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A rapid method for determining the molecular weight of a protein bound to nucleic acid in a mobility shift assay

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Mobility shift (or gel retardation) analysis is a powerful technique for detecting and characterizing proteins that bind to specific nucleic acid sequences (1, 2). The molecular weight of a protein bound directly to nucleic acid in a mobility shift gel can be determined by label transfer: the shift is performed using a ^{32}P-labeled DNA or RNA oligonucleotide, label is transferred to the bound polypeptide by UV crosslinking, and the protein is identified as a ^{32}P-labeled band upon SDS denaturing gel electrophoresis (3, 4). Although straightforward in principle, in practice it can prove difficult to determine the molecular weight of polypeptides in a shifted band due to poor recovery or to proteolysis. Here we show that the gel slice containing a crosslinked protein-DNA complex can be polymerized directly into the stacking gel of a conventional Laemmli gel. Upon electrophoresis, protein-DNA complexes migrate efficiently out of the gel slice and stack normally, allowing accurate determination of the molecular weight of the crosslinked protein.

A duplex, bromodeoxyuridine-substituted 36 bp oligonucleotide spanning the LR1 binding site from the Sγ switch region (4), labeled to a specific activity of 1×10^8 cpm/μg, was generated by extending a 10-base primer complementary to a 36-mer template. The 50 μl reaction contained 20 pmol 36-mer, 20 pmol of a 10-mer complementary to the 3′ end of the 36-mer, 1 U Klenow polymerase, 60 μM 5′ bromo-2′deoxyuridine-5′triphosphate (Sigma), 60 μM dGTP, and 70 μCi each of α-^{32}P-dATP and α-^{32}P-dCTP (Amersham; 3000 Ci/mmol). Substitution with bromodeoxyuridine was used to increase the efficiency of crosslinking (3). The modified, labeled DNA was incubated with LR1-containing protein fractions, protein-DNA complexes were resolved by mobility shift on a 1 mm thick 5% polyacrylamide-TBE gel (5), and the wet gel was autoradiographed overnight at 4°C to visualize shifted complexes. Protein-DNA complexes were irradiated in the gel (either before or after autoradiography) with a 254 run DNA Transfer Lamp (Fotodyne; 160 j/m^2) at 16 cm for 5 minutes. Bands containing protein-DNA complexes were excised from the shift gel and denatured by boiling for 5 minutes in a modified sample buffer containing DTT (1% SDS, 3 mM DTT, 125 mM Tris−HCl, pH 6.8); use of β-mercaptoethanol inhibits subsequent gel polymerization. Low molecular weight Coomassie markers (BioRad) were precast in a 1 mm thick 5% polyacrylamide gel (0.1% SDS, 125 mM Tris−HCl, pH 6.8) and denatured in parallel. After denaturation, the irradiated gel slices were placed side by side, together with a precast gel slice of similar size containing 5 μg of markers, about 1 cm from the top of a 10×11.5 cm gel plate; 1 mm spacers were then fitted and a second plate clamped to the first. An SDS−Laemmli running gel (8−20% acrylamide gradient) was poured and allowed to polymerize between the plates, leaving about 1 cm between the bottom of the gel slices and the top of the running gel; a stacking gel was then poured to fill the rest of the volume between the plates. Following electrophoresis for 3 h at 10 mA, the gel was

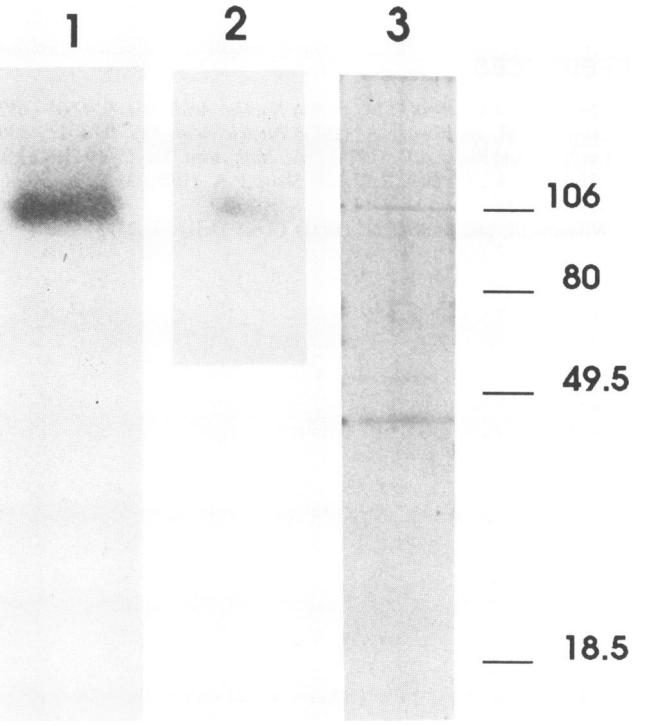

Figure. Label transfer from a ^{32}P-labeled LR1 binding site oligonucleotide to the polypeptide containing DNA binding activity. Lane 1: A gel slice containing crosslinked complexes produced after binding of crude BJAB nuclear extract to the LR1 oligonucleotide was boiled in modified sample buffer and cast directly into the denaturing SDS gel, as described in the text. Lane 2: As in lane 2, but complexes were formed using ~8000-fold purified LR1 from PD31 pre-B cells. Lane 3: Silver stained, ~8000-fold purified LR1. The sizes of molecular weight standards (in kD) are noted to the right of each panel. In all cases, protein-DNA complexes were crosslinked directly in the mobility shift gel.

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Coomassie-stained to visualize the markers, dried, and autoradiographed for 48 hours at −70°C with an intensifying screen.

The figure compares label transfer following binding of crude nuclear extract or highly purified LR1 to BrdU-substituted, ^{32}P-labeled oligonucleotide containing the LR1 binding site (4). The results show that DNA binding activity in crude extract correlates with the 10^6 kD polypeptide in the highly purified LR1 preparation.

The technique described here is rapid, because the nucleic acid binding protein is never eluted from the mobility shift gel. Additional advantages are that no material is lost upon elution or precipitation, proteolysis is minimized, and many shifted bands can readily be compared in parallel. It works for RNA binding proteins (M.Mirfakhrai and A.M.Weiner, in preparation) as well as for DNA binding proteins. In applying this method to other nucleic acid binding proteins, we have occasionally observed artefactual bands that barely enter the running or stacking gel. These bands can be eliminated by treating the gel slice with 1 mg/ml RNase A (M.Mirfakhrai and A.M.Weiner, in preparation) or 1 mg/ml DNase I for 30 min prior to denaturation.

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