Maniatis, T., Fritsch, E. F., & Sambrook, J. (1982) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
Mayaux, J.-F., Fayat, G., Fromant, M., Springer, M., Grunberg-Manago, M., & Blanquet, S. (1983) *Proc. Natl. Acad. Sci. U.S.A. 80*, 6152–6156.
Mazat, J.-P., Merle, M., Graves, P.-V., Mêrault, G., Gandar, J.-C., & Labouesse, B. (1982) *Eur. J. Biochem. 128*, 389–398.
Mechulam, Y., Dardel, F., Lecorre, D., Blanquet, S., & Fayat, G. (1991) *J. Mol. Biol. 217*, 465–476.
Mêrault, G., Graves, P. V., Labouesse, B., & Labouesse, J. (1978) *Eur. J. Biochem. 87*, 541–550.
Merle, M., Graves, P.-V., & Labouesse, B. (1984) *Biochemistry 23*, 1716–1723.
Merle, M., Trêzéguet, V., Gandar, J.-C., & Labouesse, B. (1988) *Biochemistry 27*, 2246–2252.
Myers, A. A., & Tzagoloff, A. (1985) *J. Biol. Chem. 260*, 15371–15377.
Plateau, P., Mayaux, J. F., & Blanquet, S. (1981) *Biochemistry 20*, 4654–4662.
Reinbolt, J., Hounwanou, N., Boulanger, Y., Wittmann-Liebold, B., & Bosserhoff, A. (1983) *J. Chromatogr. 259*, 121–130.
Sallafranque, M. L., Garret, M., Benedetto, J. P., Fournier, M., Labouesse, B., & Bonnet, J. (1986) *Biochim. Biophys. Acta 882*, 192–199.
Sambrook, J., Fritsch, F. F., & Maniatis, T. (1989) *Molecular Cloning; A Laboratory Manual*, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
Sander, C., & Schneider, R. (1991) *Proteins 9*, 625–637.
Sanger, F., Niclen, S., & Coulson, A. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 5463–5468.
Scheinker, V., Beresten, S. F., Degtyarev, S., & Kisselev, L. L. (1979) *Nucleic Acids Res. 7*, 625–637.
Schimmel, P. (1987) *Annu. Rev. Biochem. 56*, 125–158.
Schimmel, P., & Söll, D. (1979) *Annu. Rev. Biochem. 48*, 601–648.
Tada, M., & Tada, M. (1975) *Nature 255*, 510–512.
Trêzéguet, V., Merle, M., Gandar, J.-C., & Labouesse, B. (1986) *Biochemistry 25*, 7125–7135.
Webster, T., Tsai, H., Kula, M., Mackie, G. A., & Schimmel, P. (1984) *Science 226*, 1315–1317.
Weiss, S. B., Zachau, H. G., & Lipmann, F. (1959) *Arch. Biochem. Biophys. 83*, 101–105.
Winter, G. P., & Hartley, B. S. (1977) *FEBS Lett. 80*, 340–342.
Winter, G. P., Koch, G. L., Hartley, B. S., & Barker, D. G. (1983) *Eur. J. Biochem. 132*, 383–387.

Energetics of Subunit Dimerization in Bacteriophage λ *c*I Repressor: Linkage to Protons, Temperature, and KCl†

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ABSTRACT: A common feature of gene regulatory systems is the linkage between reversible protein oligomerization and DNA binding. Experimental dissection using temperature dependence of the subunit–subunit energetics and their linkage to processes such as ion binding and release is necessary for characterization of the chemical forces that contribute to cooperativity and site specificity. We have therefore studied the effects of temperature, proton activity, and monovalent salt on monomer–dimer assembly of the λ *c*I repressor using a recently developed gel chromatographic procedure. This technique has made possible studies in the previously inaccessible picomolar concentration ranges where the assembly reactions occur. Upon formation of the dimer interface in the range pH 5–9, we find an overall absorption of protons which is temperature-dependent. The dimerization reaction displays a large negative enthalpy of association at all conditions studied (pH 5, 7, and 9). The reaction is also dependent on monovalent salt concentration: subunit association is weaker at low-salt conditions. The results suggest that a repulsive interaction between negatively charged side chains (i.e., aspartates and glutamates) on each monomer surface is attenuated by increasing concentrations of KCl. Formation of the dimer interface may be mediated by absorption of cations which stabilize the complex.

The regulation of transcriptional initiation for many prokaryotic and eukaryotic genes is governed by the interaction of regulatory proteins and specific DNA sequences. The functional energetics of each macromolecular species are also linked to any reactions of conformational change, subunit polymerization, and ion association/dissociation that it may undergo. Experimental determination of functionally relevant protein–protein and protein–DNA energetics is necessary for characterization of the noncovalent forces which contribute to site specificity and strength of interaction. A research program of this laboratory has been focused on the energetics of interactions between λ *c*I repressor and the right operator region (O_R)[1] (Ackers et al., 1982; Shea & Ackers, 1985; Brenowitz

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[1] Abbreviations: BSA, bovine serum albumin; $NaDodSO_4$, sodium dodecyl sulfate; O_R, λ right operator; C_T, total *c*I plateau concentration (monomer units); V_e, centroid elution volume; σ_w, weight-average partition coefficient; Tris, tris(hydroxymethyl)aminomethane; Bistris, [bis-(2-hydroxyethyl)amino]tris(hydroxymethyl)methane; NBS, National Bureau of Standards.

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et al., 1986a,b; Senear et al., 1986; Senear & Ackers, 1990). The present study on dimerization of the λ *cI* repressor is directed toward that goal.

The λ right operator (O_R) has served as a prototype for studies of cooperative gene regulatory systems. This system exhibits three distinct levels of control: (1) Regulatory proteins bind at multiple sites, O_R1, O_R2, and O_R3 [see Ptashne (1986) for a review]; (2) *cI* repressor binds cooperatively to adjacent sites (Johnson et al., 1979); and (3) repressor monomers dimerize. The dimer is the active form of the repressor in binding the operator (Chadwick et al., 1970). Cooperative binding of *cI* repressor to O_R is required for maintenance of the prophage in the lysogenic state and for the switchover to a lytic developmental path. A physical–chemical model of processes controlling the switch from lysogeny to lysis has been developed (Ackers et al., 1982; Shea & Ackers, 1985).

An analytical gel chromatography procedure recently developed in this laboratory (Beckett et al., 1991) provides an especially sensitive and accurate method for studying the assembly reaction of *cI* repressor as a function of temperature, proton activity, and monovalent salt. By driving the system with different chemical potentials, it is possible to delineate the chemical nature of forces which control the dimerization reaction. When the principles of linked function theory are employed (Wyman, 1964; Wyman & Gill, 1990), it is possible to determine contributions made by protons and other ions to the self-association. van't Hoff analysis allows determination of the enthalpic and entropic contributions to the overall free energy of assembly. Results of the present study combined with information from quantitative DNase footprint titration experiments (Koblan & Ackers, 1991; Senear & Ackers, 1990) and available crystal structures (Jordan & Pabo, 1988) both motivate and constrain the development of models for this system.

Materials and Methods

Chemical Reagents. Tran ^{35}S-labeling reagent (L-methionine, L-cysteine mixture), for in vivo labeling of the *cI* repressor, was from ICN Radiochemicals (1185 Ci/mmol). Sephadex G-100 (40–120-μm bead size) and BSA were from Sigma Chemical Co. All other reagents were analytical or reagent grade.

Biological Materials. Unlabeled *cI* repressor was purified according to the procedure previously described (Johnson et al., 1980). The *cI* repressor used in this study was greater than 95% pure as judged by electrophoresis on $NaDodSO_4$ gels. The radiolabeled repressor used in this study was isolated from an overproducing plasmid according to the method described by Beckett et al. (1991). The ^{35}S-*cI* repressor was greater than 95% pure as judged by autoradiography of dried Laemmli gels. The *cI* monomer concentration of the unlabeled repressor preparation was determined by UV absorbance based on $\epsilon^{280\ nm}_{mg/mL} = 1.18$. The radiolabeled repressor monomer concentration was determined by using the Bradford microassay (Hammond et al., 1988). All repressor preparations were stored at −70 °C.

Analytical Gel Chromatography. Determination of the monomer–dimer equilibrium constants was conducted by using analytical gel chromatography (Ackers & Thompson, 1965; Ackers, 1976; Beckett et al., 1991). Large-zone experiments were performed on a jacketed (0.9 × 40 cm) Sephadex G-100 column. Temperature was controlled to within ±0.01 °C as monitored by an NBS calibrated thermometer. Flow rates were typically 0.25 mL/min, controlled by a Perpex (Pharmacia) peristaltic pump. Protein solutions, typically 35 mL per zone, were applied to establish a plateau in the elution profile of concentration vs volume (Figure 1). The centroid volume, V_e, provides a measure of the weight-average partition coefficient, σ_w, according to eq 1 where V_0 is the void volume

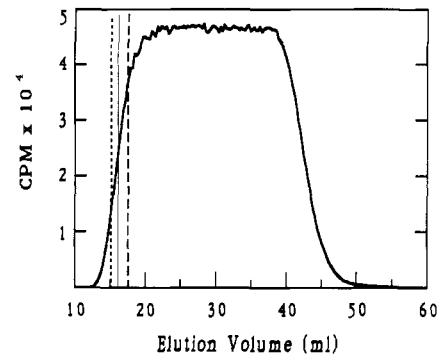

FIGURE 1: Characteristic large-zone profile for λ *cI* repressor, plateau concentration, $C_T = 7.54 \times 10^{-9}$ M, pH 7.00, $T = 20$ °C. The solid line is the centroid volume, V_e, determined for the zone by trapezoidal integration. The dashed lines indicate the elution volumes for pure dimers and monomers (left and right, respectively) determined by nonlinear least-squares analysis of σ_w vs total *cI* repressor concentration. The difference in elution volume between monomers and dimers is approximately 3 mL or 12 fractions (approximately 250 μL/fraction ±20 μL).

$$\sigma_w = (V_e - V_0)/V_i \quad (1)$$

(blue dextran) and V_i the internal volume of the column. The sum $V_i + V_0$ was determined by using L-tryptophan. Evaluation of the elution volume for each zone requires the determination of an equivalent sharp boundary (centroid) for the sharp leading or diffuse trailing edges of the solute profile (Valdes & Ackers, 1979). Experimental values of σ_w as a function of plateau concentration (units of *cI* monomer) provide an association curve for the degree of assembly (polymerization) with concentration.

The high specific activity of the radiolabeled repressor (10^{17} cpm/mol) permitted detection of the protein at monomer concentrations ranging from 6×10^{-12} to 2×10^{-6} M, far below the level of detectability for absorption spectroscopy. High concentration zones, $>10^{-9}$ M, were prepared by weight dilution of the unlabeled repressor followed by addition of the ^{35}S-*cI* repressor at a low concentration (1×10^{-10} M).

Buffers. All protein samples were prepared in buffer containing 10 mM Tris (pH 9.00, pH 8.00 ± 0.01), Bistris (pH 7.00, pH 6.00), or acetate (pH 5.00), 200 mM KCl, 2.5 mM $MgCl_2$, 1.0 mM $CaCl_2$, and 100 μg/mL BSA, $T = 20 \pm 0.01$ °C for the pH series. All buffers for the temperature study (range 5–37 °C) were adjusted with HCl to pH 7.00 ± 0.01 (as above) at the temperature of interest against NBS traceable standards. The studies as a function of added [KCl] were conducted at 10 mM Bistris (pH 7.00), 2.5 mM $MgCl_2$, 1.0 mM $CaCl_2$, and 100 μg/mL BSA, $T = 20$ °C, unless otherwise noted.

Results

Stoichiometry of Association. For each experimental condition, the values of σ_w provide an association curve which reflects the change in average molecular size with plateau concentration, C_T. A representative curve is shown in Figure 2 at pH 7.00, $T = 30$ °C. The association curves were tested against different stoichiometric models of assembly by using nonlinear least-squares methods of parameter estimation. The analysis program (Johnson et al., 1976; Johnson & Frasier, 1985) resolved the best-fit values of model parameters. For all conditions studied, a monomer–dimer model was found to

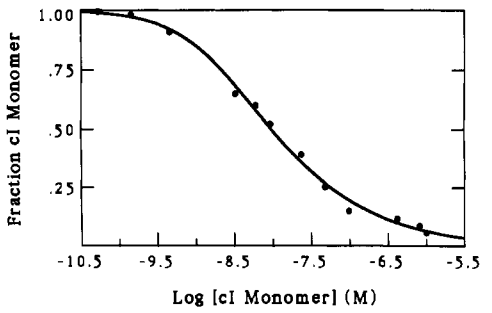

FIGURE 2: Representative dissociation curve at pH 7.00, $T = 30$ °C. Circles are experimental σ_w values. The solid line corresponds to the best resolved parameters determined by nonlinear least-squares analysis.

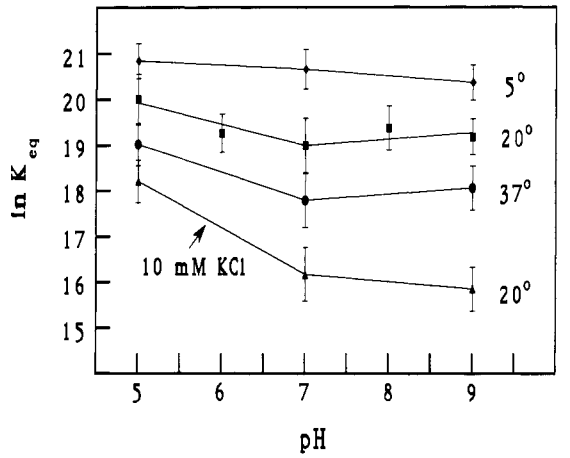

FIGURE 3: Dependence of ln K_{eq} on proton activity. Conditions were 200 mM KCl, $T = 5$ °C (♦); 200 mM KCl, $T = 20$ °C (■); 200 mM KCl, $T = 37$ °C (●); and 10 mM KCl, $T = 20$ °C (▲). The slopes of the lines correspond to the apparent number of protons absorbed, $\Delta\nu_{H^+}$, according to $\Delta\nu_{H^+} = d \ln K_{eq}/d \ln [H^+]$.

fit the data better than other monomer–n-mer models. Each data set was fit simultaneously by eq 2 and 3 where σ_M and

$$\sigma_w = \sigma_D + (\sigma_M - \sigma_D)f_M \quad (2)$$

$$f_M = [-1 + (1 + 8K_{eq}C_T)^{1/2}]/4K_{eq}C_T \quad (3)$$

σ_D are partition coefficients for monomer and dimer, respectively, and f_M is the fraction of monomer, C_T is the total protein concentration on a monomer basis, and K_{eq} is the assembly equilibrium constant. These parametric relationships were used to fit for K_{eq} and the end points σ_M and σ_D.

Proton Dependence. The pH dependence of the monomer–dimer equilibrium constant, K_{eq}, is shown in Figure 3. In the range pH 5–9, there appears to be only a slight proton linkage to the dimerization reaction, which is most pronounced below neutral pH. The direction of the effect below pH 7 indicates a net proton absorption upon formation of the dimer interface. Seperate studies conducted at low salt (10 mM KCl) and high temperature (37 °C) could not be fit by a straight line without unreasonable residuals. The data at 20 °C suggest, but do not require, similar behavior.

Temperature Dependence. Dimer formation is temperature-dependent over the range studied (Figure 4). Linearity of the van't Hoff plot could not be discredited within error limits of the data. The resulting enthalpy is large and negative, $\Delta H°_{pH7} = -15.9 \pm 2.1$ kcal/mol. Temperature studies conducted at other pHs yielded values for $\Delta H°$ which were also large and negative ($\Delta H°_{pH5} = -10.3 \pm 1.8$ kcal/mol, $\Delta H°_{pH9} = -13.0 \pm 2.3$ kcal/mol) (see Discussion).

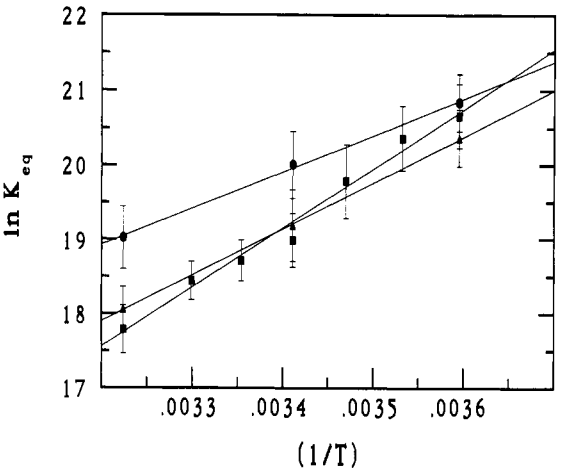

FIGURE 4: van't Hoff plots for cI repressor dimerization at pH 7.00 (■), 5.00 (●), and 9.00 (▲).

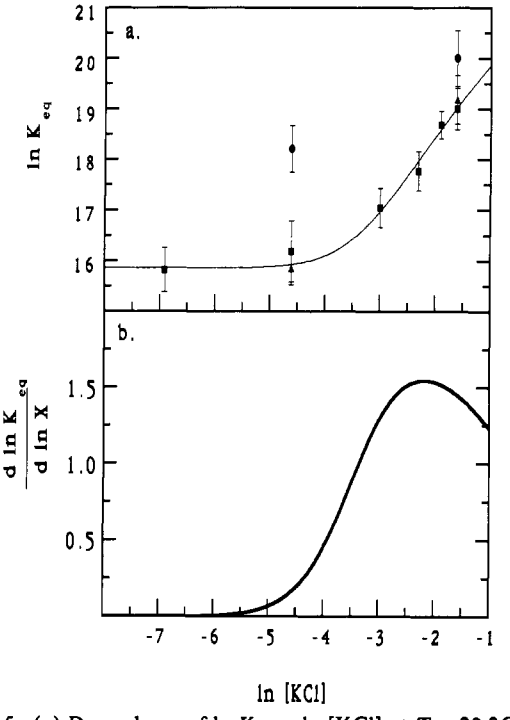

FIGURE 5: (a) Dependence of ln K_{eq} on ln [KCl] at $T = 20$ °C: pH 7.00 (■); pH 5.00 (●); and pH 9.00 (▲). The solid line represents a simple phenomenological description of the data according to the function $K_{eq} = {}^0K_{eq}[(1 + K_{dimer}[KCl]^2/(1 + K_{monomer}[KCl])^2]$. In this equation, ${}^0K_{eq}$ represents an intrinsic equilibrium constant while $K_{monomer}$ and K_{dimer} reflect overall binding to monomeric and dimeric species, respectively. This model is merely a simple description of the limited data set. Values of model parameters: ${}^0K_{eq} = (7.7 \pm 3) \times 10^6$ M^{-1}; $K_{dimer} = (1 \pm 0.5) \times 10^3$ M^{-2}; $K_{monomer} = 1.6 \pm 1.0$ M^{-1}. (b) Plot of apparent moles of linked KCl according to (a).

It is interesting to note that although the equilibrium constant changes by a factor greater than 17-fold, both the free energy and the enthalpy of dimerization remain relatively constant, $\Delta G_D = -11.2$ (±0.3) kcal/mol. Since, no curvature could be reliably detected in the van't Hoff plots, there does not appear to be any measurable change in heat capacity, ΔC_p, over this temperature range.

Salt Dependence. The equilibrium constant for cI repressor was measured at pH 7.00, $T = 20$ °C, as a function of added KCl, in the range 1–200 mM. A plot of ln K_{eq} vs ln [KCl] is nonlinear (Figure 5a) with two apparent regions of interest. Both regions of the curve indicate overall ion binding upon dimerization. In order to investigate the potential effect of

Table I: Proton, Temperature, and [KCl] Effects on Gibbs Energies of Repressor Dimerization[a]

pH	$\Delta G°$ (kcal/mol)	s	T (°C)	$\Delta G°$ (kcal/mol)	s	[KCl] (mM)	$\Delta G°$ (kcal/mol)	s
5	−11.7 ± 0.3	0.005	5	−11.4 ± 0.3	0.004	1	−9.2 ± 0.3	0.003
6	−11.2 ± 0.3	0.004	10	−11.5 ± 0.2	0.003	10	−9.4 ± 0.4	0.005
7	−11.1 ± 0.3	0.007	15	−11.3 ± 0.3	0.005	50	−9.9 ± 0.2	0.004
8	−11.3 ± 0.3	0.005	20	−11.1 ± 0.3	0.004	100	−10.4 ± 0.2	0.004
9	−11.2 ± 0.3	0.005	25	−11.1 ± 0.2	0.003	150	−10.9 ± 0.2	0.003
			30	−11.1 ± 0.2	0.003	200	−11.1 ± 0.3	0.004
			37	−11.0 ± 0.2	0.004			

[a] Errors represent 67% confidence intervals. s is the square root of the variance of fitted curves.

Table II: Resolved Parameters for Repressor Dimerization: Linkage to Protons and KCl[a]

pH	[KCl] (mM)	$\Delta G°$ (kcal/mol)	s[b]
5	10	−10.6 ± 0.3	0.005
5	200	−11.7 ± 0.3	0.005
7	10	−9.4 ± 0.4	0.005
7	200	−11.1 ± 0.3	0.004
9	10	−9.2 ± 0.2	0.004
9	200	−11.2 ± 0.3	0.005

[a] Errors represent 67% confidence intervals. [b] Square root of the variance of fitted curves.

divalent cations (Mg^{2+} or Ca^{2+}) at low concentration (1–10 mM KCl), both components were eliminated from the buffer as a control. The equilibrium constants resolved in the absence of divalent cations were identical (within the 67% confidence intervals) with those obtained in their presence (data not shown).

The overall charge state of a macromolecule in solution depends upon the pK's of amino acids and/or the extent of ion binding to specific sites on the protein. In light of earlier results from this laboratory on the proton dependence of site-specific binding (Senear & Ackers, 1990), we studied the low-salt effect (10 mM added KCl) at the extremes of pH (pH 5.00 and 9.00, T = 20 °C). The purpose was to delineate the nature of the specific ion effect, i.e., an anion vs cation binding event (Table II). We reasoned that if the protein can absorb protons in the acidic range upon formation of the dimer interface at 200 mM KCl, perhaps it would be possible to perturb that equilibrium by changing the number of counterions in the buffer. At 10 mM KCl, pH 9, the dimers form with approximately the same affinity (−9.2 kcal/mol) as at 10 mM KCl, pH 7 (−9.4 kcal/mol). However, at 10 mM KCl, pH 5, the assembly is more than 1 kcal/mol tighter, i.e., $\Delta G°$ = −10.6 kcal/mol (see Discussion).

Discussion

Information obtained from each experimental series (pH, temperature, [KCl]) combined with studies conducted at cross-points (i.e., extremes of pH and temperature) provides a systematic data base to characterize the chemical forces responsible for dimer formation in this system. While results of this study are generally consistent with earlier preliminary findings (Sauer, 1979; Johnson et al., 1980), our measurements do not support the previously drawn conclusion that the equilibrium constant is unaffected by changes in temperature, salt concentration, and pH.

The dimerization reaction is predominantly enthalpy-driven, with a standard enthalpy of $\Delta H°_{pH7}$ = −15.9 ± 2.1 kcal/mol. At the pH limits, the enthalpies of association were found to be $\Delta H°_{pH5}$ = −10.3 ± 1.8 kcal/mol and $\Delta H°_{pH9}$ = −13.0 ± 2.3 kcal/mol. These values are uniformly large and negative. A proton-linked contribution to the dimerization reaction is found to occur below neutral pH (Figure 3). Although this effect is not large, the data at other temperatures and monovalent salt concentrations do exhibit statistically significant differences between resolved equilibrium constants over the acidic pH range. Above neutral pH, there is no discernible contribution from proton ionization reactions.

Interpretation of the thermodynamic values in terms of dominant types of noncovalent processes is difficult (Sturtevant, 1977; Ross & Subramanian, 1981). While the importance of hydrophobic interactions cannot be neglected, the large negative $\Delta H°$ and significantly negative $\Delta S°$ preclude a dominant role of hydrophobic association (Privalov & Gill, 1988). Hydrogen bond formation in a medium of low dielectric constant, van der Waals interactions, and protonation events are the most important factors which contribute to observed negative values of $\Delta H°$ and $\Delta S°$ (Ross & Subramanian, 1981). Further interpretation of the differences in the enthalpies at the extremes of pH is difficult. Processes that could make positive contributions to $\Delta H°$ include hydrophobic association, charge neutralization, and/or specific dehydration events either at the surface of the repressor or at the level of co/counter ions.

Over the range studied, there is an apparent absorption of protons accompanying formation of the monomer–monomer interface as judged from the slopes of curves plotted in Figure 3: $\Delta\nu_{H^+}$ = d ln k/d ln a_{H^+} (Wyman, 1964), where k is the dimerization equilibrium constant. This reflects a shift in pK_a's toward more basic values. Senear and Ackers (1990) found approximately 2 mol of protons absorbed upon complete ligation of the three-site right operator by already dimerized repressors. The dimerization reaction is accompanied by absorption of approximately 0.3 mol of protons. In the acidic range, protons are thus seen to contribute to the formation of both dimers and specific ligation complexes (Senear & Ackers, 1990).

Although the observed proton absorptions may reflect the sum of small pK_a shifts for various ionizable groups, the overall pattern observed in this study suggests groups that titrate in the range pH 5–6. Possible candidates for these groups are quite limited in the λ repressor. Amino acids with pK_a's in the region of interest include histidine (pK_a = 6.0) and terminal amino groups (pK_a = 6.0) along with the acidic groups aspartate (pK_a = 4.5) and glutamate (pK_a = 4.6). The *c*I repressor contains only a single histidine per monomer, which is located in the amino-terminal domain (Pabo et al., 1979). The carboxy-terminal domain has been implicated in protein–protein interactions and in formation of the dimer interface. The lack of other basic groups in the carboxy terminus along with the apparent basic shift in pK_a's for proton absorption lead us to speculate that the acidic groups located in the carboxy terminus are the ionizable groups of interest.

Repressor assembly is found to be very sensitive to salt conditions: the magnitude of the assembly free energy decreases with decreasing [KCl], ΔG_D = −9.2 kcal/mol at 1 mM added KCl (Figure 5a). The curvature is indicative of a complex equilibrium in which ions exert control over the dimerization reaction. While it is difficult to interpret such a curve, there appears to be a region (1–50 mM) that is relatively

independent of added monovalent salt and a second region that is [KCl]-linked.

A reasonable conjecture is that one region is indicative of a general ionic strength "screening effect" while the other represents cations associated with a specific site or class of sites. A scenario in which negatively charged carboxylates of glutamates/aspartates repel one another on each monomer surface would fit the current data. With increasing [KCl], the repulsion between glutamates/aspartates is screened in a general ionic strength sense. If such a mechanism were at work, at the low pH and low-salt condition one would expect the monomers to associate more readily, which is the result obtained experimentally. In the acidic range, glutamates/aspartates are partially charged, thereby reducing the overall charge density at the surface. A decrease in the surface charge density allows the dimers to form more readily compared to conditions when the side chains are deprotonated (pHs 7 and 9). The data of Figure 5a were fit with a smooth function (see legend) in order to take the first derivative and estimate an apparent number of ions involved in the formation of the dimer interface. The resulting profile displays a maximum value of 1.5 mol (Figure 5b).

While the present study provides the first systematic characterization of the energetics of cI repressor dimerization, its scope has been necessarily limited. Extensions of the present work to include a wider range of conditions and ionic species will be required for a comprehensive understanding of these linkages.

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REFERENCES

Ackers, G. K. (1967) *J. Biol. Chem. 242*, 3026–3034.
Ackers, G. K. (1976) *Proteins (3rd Ed.) 1*, 1–84.
Ackers, G. K., & Thompson, T. E. (1965) *Proc. Natl. Acad. Sci. U.S.A. 53*, 342.
Ackers, G. K., Johnson, A. D., & Shea, M. A. (1982) *Proc. Natl. Acad. Sci. U.S.A. 79*, 1849–1853.
Beckett, D., Koblan, K. S., & Ackers, G. K. (1991) *Anal. Biochem.* (in press).
Brenowitz, M., Senear, D. F., Shea, M. A., & Ackers, G. K. (1986a) *Proc. Natl. Acad. Sci. U.S.A. 83*, 8462–8466.
Brenowitz, M., Senear, D. F., Shea, M. A., & Ackers, G. K. (1986b) *Methods Enzymol. 130*, 132–181.
Chadwick, P., Pirrotta, V., Steinberg, R., Hopkins, N., & Ptashne, M. (1970) *Cold Spring Harbor Symp. Quant. Biol. 35*, 283–294.
Hammond, J. B. W., & Kruger, N. J. (1988) in *Methods in Molecular Biology* (Walker, J. M., Ed.) Vol. 3, pp 25–32, Humana Press, Clifton, NJ.
Johnson, A. (1980) Ph.D. Dissertation, Harvard University, Cambridge, MA.
Johnson, A. D., Meyer, B. J., & Ptashne, M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 5061–5065.
Johnson, A., Pabo, C., & Sauer, R. T. (1980) *Methods Enzymol. 65*, 839–856.
Johnson, M. L., & Frasier, S. G. (1985) *Methods Enzymol. 117*, 301–342.
Johnson, M., Halvorson, H., & Ackers, G. K. (1976) *Biochemistry 15*, 5363–5367.
Jordan, S. R., & Pabo, C. O. (1988) *Science 242*, 893–899.
Koblan, K. S., & Ackers, G. K. (1991) *Biochemistry* (following paper in this issue).
Laemmli, U. (1970) *Nature (London) 227*, 680–685.
Pabo, C. O., Sauer, R. T., Sturtevant, J. M., & Ptashne, M. (1979) *Proc. Natl. Acad. Sci. U.S.A. 76*, 1608–1612.
Pivalov, P. L., & Gill, S. J. (1988) *Adv. Protein Chem. 39*, 191–234.
Ptashne, M. (1986) *The Genetic Switch*, Cell Press, Cambridge, MA.
Ross, P. D., & Subramanian, S. (1981) *Biochemistry 20*, 3096–3102.
Sauer, R. (1979) Ph.D. Dissertation, Harvard University, Cambridge, MA.
Senear, D. F., & Ackers, G. K. (1990) *Biochemistry 29*, 6568–6577.
Senear, D. F., Brenowitz, M., Shea, M. A., & Ackers, G. K. (1986) *Biochemistry 25*, 7344–7354.
Shea, M. A., & Ackers, G. K. (1985) *J. Mol. Biol. 181*, 211–230.
Sturtevant, J. M. (1977) *Proc. Natl. Acad. Sci. U.S.A. 74*, 2236–2240.
Valdes, R., & Ackers, G. K. (1979) *Methods Enzymol. 61*, 125–142.
Warshaw, H. S., & Ackers, G. K. (1971) *Anal. Biochem. 42*, 405–421.
Wyman, J., Jr. (1964) *Adv. Protein Chem. 19*, 224–394.
Wyman, J., & Gill, S. J. (1990) *Binding and Linkage*, University Science Books, Mill Valley, CA.