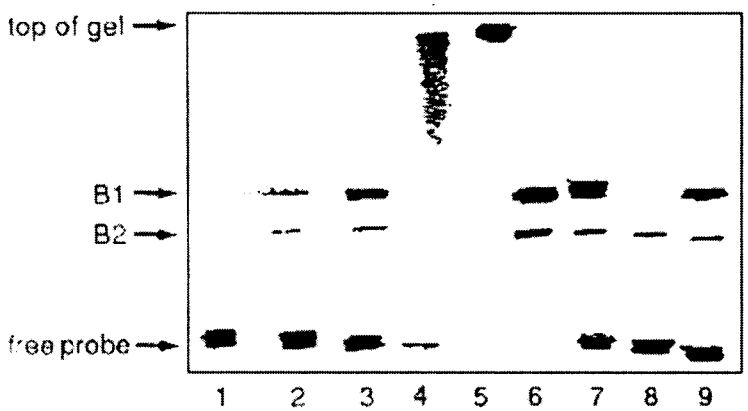
Mobility Shift DNA-Binding Assay Using Gel Electrophoresis

The DNA-binding assay using mobility shift polyacrylamide gel electrophoresis (PAGE) is a simple, rapid, and extremely sensitive method for the detection of sequence-specific DNA-binding proteins in crude extracts. This assay also permits the quantitative determination of the affinity, abundance, association rate constants, dissociation rate constants, and binding specificity of DNA-binding proteins. Proteins that bind specifically to an end-labeled DNA fragment retard the mobility of the fragment during electrophoresis, resulting in discrete bands corresponding to the individual protein-DNA complexes.

The protocol can be divided into four stages: (1) an end-labeled DNA probe containing a particular protein binding site is prepared; (2) a low-percentage, low-ionic-strength polyacrylamide gel is prepared; (3) a protein mixture is bound to the DNA probe; and (4) the binding reactions are electrophoresed through the gel, which is then dried and autoradiographed.



Hypothetical autoradiogram of a typical mobility shift DNA-binding experiment. Radioactive DNA probe and poly(dI-dC)-poly(dI-dC) were incubated with varying amounts of protein and electrophoresed from top to bottom on a low-ionic-strength polyacrylamide gel. The positions of the free probe and bound protein-DNA complexes are indicated.

Figure shows a typical autoradiogram. Lane 1 contains only the DNA probe and poly(dI-dC)-poly(dI-dC) with no added protein. A single band is seen. In lanes 2 to 5, an increasing amount of protein from a crude extract was added to a constant amount of probe and poly(dI-dC)-poly(dI-dC). In lanes 2 and 3, two discrete complexes (B1 and B2) migrate through the gel more slowly than the band corresponding to the free probe. As more protein is added to the reaction, the intensities of B1 and B2 increase while the amount of free probe decreases (compare lanes 2 and 3). As even more protein is added (lanes 4 and 5), there is little or no free probe remaining. Moreover, complexes B1 and B2 are no longer observed. Because many different nonspecific DNA-binding proteins are bound, most of the probe migrates very slowly and diffusely or does not enter the gel.

The reaction shown in lane 6 is identical to that shown in lane 5, except that a larger excess of poly(dI-dC)-poly(dI-dC) has been added. Since more protein was added in lane 6 relative to that in lane 3, the intensities of complexes B1 and B2 are greater in lane 6 than in lane 3. Note that if a more purified preparation of B1 was used in the binding reaction, all of the probe in the reaction could be

specifically bound and would migrate in complex B1, rather than at the top of the gel.

In lanes 7 to 9, a competition binding experiment determined whether complexes B1 and B2 represent specific protein-DNA complexes. Lane 7 is a standard binding reaction which contains probe, protein and poly(dI-dC)-poly(dI-dC). Lane 8 is the same as lane 7, except that a 100-fold molar excess of an unlabeled DNA fragment identical to the probe (i.e., a specific competitor) was added prior to the addition of protein. Lane 9 is the same as lane 8, except that an unlabeled DNA competitor fragment containing sequences different from the probe (i.e., a nonspecific competitor) was added.

Formation of complex B1 is competed by the specific unlabeled DNA fragment, but not by the nonspecific DNA fragment. Therefore, complex B1 results from the specific binding of a molecule in the extract to the DNA probe. Formation of complex B2 is not competed by either the specific competitor or the nonspecific competitor. Complex B2 results from the nonspecific binding of a molecule in the extract to the DNA probe. This is typical behavior for a nonspecific DNA-protein interaction.

COMMENTARY

Several methods exist for detecting the sequence-specific binding of proteins to DNA including nitrocellulose filter binding, DNase I footprinting, methylation protection, methylation interference, and mobility shift gel electrophoresis. The DNA-binding assay using mobility shift PAGE is based on the observation that DNA-protein complexes migrate through low-ionic-strength polyacrylamide gels more slowly than unbound DNA fragments. This assay is generally simpler, faster, and more sensitive than other methods. Thus, it is an ideal assay for monitoring the purification of DNA-binding proteins. More importantly, the sensitivity of this assay enables femptomole quantities of DNA-binding proteins to be detected routinely. In addition to providing quantitative information on the amount of DNA bound by the protein, the use of mobility shift PAGE provides additional information on the number and type of proteins bound. Also, each distinct species of protein bound to the probe generates a complex of distinct mobility and specificity so that interactions of several proteins binding to a single DNA fragment can be observed. Moreover, even if multiple proteins recognize overlapping sites on the DNA fragment, the complexes formed by each can be resolved and characterized.

Methylation Interference Assay for Analysis of DNA-Protein Interactions

Methylation interference identifies guanines and adenines in the DNA binding site that, when methylated, interfere with binding of the protein. The protocol described below uses a single end-labeled DNA probe that is methylated at an average of one site per molecule of probe. The labeled probe is a substrate for a protein-binding reaction. DNA-protein complexes are separated from the free probe by the mobility shift DNA-binding assay (UNIT 12.2). A DNA probe that is methylated at a position which interferes with binding will not be retarded in this assay. Therefore, the specific DNA-protein complex is depleted for DNA that contains methyl groups on purines important for binding. After gel purification, DNA is cleaved with piperidine. Finally, these fragments are electrophoresed on polyacrylamide sequencing gels and autoradiographed. Guanines and adenines that interfere with binding are revealed by their absence in the retarded complex relative to a lane containing piperidine-cleaved free probe. This procedure offers a rapid and highly analytical means of characterizing DNA-protein interactions.