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Measurement of DNA–Protein Equilibria Using Gel Chromatography: Application to the *Hin*fI Restriction Endonuclease†

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ABSTRACT: A method is described for measuring equilibrium constants of DNA–protein interactions using gel chromatography. This technique has been used to study the sequence-specific interaction of the *Hin*fI restriction endonuclease with DNA. *Hin*fI has a monomeric molecular weight of 31 000 and exists as a dimer in its active form. The protein binds to supercoiled DNA molecules containing its recognition site with an apparent free energy of −13.9 kcal/mol of sites. This interaction is highly salt sensitive and causes a release of 3.4 ion pairs. The affinity of the nuclease for its recognition site is largely independent of both pH (6.5–8.5) and temperature (7–35 °C) and was not affected by variations in the degenerate middle position of the site. Linear DNA fragments containing the *Hin*fI recognition site were bound as tightly as supercoiled molecules. Binding to nonspecific DNA sites or to methylated DNA sites was approximately 6 orders of magnitude weaker. In general, enzyme activity and binding affinity paralleled each other.

Type II restriction endonucleases provide good model systems for the study of sequence-specific recognition of DNA by proteins. One of the first steps in examining such interactions is the accurate determination of the equilibrium binding constants with specific and nonspecific DNA sites.

The most widely used method employs nitrocellulose filter binding (Riggs et al., 1970). This method is based on the observation that DNA alone will pass through a nitrocellulose filter while protein–DNA complexes are retained. A variety of other techniques have been described in addition to filter binding. Ackers et al. (1983) have developed methods for the

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