

the absence of any significant changes in the resonances corresponding to the N-terminal tyrosines when these allosteric effectors are bound.

Experiments are currently in progress to examine interactions with operator DNA, a variety of paramagnetic metals, and organic spin labels. These experiments with observations of ternary complexes of inducers and anti-inducer-repressor-operator DNA should shed more light on how this regulatory protein works.

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## ION EFFECTS ON THE *LAC* REPRESSOR-OPERATOR INTERACTION

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The effects of ions on the binding of *lac* repressor protein and operator DNA have been studied using the membrane filter technique. The association and dissociation rate constants were measured, and the equilibrium association constants calculated, as a function of

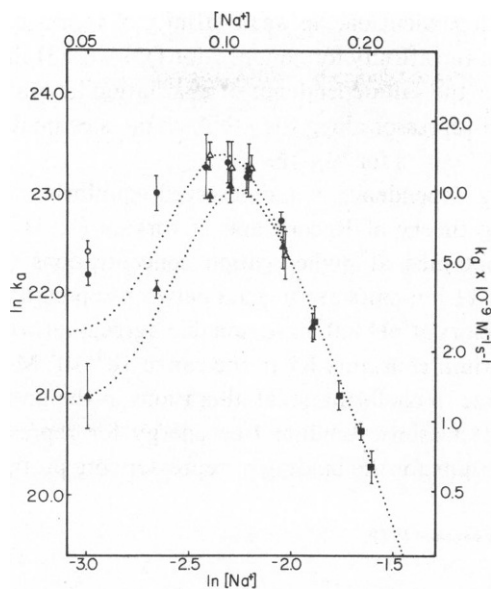


Figure 1 Dependence of the association rate constant of *lac* repressor (R) and  $\lambda$ lac DNA (O) on cation concentration for NaCl:phosphate buffer, pH 7.4, 20°C. Symbols are experimental values with S.D. indicated by error bars; some points are displaced  $\pm 0.1 \ln[\text{Na}^+]$  unit to avoid overlap. Dotted curves are theoretical values calculated according to Eqs. 4.2–4.3 and Fig. 2 of reference 4, except choosing  $\lambda = 10^4 \text{ s}^{-1}$  and using  $K_{RD} = 10^{3.1} [\text{Na}^+]^{10.3} \text{ nuc}$  from reference 2 corrected to pH 7.4. Upper curve, circles: [O] =  $5 \times 10^{-13} \text{ M}$ ; lower curve, triangles: [O] =  $1 \times 10^{-12} \text{ M}$ ; squares [O] =  $2 \times 10^{-12} \text{ M}$ . Open symbols: [R] = [O]; closed symbols: [R] = 2[O].

monovalent and divalent cation concentrations, anions, and pH. The salt dependence of the binding parameters is interpreted in light of recent theoretical analyses based on Manning's counterion condensation model (1).

The unusual dependence of the association rate constant  $k_a$  on salt concentration evidences a role for nonoperator DNA binding in the repressor's quest for its operator site on a large DNA molecule (Fig. 1). At intermediate mono- or divalent cation concentrations, the association rate goes through a maximum. At lower cation concentrations, it decreases and becomes dependent on DNA concentration; the high affinity of repressor for nonoperator DNA confines the protein to the DNA. At higher cation concentrations, the association rate

TABLE I  
*lac* REPRESSOR-OPERATOR INTERACTION

Conditions*	$D_1, \text{cm}^2 \text{s}^{-1} \ddagger$	$Z_{\text{max}} \S$	$\ln K_{\text{obs}}(1\text{M}) \parallel$	$\ln K \ddot{\parallel}$
NaCl, pH 7.4	$2.5 \times 10^{-10}$	$10.6 \pm 0.5$	$11.9 \pm 0.7$	14.1
pH 8.0	$4.5 \times 10^{-10}$	$8.8 \pm 0.3$	$14.1 \pm 0.5$	15.9
NaCH <sub>3</sub> CO <sub>2</sub> , pH 8.0	$4.0 \times 10^{-10}$	$8.5 \pm 0.3$	$17.9 \pm 0.5$	19.7
MgCl <sub>2</sub> , pH 8.0	$1.5 \times 10^{-9}$	$8.7 \pm 0.4$	$11.7 \pm 0.9$	12.9

\*Measurements at 20°C.

$\ddagger D_1$  is the diffusion constant of repressor along the DNA chain, estimated as indicated in Fig. 1.

$\S Z_{\text{max}}$  is the number of ion-pairs in repressor-operator complex, calculated according to Eqs. 12 or 19 of reference 3 in the limit of no anion binding.

$\parallel K_{\text{obs}}(1\text{M})$  is the observed equilibrium constant at  $[\text{M}^{+N}] = 1\text{M}$ , estimated by extrapolation of the  $\ln$ - $\ln$  plot of  $K_{\text{obs}}$  vs.  $[\text{M}^{+N}]$ .

$\ddot{\parallel} K$  is the thermodynamic equilibrium constant, calculated according to Eq. 7.17 of reference 5.

decreases and becomes dependent on the weak affinity of repressor for nonoperator DNA. Using published values of the affinity for nonoperator DNA (2, 3) the data are fit to Berg and Blomberg's (4) theory for the salt dependence of association kinetics with coupled diffusion. The diffusion constant of repressor along the DNA chain is estimated to be  $\sim 3 \times 10^{-10}$  cm<sup>2</sup>/s for NaDNA and  $\sim 1 \times 10^{-9}$  cm<sup>2</sup>/s for MgDNA.

The ion concentration dependence of the observed equilibrium constant  $K_{\text{obs}}$  is analyzed according to the binding theory of Record and coworkers (3, 5), and the predicted linear log-log dependence is obtained at higher cation concentrations (Table I). In repressor-operator complex, about 11 ion-pairs are formed between repressor and DNA phosphates at pH 7.4 and about 9 ion-pairs at pH 8.0, in reasonable agreement with previous estimates (6). Thermodynamic equilibrium constants  $K_f^0$  in the range  $10^6$ – $10^8$  M<sup>-1</sup> are found to depend on the anion, presumably due to conformational alterations in the protein. This corresponds to 9–12 kcal/mol of nonelectrostatic binding free energy for repressor-operator complex, in agreement with recent results for the binding of repressor core protein and operator (7).

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## RECONSTITUTION AND ELECTRON SPIN RESONANCE SPIN LABELING STUDIES OF NUCLEOSOMES

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The spin label, N-(2,2,5,5-tetramethyl-3-carbonylpyrrolidine-1-oxyl)-imidazole, a tyrosine specific label (1), was used to study the mode of reconstitution of nucleosome core particles. The histone cores in 2 M NaCl were first reacted with the imidazole spin label. After the removal of unreacted label, the histone cores were mixed with purified core DNA (145 base pairs) in 2 M NaCl. The mixture was then reconstituted by salt step-gradient dialysis according to Tatchell and Van Holde (2). At each step of the dialysis, an electron spin resonance (ESR) spectrum of the labeled tyrosyls was recorded and the correlation time of the label determined. As the ionic strength was gradually decreased, the correlation time of the