119°C and $\Delta S_{\text{p}}^\circ_{\text{obs}}/\Delta C_{\text{p}}^\circ_{\text{obs}}$ in the range ($-0.21$) to ($-0.31$) at 25°C. However, one cannot draw conclusions based on these observations until the contribution to $\Delta S_{\text{p}}^\circ_{\text{obs}}$ from the polyelectrolyte effect has been determined.

**Differences in Thermodynamics Can Be Rationalized Based on Structural Data.** Recent structural data of GR DBD and ER DBD in complex with DNA (2, 3, 5, 6) show that the origin of sequence specificity is not a simple matter of exchanging functional groups at the interacting surfaces. It seems crucial to consider DNA as an active binding partner and to closely examine changes in local DNA structure as a function of sequence. There could also be entropic penalties associated with excess water trapped at the interface (5, 21), although water molecules can serve as extensions of functional hydrogen-bonding groups and fill any cavities at the DNA–protein interface.

The possible importance of water release on DNA recognition by GR DBD was also realized in our previous study (13). The difference between the pGRE and pGRE2 sequences is that an AT base pair is replaced by a GC base pair as shown in Fig. 1. The resulting difference in the major groove where the protein binds is that the bulky methyl group at the Thy position four is removed and the guanine backbone hydrogen bonding carbonyl and amino groups are switched. We suggested that the entropy-driven specificity for binding to pGRE compared to pGRE2 was due to the removal of the Thy methyl, which creates a cavity between the interacting surfaces. This cavity could be occupied by an immobilized water molecule that forms hydrogen bonds with the Cyt carbonyl and other functional groups at the interface. We now find that complexes with DNA sequences lacking this methyl group are enthalpically favored over sequences containing the methyl (Fig. 4), which might indicate a difference in the number of favorable hydrogen bonds. We also measure an entropic penalty for the removal of the methyl at both 10°C and 34°C ($\simeq 1$ kcal mol$^{-1}$ per methyl). The entropic penalty for the immobilization of a water molecule or a cavity filled with a crystalline salt has been estimated to a maximum of 2 kcal mol$^{-1}$ at 300 K (34). The water release mechanism is thus well-supported by thermodynamic data and by recently determined structures in which additional waters are found in the region of this base pair in both specific and semispecific complexes that lack the Thy methyl.

However, it is clear that a methyl group does not fully account for the difference in thermodynamics for binding to pGRE compared with pGRE2. The binding to half-sites containing Thy or Ura at position four is enthalpically unfavorable but entropically favorable compared with half-sites that contain Cyt or Cyt at the same position (Fig. 4). The structures of GR DBD and ER DBD in complexes with DNA show that there is a difference in local DNA conformation between hexameric GRE and ERE half-sites (5), although these differ in only two central base pairs (TGTTCT for GRE compared with TGACCT for ERE). The ERE sequence has a more narrow major groove and this places the functional groups of the bases closer to side chains in the protein recognition helix. The effect results in additional DNA–protein hydrogen bonds at the interface of complexes with an ERE binding site, especially in the region of the two discriminating base pairs. Moreover, additional waters are trapped at the interface close to the two central base pairs of complexes.
with an ERE binding site. The absence of water molecules and DNA–protein hydrogen bonds in this region of the GR DBD-GRE complex is also supported by a molecular dynamics simulation (4).

The present thermodynamic data can be rationalized as follows. If one assumes that the replacement of only one of the discriminating base pairs (as in this study) results in some intermediate DNA conformation, then the side chains of the GR DBD recognition helix might be placed closer to functional groups of half-sites containing Cyt or Cyt5 in place of Thy or Ura at position four. Such a conformational change in DNA with the subsequent formation of additional favorable hydrogen bonds would explain the more favorable enthalpy observed when GR DBD binds to half-sites containing Cyt or Cyt5 compared with Ura or Thy at position four (Fig. 4). Furthermore, a DNA conformation that resembles the ERE half-site could result in the entrapment of additional waters at the interface. A less optimal dehydration of the interface could explain the less favorable entropy of binding to half-sites containing Cyt or Cyt5 compared with Ura or Thy at position four (Fig. 4).

Thus, for GR DBD binding to sequence-specific DNA, there seems to be a delicate balance between a dehydration of the DNA–protein interface to gain entropy and the use of bridging water molecules to fulfill the hydrogen bonding needs of polar functional groups. Waters are left at the interface when necessary to fill gaps, but the immobilization of too many waters will attenuate the affinity. This is also illustrated by the structure (5) of a complex between a GR DBD mutant, which binds with lower affinity to pGRE than the wild-type GR DBD (13), and a GRE. The number of DNA–protein hydrogen bonds in this semispecific complex is equal to or even exceeds those found in the specific complexes but there is a large number of water molecules left at the interface. The lower affinity of this mutant might then be a result of a less optimal dehydration of the DNA–protein interface as noted (5, 6).

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