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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REFERENCES

1. Jacob, F., and J. Monod. 1961. Genetic regulatory mechanism in the synthesis of proteins. *J. Mol. Biol.* **3**:318–356.
2. Miller, J. H., and W. S. Reznikoff. 1978. The Operon. Cold Spring Harbor Laboratory. Cold Spring Harbor, New York.
3. Farabaugh, P. J. 1978. Sequence of the *lac I* gene. *Nature (Lond.)* **274**:765–769.
4. Beyereuther, K. 1979. Revised sequence for the *lac* repressor. *Nature (Lond.)*. **274**:767.
5. Gilbert, W., and A. Maxam. 1973. The nucleotide sequence of the *lac* operator. *Proc. Natl. Acad. Sci. U.S.A.* **70**:3581–3584.
6. Miller, J. H., C. Coulondre, M. Hofer, U. Schmeissner, H. Sommer, A. Schmitz, and P. Lu. 1979. Genetic studies of the *lac* repressor. IX. Generation of altered proteins by the suppression of nonsense mutations. *J. Mol. Biol.* **131**:191–222.
7. Lu, P., M. C. Jarema, K. Mosser, and W. E. Daniel. 1976. *Lac* repressor: 3-fluorotyrosine substitution for nuclear magnetic resonance studies. *Proc. Natl. Acad. Sci. U.S.A.* **73**:3471–3475.
8. Adler, K., K. Beyreuther, E. Fanning, N. Geisler, B. Gronenborn, A. Klemm, B. Muller-Hill, M. Pfahl, and A. Schmitz. 1972. How *lac* repressor binds DNA. *Nature (Lond.)* **237**:322–327.
9. Chandrasekaran, R., T. Jardetzky. 1979. The delta helix-a possible left-handed stable polypeptide structure in the N-terminal segment of the *lac* repressor. *FEBS (Fd. Eur. Biochem. Soc.) Lett.* **101**:11–14.
10. Miller, J. H. 1972. Experiments in Molecular Genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
11. Platt, T., J. G. Files, and K. Weber. 1973. *Lac* repressor, specific proteolytic destruction of the NH_2-terminal region and the loss of the DNA binding activity. *J. Biol. Chem.* **248**:110–121.
12. Hull, W. E., and B. D. Sykes. 1975. Fluorotyrosine alkaline phosphatase: internal mobility of individual tyrosines and the role of chemical shift anisotropy as a ^{19}F nuclear spin relaxation mechanism in proteins. *J. Mol. Biol.* **98**:121–153.
13. Coleman, J. E., and J. M. Armitage. 1978. Tyrosyl-base-phenylalanyl intercalation in gene 5 protein-DNA complexes: proton nuclear magnetic resonance of selectively deuterated gene 5 protein. *Biochemistry.* **17**:5038–5045.
14. Buck, F., H. Ruterjahns, and K. Beyreuther. 1978. ^1H NMR study of the lactose repressor from *E. coli*. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* **96**:335–338.
15. Wade-Jardetzky, N., R. P. Brady, W. W. Conover, O. Jardetzky, N. Geisler, and K. Weber. 1979. Differential mobility of the N-terminal headpiece in the *lac*-repressor protein. *J. Mol. Biol.* **128**:259–264.

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

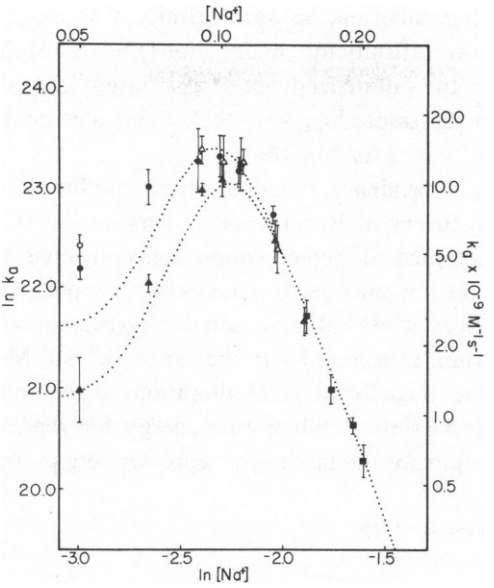

Figure 1 Dependence of the association rate constant of *lac* repressor (R) and λ*plac* DNA (O) on cation concentration for NaCl:phosphate buffer, pH 7.4, 20°C. Symbols are experimental values with S.D. indicated by error bars; some points are displaced ±0.1 ln[Na^+] unit to avoid overlap. Dotted curves are theoretical values calculated according to Eqs. 4.2–4.3 and Fig. 2 of reference 4, except choosing $\lambda = 10^4$ s^{-1} and using $K_{RD} = 10^{3.1}$ $[Na^+]^{10.3}$ nuc from reference 2 corrected to pH 7.4. Upper curve, circles: [O] = 5×10^{-13} M; lower curve, triangles: [O] = 1×10^{-12} M; squares [O] = 2×10^{-12} 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*	$D_1 cm^2 s^{-1}$‡	Z_{max}§	ln $K_{obs}(1M)$‖	ln K_T°¶
NaCl, pH 7.4	2.5×10^{-10}	10.6 ± 0.5	11.9 ± 0.7	14.1
pH 8.0	4.5×10^{-10}	8.8 ± 0.3	14.1 ± 0.5	15.9
$NaCH_3CO_2$, pH 8.0	4.0×10^{-10}	8.5 ± 0.3	17.9 ± 0.5	19.7
$MgCl_2$, pH 8.0	1.5×10^{-9}	8.7 ± 0.4	11.7 ± 0.9	12.9

*Measurements at 20°C.

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

§Z_{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.

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

¶K_T° 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^2/s for NaDNA and $\sim 1 \times 10^{-9}$ cm^2/s for MgDNA.

The ion concentration dependence of the observed equilibrium constant K_{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_T° in the range 10^6–10^8 M^{-1} 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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REFERENCES

1. Manning, G. S. 1978. The molecular theory of polyelectrolyte solutions with applications to the electrostatic properties of polynucleotides. *Quart. Rev. Biophys.* **11**:179–246.
2. Revzin, A., and P. H. von Hippel. 1977. Direct measurement of association constants for the binding of *Escherichia coli lac* repressor to non-operator DNA. *Biochemistry.* **16**:4769–4776.
3. deHaseth, P. L., T. M. Lohman, and M. T. Record, Jr. 1977. Nonspecific interaction of *lac* repressor with DNA: an association reaction driven by counterion release. *Biochemistry.* **16**:4783–4790.
4. Berg, O. G., and C. Blomberg. 1978. Association kinetics with coupled diffusion. III. Ionic-strength dependence of the *lac* repressor-operator association. *Biophys. Chem.* **8**:271–280.
5. Record, M. T., Jr., C. F. Anderson, and T. M. Lohman. 1978. Thermodynamic analysis of ion effects on the binding and conformational equilibria of proteins and nucleic acids: the roles of ion association or release, screening, and ion effects on water activity. *Quart. Rev. Biophys.* **11**:103–178.
6. Record, M. T., Jr., P. L. deHaseth, and T. M. Lohman. 1977. Interpretation of monovalent and divalent cation effects on the lac repressor-operator interaction. *Biochemistry.* **16**:4791–4796.
7. Matthews, K. S. 1979. Tryptic core protein of lactose repressor binds operator DNA. *J. Biol. Chem.* **254**:3348–3353.

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