Ion Concentration and Temperature Dependence of DNA Binding: Comparison of PurR and LacI Repressor Proteins†

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Received December 18, 2000; Revised Manuscript Received April 17, 2001

ABSTRACT: Purine repressor (PurR) binding to specific DNA is *enhanced* by complexing with purines, whereas lactose repressor (LacI) binding is *diminished* by interaction with inducer sugars despite 30% identity in their protein sequences and highly homologous tertiary structures. Nonetheless, in switching from low- to high-affinity DNA binding, these proteins undergo a similar structural change in which the hinge region connecting the DNA and effector binding domains folds into an α-helix and contacts the DNA minor groove. The differences in response to effector for these proteins should be manifest in the polyelectrolyte effect which arises from cations displaced from DNA by interaction with positively charged side chains on a protein and is quantitated by measurement of DNA binding affinity as a function of ion concentration. Consistent with structural data for these proteins, high-affinity operator DNA binding by the PurR·purine complex involved ∼15 ion pairs, a value significantly greater than that for the corresponding state of LacI (∼6 ion pairs). For both proteins, however, conversion to the low-affinity state results in a decrease of ∼2-fold in the number of cations released per dimeric DNA binding site. Heat capacity changes (ΔC_p) that accompany DNA binding, derived from buried apolar surface area, coupled folding, and restriction of motional freedom of polar groups in the interface, also reflect the differences between these homologous repressor proteins. DNA binding of the PurR·guanine complex is accompanied by a ΔC_p (−2.8 kcal mol^{-1} K^{-1}) more negative than that observed previously for LacI (−0.9 to −1.5 kcal mol^{-1} K^{-1}), suggesting that more extensive protein folding and/or enhanced structural rigidity may occur upon DNA binding for PurR compared to DNA binding for LacI. The differences between these proteins illustrate plasticity of function despite high-level sequence and structural homology and undermine efforts to predict protein behavior on the basis of such similarities.

Efforts to elucidate detailed mechanisms for high-affinity protein·DNA binding and specific sequence recognition suggest that structural information alone is insufficient for deciphering the atomic contributions to these processes (*1*−*12*). Adaptability of protein·DNA interfaces to altered sequence of either partner, combined with their interactions with water and solutes, generates highly complex thermodynamic and kinetic systems (*1*−*12*). Consequently, measurements of binding affinity under different conditions are necessary for comprehensive analysis of a specific protein·DNA interaction. A central influence on DNA behavior, and therefore on protein·DNA interactions, arises from its high density of negative charges, each of which can bind tightly to cations in the solvent. For DNA alone in solution, these ions are not released even when the solvent ion concentration approaches zero (*1*). However, upon binding protein, cations in the DNA binding site may be replaced by positively charged amino acid side chains. A strong theoretical basis has been established for interpreting the dependence of protein·DNA affinity on salt concentration, termed the "polyelectrolyte effect" (reviewed in refs *1* and *13*−*17*). Measurement of the monovalent cation dependence over its linear range can be utilized to estimate the number of cations displaced by—and presumably therefore the number of ion pairs involved in—the interaction (reviewed in refs *1*, *2*, *4*−*6*, *11*, and *13*−*17*).

Similarly, thermodynamic parameters derived from the temperature dependence of protein·DNA binding have been interpreted in terms of structural changes. Several processes may contribute to these energetic changes: decrease in nonpolar surface area exposed to water (termed the "hydrophobic effect"), creation and/or neutralization of charged groups, changes in internal vibrational modes, effects of temperature on conformational equilibria, and differential temperature effects between free and complexed forms (*1*, *2*, *4*−*10*, *12*, *18*−*23*). The large negative ΔC_p commonly associated with protein·DNA binding has been ascribed primarily to the hydrophobic effect and derives from decreased solvent accessible surface area and coupled protein folding that is concomitant with DNA binding (*1*, *2*, *8*, *12*).

We sought to compare the polyelectrolyte and hydrophobic effects for the highly homologous purine and lactose repressors, both members of the extended LacI family of regulatory proteins (*24*, *25*). Purine repressor (PurR)[1] is the key element for regulating expression of a number of genes involved in purine biosynthesis (*26*, *27*), whereas LacI is the regulator

† This work was supported by grants from the National Institutes of Health (GM22441) and the Robert A. Welch Foundation (C-576) and utilized facilities of the W. M. Keck Center for Computational Biology.

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10.1021/bi0028643 CCC: $20.00
Published on Web 06/14/2001

for expression of genes involved in lactose metabolism and controls a catabolic rather than anabolic pathway (*28*). These proteins share high levels of sequence and structural homology yet exhibit opposite functional modes (*24*, *25*, *27*−*32*). PurR performs its biological function by binding to specific sequences upon complex formation with purine corepressors (*33*). This response is the opposite of that for LacI, where a decrease in DNA binding affinity occurs upon complex formation with sugar inducer molecules (*34*, *35*). Interestingly, however, a small set of sugars termed anti-inducers elicit an effect in LacI similar to that in PurR: these ligands increase the affinity of LacI for its operator DNA sequences (*35*).

The structure of the 341-amino acid purine holorepressor (PurR·purine·*pur*F) has been determined by X-ray crystallography (*29*, *36*). The protein is dimeric, and each monomer contains a C-terminal corepressor binding domain (residues ∼60−341) and a smaller N-terminal DNA binding domain (residues 1−60). The N-terminal domain contains a helix−turn−helix motif that binds in the DNA major groove and a hinge helix that inserts into the minor groove, introducing a bend in the target DNA (*29*, *36*). This hinge helix covalently connects the DNA and corepressor binding domains (*29*, *36*) and contributes to DNA specificity (*37*). NMR studies demonstrate that this hinge segment is unfolded in the isolated PurR N-terminal domain (*38*); proteolytic studies indicate that this region is susceptible to cleavage in the absence of DNA (*39*), and crystallographic structures of the aporepressor resolve only the core domain (*30*). These results suggest that the hinge helix is folded only when PurR·purine forms a high-affinity complex with DNA.

Structures for LacI from both X-ray and NMR analyses exhibit significant similarity to the PurR protein (*31*, *32*, *40*−*43*), as anticipated on the basis of their sequence homology (*24*, *25*). LacI also has a C-terminal ligand binding domain (residues ∼60−340) and an N-terminal DNA binding domain (residues ∼1−60) (*31*, *32*). However, LacI is one of the few members of this family that is tetrameric; this higher-order assembly allows looped complexes with DNA containing multiple operator sequences and is mediated by the C-terminal region (residues ∼340−360) (*28*). In complex with DNA, this protein also contains a hinge helix that inserts into the minor groove in a fashion analogous to that of the PurR protein, and the hinge helix appears to require the specific complex with DNA for effective folding (*31*, *32*, *41*−*43*). The central difference from PurR is that LacI·DNA complex formation occurs in the *absence* of ligand and is interrupted by the presence of inducer sugars (*34*, *35*).

The polyelectrolyte and hydrophobic effects (reviewed in ref *1*) have been explored for wild-type LacI binding to DNA (*1*, *2*, *7*, *8*, *11*, *12*, *14*−*17*, *44*−*48*). To compare PurR behavior to that of its homologue, LacI, we have examined ion concentration and temperature dependences of DNA binding. These studies demonstrate that specific DNA binding of PurR is strongly ion concentration-dependent, with ∼15 ion pairs formed upon DNA binding in the presence of guanine compared to ∼6 for LacI. This difference arises primarily from ion pairs that can be identified in the high-resolution X-ray structures for PurR and LacI (*29*, *32*, *36*). Similarly, the temperature dependence of DNA binding indicates a significantly greater effect on PurR·guanine·DNA complex formation than on LacI·DNA binding under high-affinity conditions. These differences underscore the functional distinctions that can be achieved with similar sequences and folds and emphasize the importance of thermodynamic as well as structural studies for achieving insight into protein function.

MATERIALS AND METHODS

Protein Preparation. Purification protocols followed those described previously for PurR (*49*), wild-type LacI (*3*), −11aa LacI (*50*), and H74W LacI (*51*). DNA binding activity for each of these proteins was determined by specific operator binding under stoichiometric conditions. The activity level measured was greater than 90% for all proteins that were examined.

DNA Binding. The target DNA sequence used for PurR·DNA binding experiments was a 30 bp sequence corresponding to the *pur*F operator. The sequences of the two strands were as follows: 5′-GAATCCCTACGCAAACGTTTGCGTTTTCTG-3′ and 5′-GACAGAAAACGCAAACGTTTGCGTAGGGAT-3′. After hybridization, a two-base single-stranded overhang remains on both the top and bottom strand for potential labeling with the fill-in reaction using [α-^{32}P]ATP. The operator used for DNA binding of H74W, −11aa, and wild-type LacI was a 40 bp sequence containing the O^1 operator sequence (5′-TGTTGTGTGGAATTGTGAGCGGATAACAATTTCACACAGG-3′) and its complementary strand. The apparent dissociation constant for protein·DNA binding (K_d) was determined by titrating a constant concentration of labeled DNA (at concentrations well below the K_d) with a wide range of protein concentrations. DNA was labeled with [γ-^{32}P]ATP using polynucleotide kinase (*52*). Use of 40 bp sequences containing *pur*F operator or 30 bp *lac*O^1 sequences did not yield significant differences in behavior (data not shown).

Ion Concentration and Temperature Dependence Binding Assays. The reactions for assessing the ion concentration dependence of DNA binding were performed in 10 mM Tris-HCl (pH 7.6), 5% (v/v) DMSO, 0.1 mg/mL BSA, and a variable KCl concentration. The reactions for the temperature dependence of DNA binding were performed in 10 mM Tris-HCl (pH 7.6, adjusted at the temperature for the experiment), 250 mM KCl, 5% (v/v) DMSO, and 0.1 mg/mL BSA. For specific DNA binding by PurR, the final guanine concentration was $\sim 4 \times 10^{-6}$ M. The concentration of IPTG or ONPF used in LacI·DNA binding experiments was 2×10^{-3} M. The DNA concentration was maintained at $\leq 10^{-12}$ M for high-affinity binding (presence of guanine for PurR and absence of IPTG for LacI proteins) and $\leq 10^{-11}$ M for low-affinity binding reactions (absence of guanine for PurR and presence of IPTG for LacI proteins). In experiments with a varying ion concentration, reaction mixtures were incubated at room temperature followed by filtration on nitrocellulose. For the temperature dependence experiments, after equilibration the samples were transferred and filtered rapidly to minimize any temperature fluctuations that might occur due to heat transfer. The temperature change observed was less than 1 °C over the course of transfer and filtration. The

[1] Abbreviations: BSA, bovine serum albumin; IPTG, isopropyl β-D-thiogalactoside; LacI, *lac* repressor; ONPF, *o*-nitrophenyl β-D-fucoside; PurR, purine repressor.

equilibration time was 45 min for high-affinity binding and 1.5 h for low-affinity binding for each of the proteins. For all experiments, filters were dried following filtration and were exposed on a phosphorimager plate (Fuji) overnight. The retained radioactivity was analyzed by MacBas version 2 (Fuji).

Data Analysis. Under nonstoichiometric conditions, the apparent dissociation constant was determined by fitting the DNA binding data to the following equation:

$$Y_i = Y_0 + Y_m \frac{[P]}{[P] + K_d} \quad (1)$$

where Y_i corresponds to the total signal from bound DNA at a given protein concentration, Y_0 is the background signal, Y_m is the range of the binding signal ($Y_{max} - Y_0$), [P] is the protein concentration (dimeric for PurR and −11aa LacI protein, tetrameric for wild-type and H74W LacI proteins), and K_d is the apparent dissociation constant. All references to K_d are apparent values. Data are presented as fractional saturation [$R = (Y_i - Y_0)/Y_m$] as a function of log[P]. Individual experiments utilized duplicate determinations for each data point to measure the apparent dissociation constant (except for wild-type LacI in the presence of IPTG). At least three independent measurements of K_d were performed to allow appropriate error analysis. Since all of the DNA binding experiments were conducted under nonstoichiometric conditions with the protein concentration greatly exceeding the DNA concentration, no involvement of the second DNA binding site of the tetrameric LacI proteins was possible. We therefore monitored and reported the cation release and heat capacity change per dimeric protein unit for better comparison of PurR and LacI.

The ion concentration dependence of DNA binding at constant temperature and pressure was analyzed according to the following equation, assuming that water dependence is negligible compared to the displacement of ions, as demonstrated in model studies (*1*, *11*, *13*):

$$\frac{d(-\log K_d)}{d(\log[KCl])} = -(a + c) \quad (2)$$

where a and c are the stoichiometries of cation and anion release, respectively. This equation is further simplified by assuming that $c \ll a$. This assumption holds for most protein·DNA interactions given the polyanionic character of DNA and tight binding of cations versus the generally weak binding of ions to protein side chains so that

$$\frac{d(-\log K_d)}{d(\log[KCl])} = -a = -0.88Z \quad (3)$$

where Z is the number of positive charges on the protein that are involved in neutralizing DNA backbone phosphates (*1*, *11*, *13*). The factor of 0.88, an experimentally determined coefficient for conversion to the number of ion pairs involved in the interaction, was obtained from binding of a variety of oligocations to DNA (*13*).

The temperature dependence of DNA binding was analyzed using van't Hoff analysis and fitting for ΔC_p, T_H, and T_S, using the following equation modified from ref *12* that assumes a large negative heat capacity change:

$$-\log K_d = \frac{\Delta C_p}{2.303R}\left[\frac{T_H}{T} - \ln\left(\frac{T_S}{T}\right) - 1\right] \quad (4)$$

where ΔC_p is the heat capacity change and T_H and T_S define the characteristic temperatures where the enthalpic (ΔH) and entropic (ΔS) contributions to the free energy change are zero, respectively. Data analysis for all experiments was conducted with Igor Pro (Wavemetrics).

RESULTS

PurR Ion Concentration Dependence. To investigate the ion concentration dependence of PurR binding to DNA, we determined the equilibrium dissociation constant (K_d) over a range of KCl concentrations utilizing nitrocellulose filter binding. Most proteins exhibit curvature in plots of log K_d versus log[salt], with a maximum in affinity and decreasing affinity at very low and elevated salt concentrations (*48*). Measurements to determine the extent of cation release associated with complex formation require establishing the linear range for K_d dependence on ion concentration. The linear range for PurR dependence was identified as 200−400 mM KCl, and this region was utilized for detailed measurements to determine the extent of cation release. Of note, this range for PurR is higher than that observed for LacI and many other proteins, suggesting the possibility that a greater number of ion pairs are involved in this interaction. Under all conditions, the DNA concentration was maintained below the estimated K_d and titrated with increasing PurR concentrations until a saturating plateau was reached. The guanine concentration was set so that PurR was saturated, and the experimental design introduced guanine prior to DNA exposure. A series of representative titrations is presented in Figure 1 for high-affinity (presence of guanine) and low-affinity (absence of guanine) DNA binding. For both binding modes, increasing the KCl concentration resulted in decreased DNA binding affinity (Figure 2). In this linear range, the slope of the data for ion concentration dependence is directly related to the number of cations released during protein·DNA complex formation and can be converted to the number of ion pairs that are formed (Table 1). The ion concentration effect is more pronounced for high-affinity binding of DNA by the PurR·guanine complex, with a differential of >5000 between 200 and 400 mM KCl; over the same KCl concentration range, the K_d for low-affinity PurR binding (no guanine) decreased only ∼50-fold. Similar ion concentration behavior but much lower affinity was found for PurR·guanine binding to *lac*O^1 DNA than for PurR·*pur*F binding (data not shown). Interestingly, because of the differences in the slopes for the two binding modes, affinities for both PurR·DNA binding modes converge at ∼400 mM KCl.

LacI Ion Concentration Dependence. To compare the ion concentration dependence results for PurR·DNA binding to its homologue LacI, we measured DNA binding affinity for a series of LacI proteins under conditions that monitor interaction between a single dimeric DNA binding site and *lac*O^1 DNA. The linear region for LacI and its variants was 100−300 mM KCl, except for the case of wild-type LacI low-affinity binding in the presence of inducer (IPTG), where a lower range of 50−150 mM KCl was necessary to achieve measurable K_d values. We also determined the ion concen-

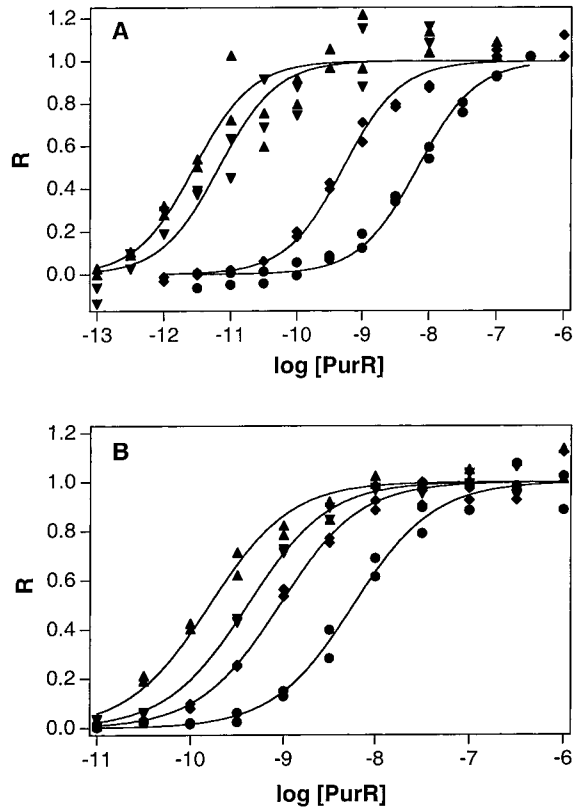

FIGURE 1: Binding isotherms for PurR binding to DNA in the presence (A) and absence of guanine (B) at various KCl concentrations [(▲) 200, (▼) 250, (◆) 316, and (●) 400 mM]. Titrations were performed under nonstoichiometric conditions with a DNA concentration of $\leq 10^{-12}$ M in the presence of guanine and $\leq 10^{-11}$ M in the absence of guanine. The lines represent the best fit according to eq 1 in Materials and Methods. Data represent a single experiment. For analysis of cation release, K_d values from at least three experiments were averaged.

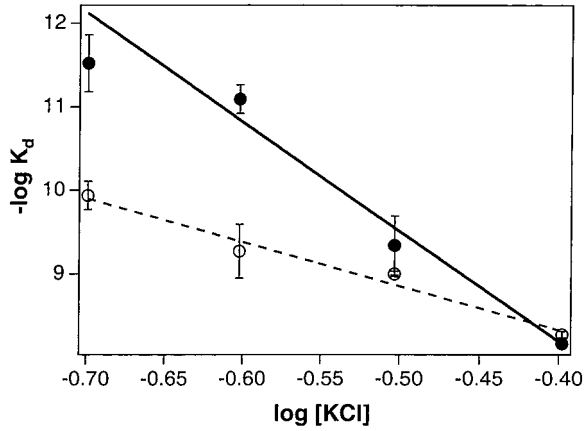

FIGURE 2: KCl concentration dependence of PurR binding in the presence of guanine (●) and in the absence of guanine (○). The solid and dashed lines correspond to weighted linear fits of the DNA binding data. Each data point represents an average of three to four independent experiments, and each error bar represents one standard deviation.

tration dependence for DNA binding in the presence of the anti-inducer ONPF, which increases the affinity of LacI for the operator (*35*, *53*). These experiments were necessary to correlate the number of cations released with specific protein DNA contacts in the recently determined X-ray structure of the dimeric LacI·ONPF·O^{sym} complex (*32*). To provide a wider range of proteins for interpreting the results of these

Table 1: Summary of Ion Pairs Involved in DNA Binding

high-affinity binding		low-affinity binding	
protein complex	no. of ion pairs formed[a]	protein complex	no. of ion pairs formed[a]
PurR·guanine·*pur*F	14.9 ± 0.8	PurR·*pur*F	5.9 ± 0.7
LacI·O^1	6.4 ± 0.2[b]		
LacI·ONPF·O^1	6.4 ± 1.0	LacI·IPTG·O^1	3.1 ± 0.4
H74W LacI·O^1	8.1 ± 0.9	H74W LacI·IPTG·O^1	4.5 ± 0.2
−11aa LacI·O^1	4.3 ± 0.4	−11aa LacI·IPTG·O^1	ND[c]

[a] Calculated from eq 3 in Materials and Methods. [b] Data are from ref *47* and are consistent with measurements reported by Winter et al. (*48*) and by Frank et al. (*2*) for 40 bp operator fragments. [c] Not determined due to an affinity below the range that can be accessed by the assay.

measurements, we also examined the ion concentration dependence of H74W LacI, a mutant deficient in inducer response (*51*), and a LacI dimeric mutant (−11aa LacI) that exhibits thermodynamic linkage of protein assembly (monomer association to dimers) and DNA binding (*54*). For all of these LacI proteins, increased KCl concentration resulted in decreased DNA binding affinity. Interestingly, the largest change was observed for H74W LacI·DNA binding (~2000-fold from 100 to 300 mM KCl) and the smallest for H74W LacI binding in the presence of IPTG (~100-fold from 100 to 300 mM KCl). In general, the ratio of high-affinity to low-affinity binding constants also decreased as the ionic strength increased.

The significant effect of inducer on operator DNA binding by wild-type LacI is illustrated by the differential between the two sets of data shown in Figure 3A. This differential is diminished for H74W LacI (Figure 3B), as anticipated on the basis of the resistance of this mutant protein to induction. For H74W, the affinities almost converge at ~300 mM KCl, a pattern that mirrors PurR behavior except for the opposite effects of effector ligand on DNA binding affinity. The dimeric deletion protein, −11aa LacI, exhibited the lowest affinity and the least ion concentration dependence for DNA binding of LacI proteins (Figure 3C). The ion concentration dependence of LacI·ONPF binding to DNA follows a pattern similar to that of wild-type LacI (Figure 3C). Measurement of the LacI binding affinity to *pur*F DNA yielded behavior comparable to that of LacI·IPTG binding to *lac*O^1 DNA (data not shown).

Cation Release by PurR and LacI Proteins. A summary of the derived slopes and calculated number of ion pairs involved in the binding interactions according to eq 3 is presented in Table 1. When PurR holorepressor binds DNA, ~15 ion pairs are formed, more than double the amount for low-affinity PurR·*pur*F DNA binding (~6 ion pairs). The difference in the slope of the salt dependence results in the intersection of the two binding modes at ~400 mM KCl. These results are consistent with previous observations using buffer systems at different ionic strengths (*33*). Wild-type LacI high-affinity DNA binding, in the absence or presence of the anti-inducer ONPF, involved ~6 ion pairs. Results for wild-type LacI binding to O^1 in the presence of IPTG or to *pur*F DNA yielded only ~3 ion pairs. Previous work under conditions where both DNA binding sites in the tetramer are occupied yielded ~10−12 ion pairs per tetramer (*14*, *15*, *44*), ~5−6 pairs per dimeric DNA binding site. These experiments employed much longer DNA sequences, and

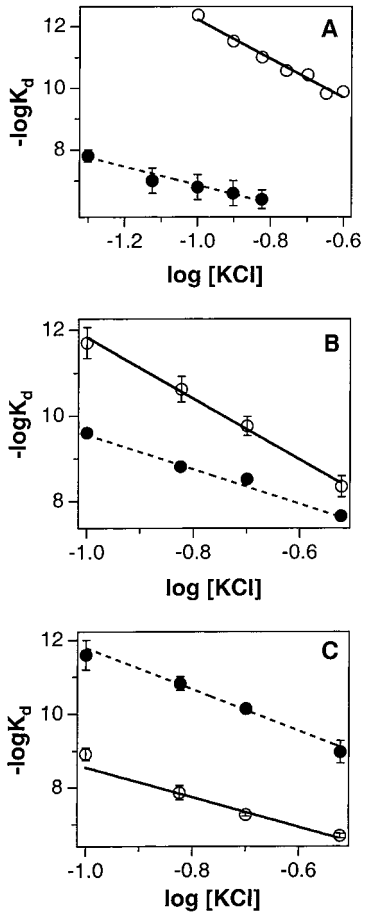

FIGURE 3: KCl concentration dependence for DNA binding by LacI proteins. The solid and dashed lines represent best weighted linear fits to the data. Binding data in panels A and B are shown for measurements in the absence (○) and presence of IPTG (●): (A) wild-type LacI [data for LacI in the absence of IPTG are from Whitson et al. (*47*)], (B) H74W LacI, and (C) −11aa LacI (○) and wild-type LacI in the presence of ONPF (●). Each data point represents an average of three to four independent experiments, and each error bar represents one standard deviation.

Table 2: Summary of Temperature Dependence for DNA Binding of PurR

	−log K_d	
temp (°C)	high-affinity binding (with guanine)	low-affinity binding (without guanine)
1	9.6 ± 0.3	7.7 ± 0.1
12	10.5 ± 0.1	8.1 ± 0.1
22	11.1 ± 0.2	9.3 ± 0.3
28	11.1 ± 0.4	8.5 ± 0.1
33	10.7 ± 0.3	not determined
37	9.8 ± 0.2	8.3 ± 0.2

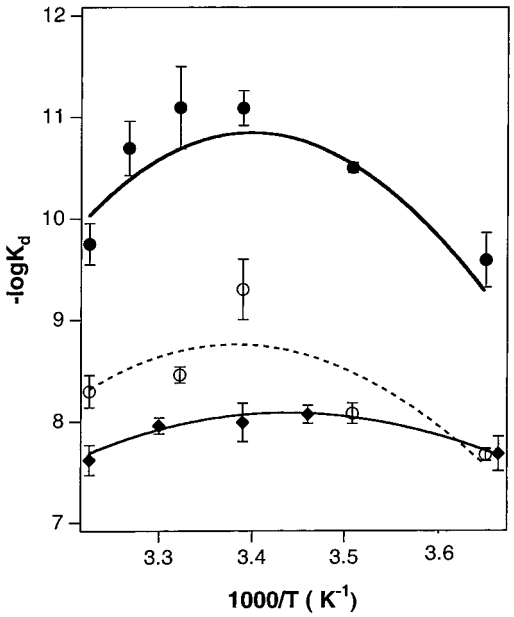

FIGURE 4: van't Hoff plot of binding data for LacI and PurR. Data are presented for −11aa LacI (◆) and PurR in the presence of guanine (●) and in the absence of guanine (○). The points represent the average from three to four experiments under each condition, and the error bars represent one standard deviation. The solid and dashed curves correspond to the best nonlinear weighted fits according to eq 4 (see Materials and Methods).

the results are therefore consistent with the observation of a lower number of ion pairs (∼3 per dimer) on the shorter DNA sequences.

None of the LacI proteins under any conditions (wild-type LacI, H74W LacI, or −11aa LacI) exhibited ion pair formation comparable to PurR·guanine binding to DNA. For the variant LacI proteins in the absence of inducer, the number of ion pairs that were formed varied from ∼4 (−11aa LacI) to ∼8 (H74W LacI). Like the wild-type behavior, but with different absolute values, high-affinity DNA binding by H74W LacI involved ∼2-fold more ion pairs than H74W LacI in the presence of IPTG. Attempts to measure the level of cation release for −11aa LacI in the presence of IPTG were unsuccessful due to the low affinity of this complex for operator DNA. LacI binding for mutant and wild-type proteins mirrors PurR behavior, albeit with a lower overall level of cation release and hence ion pair formation; high-affinity binding involves an ∼2-fold greater level of cation release than low-affinity binding. The inverse relationship between effector ligand response for PurR and LacI does not alter this relationship.

Temperature Dependence. The temperature dependence of PurR·DNA binding in both high- and low-affinity modes was examined by incubating the mixtures for the binding reactions at temperatures ranging from 1 to 37 °C in 250 mM KCl. A summary of the data over this temperature range is presented in Table 2. At all temperatures, PurR holorepressor binds DNA with higher affinity than PurR alone. The maximum difference between these binding affinities lies in the middle of the examined temperature range. The temperature dependence of PurR·DNA binding was analyzed by using a van't Hoff plot (−log K_d vs $1/T$) as shown in Figure 4. The observed nonlinear behavior results from a large negative heat capacity change that can be calculated from the data using an empirical formula (*12*) shown as eq 4 in Materials and Methods. A summary of the calculated thermodynamic parameters describing the temperature dependence of the binding affinities is given in Table 3.

PurR high-affinity DNA binding (in the presence of corepressor) is accompanied by a large negative heat capacity change (−2.8 ± 0.5 kcal mol^{-1} K^{-1}), with a much smaller absolute value for ΔC_p observed in the absence of corepressor (−0.8 ± 0.3 kcal mol^{-1} K^{-1}). LacI high-affinity binding to O^1 in the absence of inducer yields a ΔC_p of −0.9 ± 0.1 kcal mol^{-1} K^{-1} (*8*, *12*). These measurements are not feasible for LacI low-affinity DNA binding (in the presence of IPTG).

Table 3: Thermodynamic Parameters Derived from van't Hoff Analysis

protein·DNA complex	ΔC_p (kcal mol^{-1} K^{-1})	T_H (K)	T_S (K)
PurR·guanine·*pur*F	-2.8 ± 0.5	294 ± 1	300 ± 1
PurR·*pur*F	-0.8 ± 0.3	303 ± 5	314 ± 10
−11aa LacI·O^1	-0.9 ± 0.3	291 ± 2	303 ± 4
wild-type LacI·O^1	-0.9 ± 0.1[a]	294 ± 1[a]	312 ± 2[a]
wild-type LacI·O^{sym}	-1.3 ± 0.3[b]	293 ± 1[b]	305 ± 3[b]
	-1.5 ± 0.2[c]	290[c]	294[c]

[a] From refs *8* and *12* based in part on data reported in ref *47*. [b] From refs *8* and *12*. [c] From ref *2*.

However, further *increasing* the affinity of LacI for operator DNA may be accomplished by substituting the natural operator O^1 with the symmetric operator O^{sym}, more similar to the symmetry in the *pur*F operator. Measurements for LacI binding to O^{sym} by others have yielded ΔC_p values of -1.3 ± 0.3 (*8*, *12*) and -1.5 ± 0.2 kcal mol^{-1} K^{-1} (*2*). Therefore, the magnitude of the ΔC_p alteration with changes in DNA binding affinity is parallel for these two repressor proteins. We utilized the −11aa LacI protein (*50*) to explore whether thermodynamic linkage of assembly and DNA binding (*54*) is reflected in the ΔC_p value. The −11aa LacI protein binding to *lac*O^1 DNA yielded a value for ΔC_p of -0.9 ± 0.3 kcal mol^{-1} K^{-1}, comparable to the values measured for wild-type LacI (*8*, *12*), indicating that coupled assembly and O^1 DNA binding does not necessarily impact the heat capacity change. Further, detailed analyses of wild-type LacI operator binding (*55*) and PurR·guanine binding to *pur*F DNA at various temperatures (data not shown) have demonstrated no apparent linkage of assembly with DNA binding. In summary, PurR holorepressor·DNA binding generated a significantly higher absolute value for the observed heat capacity change than LacI in a dimeric or tetrameric form. The remaining parameters (T_H and T_S) had values of ∼21 and ∼27 °C, respectively, for PurR·guanine·DNA binding. The corresponding values for LacI are ∼21 and ∼39 °C, respectively, for high-affinity binding (*12*). For PurR low-affinity binding, a shift to higher temperatures was observed for these values (Table 3).

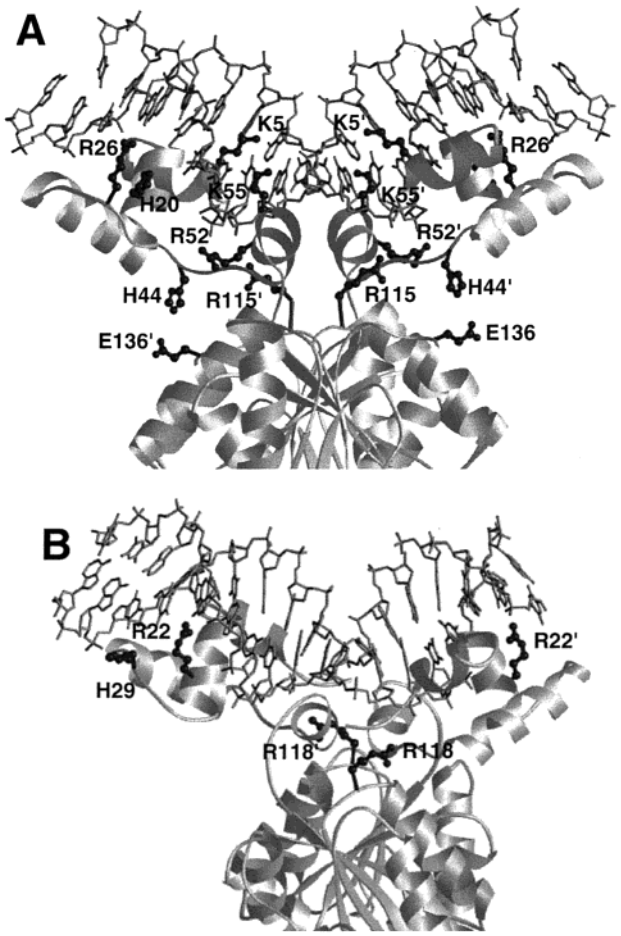

FIGURE 5: Positively charged side chains in the DNA binding site for repressor proteins. Using the structures of the PurR·guanine·*pur*F (*36*) and dimeric LacI·ONPF·O^{sym} (*32*) complexes, the ring nitrogen of histidine and nitrogens in side chains of arginine and lysine that are within 7.5 Å of DNA phosphate oxygens were identified. These are illustrated for PurR (A) and LacI (B) and are listed in Table 4. The side chain for H29 for LacI is not resolved on one of the two N-terminal domains within the dimer, and H20 in PurR is obscured by the backbone (*32*, *36*); these residues are therefore missing in one monomer of each structure in the figure. The structures were rendered from PDB entries 1WET (PurR) and 1EFA (LacI) using the program Ribbons (*60*).

DISCUSSION

Ion Concentration Dependence. For DNA binding proteins, the observed equilibrium dissociation constant, K_d, depends on the specific conditions of the experimental system, including salt concentration, pH, and temperature. Previous studies demonstrated that increasing the ion concentration primarily resulted in diminished protein·DNA affinity, for prokaryotic and eukaryotic proteins and for both specific and nonspecific DNA binding (*1*, *2*, *4*, *5*, *7*, *11*, *13*−*17*, *44*−*48*). This phenomenon has been attributed to the polyelectrolyte effect, the entropically favored replacement of cations from the negatively charged surface of the nucleic acid with positively charged side chain groups from the interacting protein (reviewed in ref *1*). The theoretical background for analysis of ion dependence experiments based on the polyelectrolyte effect has been formulated (reviewed in ref *1*). In our study, we have used a simplified equation (*1*, *11*, *13*, *47*) that assumes the release of anions from the protein's positively charged groups and water from the protein·DNA interface is negligible relative to the release of cations. Under this assumption, the number of cations released upon protein·DNA complex formation can be determined from the slope of a plot of $-\log K_d$ versus the logarithmic concentration of the monovalent salt (*1*).

Correlation of Ion Concentration Dependence with Structural Data for PurR. The high-resolution X-ray structure for the PurR·guanine·*pur*F complex (*36*) allows correlation of cation release, as deduced from the ion concentration dependence data, with positively charged amino acid side chains that interact with DNA. Each PurR monomer makes three contacts to the DNA by basic residues within the helix−turn−helix domain (K5, H20, and R26), two additional contacts by residues within the hinge helix (R52 and K55), and one contact from the core domain (R115) as shown in Figure 5 and summarized in Table 4 for a total of 12 ion pairs per dimeric site. The role of K55 has been confirmed by mutational studies (*37*). In our studies, high-affinity PurR·guanine·DNA binding was accompanied by formation of ∼15 ion pairs, only slightly larger than the value deduced from the structural analysis. In contrast, low-affinity binding

Table 4: Candidates for Ionic Interactions from DNA-Bound X-ray Structures

	charged side chains within 7.5 Å of phosphate oxygens on DNA[a]		
protein complex	helix−turn−helix motif	hinge helix and core domain	side chain contacts between subunits
PurR·*pur*F·guanine[b]	K5	R52	H44−E136′
	H20	K55	
	R26	R115	
LacI·O^{sym}·ONPF[c]	R22	R118	
	H29		

[a] Interactions per monomeric subunit. [b] Based on PDB entry 1WET (*36*). [c] Based on PDB entry 1EFA (*32*).

in the absence of guanine involved ∼6 ion pairs. These electrostatic contacts for DNA binding in the absence of guanine presumably originate from the three helix−turn−helix domain interactions per monomer, specifically the contacts of K5, H20, and R26, for a total of six per dimer, similar to the value observed experimentally. The inability to resolve the hinge structure in crystallographic studies, evidence from NMR studies, and the proteolytic susceptibility of this region in the absence of DNA indicate that the hinge folds only in the PurR holorepressor complex with DNA (*30*, *38*, *39*). Thus, these contacts would not contribute to the ion concentration dependence of DNA binding in the absence of guanine, and the consequent separation of the core domain from the DNA binding would preclude R115 interaction. Thus, contacts of positively charged amino acids to the DNA backbone observed in the structure account for the overwhelming majority of ion pairs formed in the complex. The differential between experimental and structural values may arise from release of other tightly bound ions upon complex formation. One potential site for this effect is at the interface between the N-terminal DNA binding domain and partner core. An ion pair has been identified that forms only in the holorepressor complex and involves H44 and E136′ in the core of its partner. This ion pair may stabilize and orient the N-terminal DNA binding domain for specific contacts; if the side chains bind ions tightly in the absence of DNA, complex formation would be accompanied by a number of ion pairs larger than the number identified as protein·DNA contacts.

Correlation of Ion Concentration Dependence with Structural Data for LacI. Structural data from both NMR studies (*40*−*43*) and X-ray crystallography (*32*) can be utilized to identify positively charged side chains of LacI that make contacts in the high-affinity complex. Using NMR data on the isolated N-terminal DNA binding domain, Record and colleagues (*2*, *56*) have suggested that each of the monomeric N-termini of LacI utilizes R22, H29, R51, and possibly K59 to contact phosphates on DNA. However, D8 is within 6 Å of R51, suggesting that R51 may be partially neutralized in the isolated N-terminus, consistent with ∼6 ion pairs formed upon LacI complex formation (*2*, *56*). The resolution of the X-ray structure of the high-affinity LacI·DNA complex is insufficient to perform a similar structural analysis (*31*); however, the recently determined X-ray structure of the LacI dimer complexed to operator DNA in the presence of the anti-inducer ONPF (*32*) shows that R22 and H29 are in the proximity of the negatively charged phosphate groups of the major groove (Figure 5 and Table 4). Although its backbone is within ∼6 Å of phosphate residues, the K59 side chain is directed away from the DNA, and the side chain of R51 in this structure is further than 7.5 Å from phosphate oxygens, presumably due to reorientation elicited by the presence of the hinge helix. The backbone of K2 is within ∼5 Å of the phosphate backbone, although the side chain is not resolved, and this residue may also contribute a partial charge to the interaction. Moreover, this structure indicates that the side chain of R118 from the core domain interacts with phosphates in the DNA minor groove (*32*). Thus, analysis of either NMR or crystallographic structures independently is consistent with the ∼6 ion pairs formed on specific DNA complex formation (whether the anti-inducer is present or not), and the discrepancy between the structures may reflect the inherent flexibility in the specific contacts made by this protein under different conditions, particularly in the absence of the core domain.

Interestingly, flexibility in binding has been invoked to explain other LacI·DNA binding experiments. Wild-type LacI binding to sequences that deviated stepwise from O^1 or O^{sym} DNA released more cations than the parent DNA sequence (*2*, *7*). As DNA binding becomes less favorable, the protein·DNA interface apparently shifts to increase the number of electrostatic interactions and thereby enhance affinity, a process termed adaptability (*7*). To further explore this issue, we examined H74W, a mutant with significantly lower IPTG responsiveness and an ∼4-fold higher affinity for O^1 than wild-type protein (*51*). Our goal was to determine whether the preference for the high-affinity protein form, even in the presence of inducer, would be reflected in the ionic strength dependence. The results indicated that ∼8 ion pairs were formed compared to ∼6 for wild-type LacI. In the presence of IPTG, H74W LacI involved ∼4.5 ion pairs, a decrease of ∼2-fold, but still an absolute value greater than that observed for wild-type LacI.

This increased level of cation release for both forms of H74W LacI may reflect "adaptation" of the protein·DNA interface to enhance binding affinity by increasing the number of electrostatic interactions. This process could engage several potential side chains, including K2, R51, or K59. The elevated level of cation release for H74W in the presence of inducer might derive from a combination of adaptability and an incomplete transition to the low-affinity conformation. Interestingly, however, the 2-fold decrease in the level of cation release for low-affinity binding is consistent with the pattern observed for both wild-type LacI and PurR proteins. In the low-affinity form, these proteins make fewer contacts that displace cations from the DNA backbone, presumably because the N-terminal domains are not well aligned and the hinge helix is not present.

Temperature Dependence. Protein·DNA complex formation often results in large, negative heat capacity changes (*1*, *2*, *6*−*10*, *12*, *18*−*20*). This phenomenon has been attributed to the hydrophobic effect, the burial of apolar protein surfaces that were exposed prior to protein·DNA complex formation (*8*, *12*). To explain the unexpectedly large, negative value for ΔC_p observed in many cases of protein·DNA binding, linkage of protein domain folding with complex formation has been suggested (*8*, *12*).

The folding of the hinge helices has been suggested as a key difference between the high-affinity and low-affinity forms of the PurR (*29*, *30*, *38*, *39*) and LacI proteins (*31*, *32*, *41*−*43*). Record and colleagues (*2*) indicated that folding

of the two hinge helices (one per monomer) could account for an approximately −1.0 kcal mol^{-1} K^{-1} change in the heat capacity for LacI or PurR, consistent with the magnitude of the LacI dimer and tetramer ΔC_p values. In contrast, van't Hoff analysis of the temperature dependences of DNA binding showed that PurR·guanine·DNA complex formation was accompanied by a very large negative heat capacity change of −2.8 ± 0.5 kcal/mol, whereas in the absence of guanine, a less negative ΔC_p is observed (−0.8 ± 0.3 kcal mol^{-1} K^{-1}). This result is consistent with the ion concentration dependence observations reported here that indicate that the hinge helix ionic contacts do not occur unless guanine is present. More extensive folding of the hinge helices in PurR than in LacI can result in a more negative value of ΔC_p for PurR holorepressor·DNA binding than LacI binding. This explanation is consistent with detection of hinge helix folding and DNA binding of the isolated N-terminal domains of LacI, but not for PurR (*38*, *41*−*43*).

Increased restriction of internal vibrational modes of polar elements can also contribute to the observed large negative ΔC_p values (ref *10* and examples in refs *18*, *19*, and *57*−*59*). This contribution would be greater in PurR holorepressor·DNA than in PurR aporepressor·DNA or LacI·DNA binding, because of the large number of electrostatic interactions in PurR holorepressor. Consistent with this possibility, a smaller *R* factor, denoting decreased motional flexibility in the structure, was reported for PurR·guanine·*pur*F crystal structure ($R = 0.18$) (*36*) than for the LacI·ONPF·O^{sym} structure ($R = 0.25$) (*32*), despite a similar resolution (2.6 Å).

Conclusion. The ion concentration and temperature dependences of PurR are strikingly different compared to those of LacI despite the high degree of sequence and structural homology of these proteins. In the high-affinity forms, PurR holorepressor binding to DNA involves ∼15 ion pairs compared to only ∼6 for LacI. In general, conversion to the low-affinity form (either by elimination of corepressor for PurR or addition of inducer for LacI) results in an ∼2-fold decrease in the level of cation release. The distinctions in the ion concentration dependence observed for PurR versus LacI can be rationalized by differences in the DNA and interdomain contacts of these proteins based on the available structures. The larger negative value of ΔC_p measured for PurR versus LacI high-affinity binding may derive from differences in hinge helix folding coupled with DNA binding and enhanced structural rigidity on DNA binding. These distinct behaviors of the highly homologous LacI and PurR proteins illustrate an increasingly apparent phenomenon: significant plasticity in protein structure and function. The behavior of these repressor proteins demonstrates clearly that similar folds, even those with very high levels of sequence similarity, undergo structural shifts that may substantially alter thermodynamic behavior and allow them to perform distinct functions.

ACKNOWLEDGMENT

We thank Drs. Liskin Swint-Kruse, Catherine Falcon, and Mary Purugganan for review of the manuscript and fruitful discussions.

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BI0028643