

Binding of hairpin and dumbbell DNA to transcription factors

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In gel-shift assays of the binding of transcription factors to short specific oligonucleotide sequences in double-helical DNA it is usual to prepare two complementary DNA sequences and to anneal them to form the target DNA. This is inconvenient because an excess of the unlabelled strand must be used to ensure that the labelled strand is completely annealed, or the double-helical species must be separated and purified. Strand separation is another potential problem.

We prepared a hairpin sequence CRE II and a ligated circular (dumbbell) sequence CRE IV that contains the CREB binding sequence CRE (1, 2) and a dumbbell sequence TRE IV that contains the JUN binding sequence TRE (3) (Figure 1). We find that they can replace the double-stranded sequences CRE I and TRE I in gel-shift assays with equal or greater sensitivity (Figures 2A and 3A). The binding of [^{32}P]-labelled dumbbell CRE IV to CREB could be suppressed 75% by a 150-fold excess of unlabelled dumbbell CRE (Figure 2B, lane 2), but not by a 200-fold excess of a dumbbell DNA that did not contain the CRE sequence (results not shown). Similarly, binding of [^{32}P]-labelled dumbbell TRE IV to JUN could be reversed by the presence of a 50-fold excess of unlabelled TRE IV (Figure 3B, lane 2), but not by a 100-fold excess of a random dumbbell sequence (Figure 3, lane 3).

Dumbbell structures are particularly advantageous where double-stranded DNA is at risk of exonuclease degradation. We incubated [^{32}P]-labelled CRE-containing oligomers I–IV with two independently purchased samples of fetal calf serum (10% in RPMI media) and with two independently collected samples of 100% human serum. In every case we found that the rate of degradation of the ligated dumbbell sequence CRE IV was much lower than that of the hybridized double-stranded form CRE I. The hairpin sequence CRE II had intermediate stability.

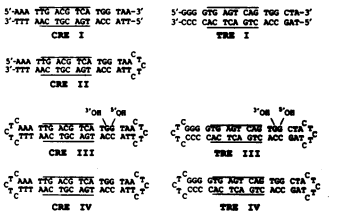

Figure 1. Structures of DNAs containing double-stranded CRE and TRE sequences. The underlined sequences are the core CRE and TRE octamer recognition sequences. I. Hybridized single strands. II. A 41mer sequence that folds into a hairpin. The double-stranded stem is identical in sequence to the hybridized sequence in CRE I above. III. 46mer oligonucleotides that fold into dumbbells. The double-stranded stems are identical in sequence to the hybridized sequences in I above. IV. The ligated forms of III above. The cyclic 46mers IV were obtained by phosphorylating III with ATP and polynucleotide kinase and subsequently treating with DNA ligase in buffer containing 50 mM Tris (pH 7.6), 10 mM $MgCl_2$, 1 mM ATP, 1 mM DTT and 5% polyethylene glycol overnight at room temperature. The ligated products were separated from the unligated starting materials by electrophoresis on a 12% denaturing gel.

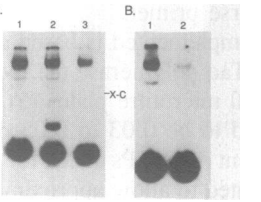

Figure 2. Autoradiogram of a 6% non-denaturing gel showing: A. binding of 200 ng CREB protein to 0.015 pmol of: lane 1, [^{32}P]-dumbbell CRE IV; lane 2, [^{32}P]-hairpin CRE II; lane 3, [^{32}P]-double-stranded 18mer CRE 1. B. Binding of 100 ng CREB to: lane 1, 0.015 pmol [^{32}P]-dumbbell CRE IV; lane 2, same as in lane 1 but with a 150-fold excess of unlabelled dumbbell CRE IV. Binding was carried out for 15 minutes at room temperature in 10 µl buffer containing 15 mM Tris (pH 7.5), 50 mM KCl, 0.1 mM EDTA, 0.5 mM DTT, 5% glycerol, 9 mg/ml acetylated BSA and 25 ng/µl polydI/dC.

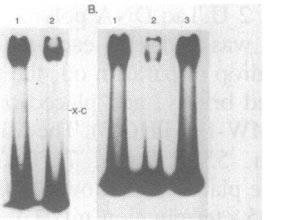

Figure 3. Autoradiogram of a 6% non-denaturing gel showing A. Binding of 200 ng JUN protein to 0.015 pmol of: lane 1, [^{32}P]-dumbbell TRE IV; lane 2, [^{32}P]-double-stranded 18mer TRE I. B. Binding of 200 ng JUN to: lane 1, 0.015 pmol [^{32}P]-dumbbell TRE IV; lane 2, same as in lane 1, but in the presence of a 50-fold excess of unlabelled dumbbell TRE IV; lane 3, same as lane 1 but in the presence of a 100-fold excess of a non-specific dumbbell sequence. Binding was carried out for 15 minutes at room temperature in 20 µl buffer containing 50 mM Tris (pH 7.9), 100 mM KCl, 1 mM EDTA, 1 mM DTT, 125 mM $MgCl_2$, 20% glycerol and 50 ng/µl polydI/dC.

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