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).

Received for publication 17 December 1979.

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

Daniel C. F. Chan, Thomas A. Grover, and Lawrence H. Piette, *University of Hawaii at Manoa, Cancer Center of Hawaii, Honolulu, Hawaii 96813 U.S.A.*

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

spin label increased. This is in contrast to what we observed previously (1) for the histone core alone, in which a decrease in the ionic strength caused the histone core (in the absence of DNA) to dissociate, freeing up the label and decreasing its correlation time. Judging from the change in rotational correlation times for the spin label, we concluded that the histone core binds progressively to the DNA in the range of 2 M–0.3 M NaCl. When the ionic strength is <0.3 M, full association between the histone core and DNA takes place. These reconstituted spin labelled nucleosome core complexes, purified by isokinetic sucrose gradient, were found to have identical physical properties (histone content, sedimentation coefficient, thermal melting profile, and circular dichroism) as the native particle.

The specific role of the tyrosyl residues in th reassociation process for the nucleosome core has also been investigated. The nucleosome core particle was first dissociated in 2 M NaCl and labeled with the imidazole spin label to varying degrees. The mixture of labeled core protein and endogenous DNA was reassociated by salt step-gradient dialysis and purified by isokinetic sucrose gradient. A major monomer peak, which sedimented at a position equivalent to the native nucleosome core particle ($S_{20,w}$ = 11), was found for all the reassociated samples, independent of the degree of labeling. This suggests that complete reassociation can be attained even when most of the surface tyrosines on the histone core have been labeled. However, when labeling was carried out under denaturing conditions by exposure of the histone core to urea so as to label those tyrosines that may be buried in the native structure, additional histone-DNA complexes, as measured by sucrose gradient, were formed with properties different from those of the native nucleosome core. This suggests that some of these "buried" tyrosines are essential for the specific histone-histone interactions that lead to the stable histone core structure. Spin labeling the "buried" tyrosines prevents the compact wrapping of DNA into the nucleosome core particle. If, however, the nucleosome core is dissociated and treated with urea without labeling of the tyrosyls, complete reassociation can take place yielding complexes identical to the native structure.

Distribution of the labeled tyrosines in the histone pairs H2A + H2B and H3 + H4 purified from the spin labeled nucleosome or reconstituted core complex by hydroxylapatite column chromatography (3) was analyzed. The four labeled tyrosines in the nucleosome are distributed in almost equal amounts among the histone pairs H2A + H2B and H3 + H4. In the reconstituted complex, in which the native sample was previously dissociated in 2 M NaCl and only the easily accessible or surface tyrosines on the histone core are labeled, specifically under these conditions, we find that 2 out of 8 of the tyrosines in the histone pair H2A + H2B have been labeled while 3 out of 7 tyrosines in H3 + H4 have been modified.

Received for publication 17 December 1979.

REFERENCES

1. Chan, D. C. F., and L. H. Piette. 1980. ESR spin label studies of the nucleosome core particle and histone core. *Biochim. Biophys. Acta.* In press.
2. Tatchell, K., and K. E. Van Holde. 1977. Reconstitution of chromatin core particles. *Biochemistry.* 16:5295–5303
3. Simon, R. H., and G. Felsenfeld. 1979. A new procedure for purifying histone pairs H2A + H2B and H3 + H4 from chromatin using hydroxylapatite. *Nucl. Acids Res.* 6:689–696.