A COMPARISON OF LAC REPRESSOR BINDING TO OPERATOR AND TO NONOPERATOR DNA

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SUMMARY. We have compared the operator and nonoperator DNA binding activities of the lac repressor with respect to inactivation or inhibition by trypsin, heat, actinomycin, and isopropylthiogalactoside. The two DNA binding activities were found to differ only in their sensitivity to the inducing ligand isopropylthiogalactoside. Repressor binding to poly(dT-dT-dG)·poly(dC-dA-dA) was shown not to be affected by isopropylthiogalactoside.

INTRODUCTION. The lac repressor has two DNA binding activities. The first is strong binding to the lac operator (1-3); the second is relatively weak binding to nonoperator DNA (4,5). It is the purpose of this paper to compare these activities. The binding of repressor to nonoperator DNA is highly relevant for the in vivo regulation of the lac operon (5,6) and for explaining certain aspacts of in vitro studies, such as the paradoxically fast kinetics of repressor binding to operator (3,5,7,8).

Until recently, the binding of lac repressor to nonoperator DNA could not be studied directly by nitrocellulose filter assays because the interaction was too weak to cause efficient filter retention. Competition assayswere done (4,9) but any treatment of repressor that inhibited operator binding activity rendered the measurement of competition impossible. Fortunately we found that UV irradiation of [BrdU]DNA-repressor complexes in low salt causes the covalent attachment of repressor to both operator and nonoperator DNA (10). By using this photochemical attachment procedure, direct assays of nonoperator binding activity can be done and we report here that both operator and nonoperator binding activities are similar in their sensitivity to trypsin, heat, and actinomycin.

but they differ in response to isopropylthiogalactoside (IPTG)†.

MATERIALS AND METHODS. Lac repressor (i^{superq}) was prepared from strain M96 according to published procedures (10). SDS-gel electrophoresis gave a single band and all DNA binding activity sedimented as lac repressor in a glycerol gradient. The preparation of λh80dlac[^{32}P]DNA and wild type λh80[^{32}P]DNA (both normal and BrdU-substituted) has been described (10). Poly[d(TTG)·d(CAA)]

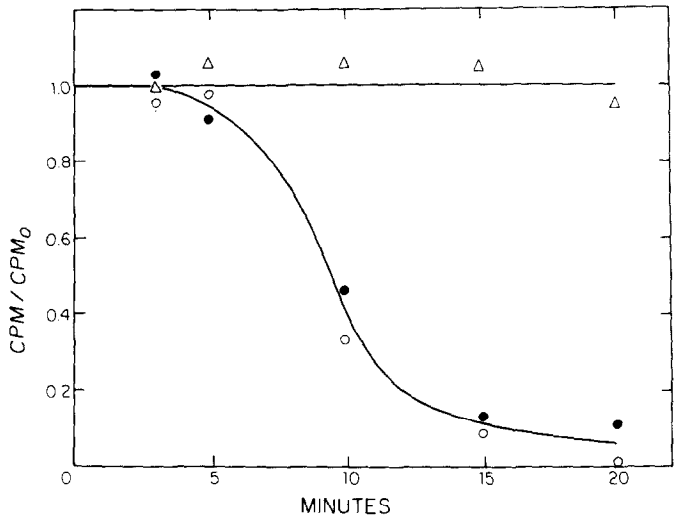

Figure 1. Inactivation of operator, nonoperator, and IPTG binding activities of lac repressor by trypsin. Lac repressor (930 μg/ml protein) was treated with trypsin (0.14 μg/ml) at 37° C as in reference 11. At the indicated times, aliquots of the treated repressor were removed and trypsin action stopped by phenylmethylsulfonyl fluoride and bovine serum albumin as in Platt, et al. (11). Operator, nonoperator, and IPTG binding activities were measured as described in Materials and Methods. For operator binding, 10-μl aliquots were diluted 30 times and then 20 μl added to 0.5 ml of buffer I containing 50 ng of λh80dlac[^{32}P]DNA. For nonoperator DNA binding, 50 μl of the diluted repressor was added to 0.5 ml of buffer I containing 50 ng of BrdU-substituted λh80 wild type [^{32}P] DNA. For both operator and nonoperator DNA binding, the concentration of repressor was chosen, on the basis of complete binding curves, to be 20 times that needed to give half maximum filter retention. For IPTG binding, 40 μl of the treated repressor was added to 140 μl of buffer I containing 0.26 M KCl and 4.6 X 10^{-6}M of [^{14}C]IPTG. The data were normalized by CPM_0, the filter-bound counts obtained with no trypsin treatment. Δ-Δ, [^{14}C]IPTG; ●-●, λh80dlac[^{32}P]DNA: O-O, BrdU-substituted λh80 wild type [^{32}P]DNA.

† Abbreviation: IPTG, isopropyl-1-thio-β-D-galactoside.

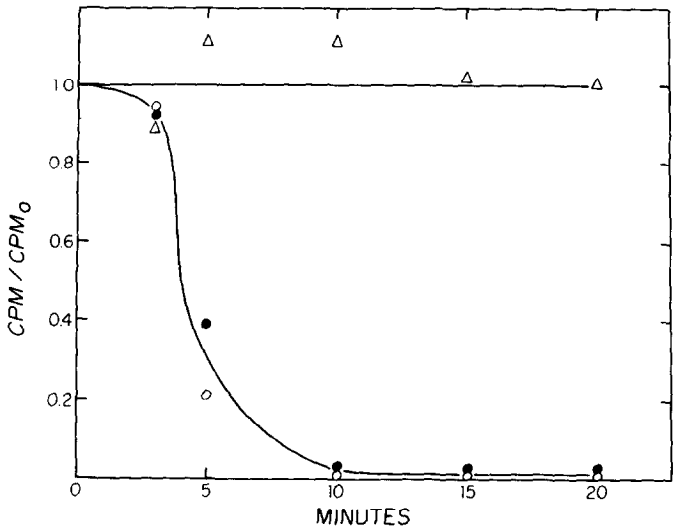

Figure 2. Heat inactivation of lac repressor activities. ^{35}S-labeled lac repressor (49 µg/ml protein, 1500 cpm/µg) was heated at 60° C in 0.01M Tris HCl, pH 7.4. Aliquots were taken at various times, diluted in buffer I, and operator and nonoperator DNA binding activities measured. For operator binding assays, 33 ng of repressor protein was added to 50 ng of λh80dlac[^{32}P]DNA in 0.52 ml of buffer I. For nonoperator binding assays, 85 ng of repressor protein was added to 50 ng of BrdU-substituted λh80 wild type [^{32}P]DNA in 0.55 ml of buffer I. The ^{35}S counts in the repressor were sufficiently low as not to interfere with the DNA binding assays. [^{35}S]repressor was used for the control to show that heat treatment was not reducing the retention of repressor protein by nitrocellulose filters. ●-●, λh80dlac[^{32}P]DNA; O-O, BrdU-λh80 wild type [^{32}P]DNA; Δ-Δ, heat treated [^{35}S]repressor mixed with λh80dlac DNA and filtered.

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was a gift from R. D. Wells (see ref. 9). Actinomycin D and trypsin were from Merck and Sigma Companies respectively. Lac repressor was treated with trypsin according to Platt, et al. (11).

Operator and [^{14}C]IPTG binding activities were measured by standard nitrocellulose filter assays (12,13) except that 0° C was used. The binding of repressor to BrdU-substituted nonoperator DNA (BrdU-λh80[^{32}P]DNA) was measured by the photochemical attachment technique (10). UV irradiation was at 0° C and at 9 cm distance from the lamp. Buffer I has been described (10).

RESULTS. Platt, et al. (11) have shown that trypsin treatment of the lac repressor destroys operator binding activity much more

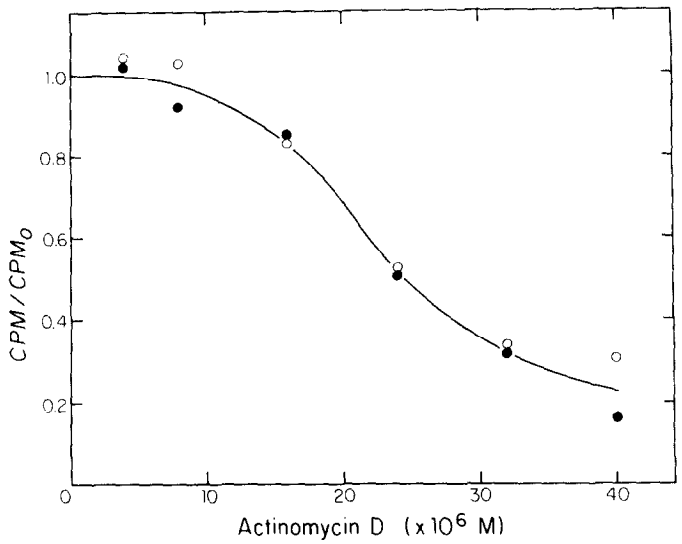

Figure 3. Actinomycin inhibition of operator and nonoperator DNA binding activities. Repressor (23 ng protein) was mixed with 25 ng of BrdU-DNA (either wild type or dlac) in 0.5 ml of buffer I and the indicated concentration of actinomycin D. Operator binding and nonoperator binding were then ascertained as in Materials and Methods. ●-●, BrdU-substituted λh80dlac[^{32}P]DNA. O-O, BrdU-substituted λh80 wild type [^{32}P]DNA.

rapidly than IPTG binding activity. The data shown in figure 1 confirm these observations. Figure 1 also shows that trypsin inactivates operator and nonoperator binding activities at similar rates. Figure 2 shows that operator and nonoperator binding activities are inactivated at similar rates by heat treatment.

Actinomycin D binds in the minor groove of double-stranded DNA preferentially at the sequence dG-dC (14). The lac operator sequence (15) has one actinomycin binding site, and actinomycin is known to inhibit repressor binding to operator (12). If nonoperator binding were by a different mechanism than operator binding (perhaps involving only interactions with the phosphate backbone) and not sensitive to changes in the minor groove, then inhibition studies with actinomycin might have shown an interesting differential response. We report here that such is not the case; operator and nonoperator binding activities are equally sensitive

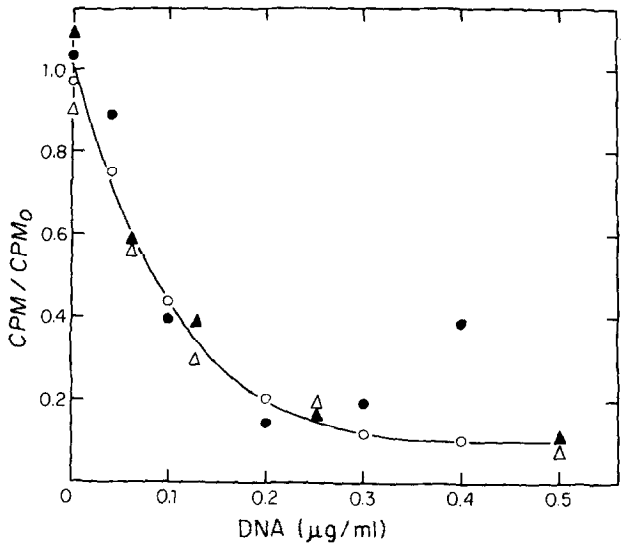

Figure 4. The effect of IPTG on competition by synthetic DNAs. Competing DNA was mixed with 25 ng of BrdU-λh80[^{32}P]DNA in 0.4 ml of buffer I and then sufficient repressor was added to give maximum retention in the absence of competing DNA. After at least 15 min to reach equilibrium, the solution was UV irradiated as in Materials and Methods for 15 min. Aliquots (0.15 ml) were then filtered in duplicate. When present, IPTG was at 1 mM through the procedure. (▲), poly(dA-dT)·poly(dA-dT), no IPTG. (Δ), poly(dA-dT)·poly(dA-dT) plus IPTG. (●), poly(dT-dT-dC)·poly(dC-dA-dA), no IPTG. (○), poly(dT-dT-dG)·poly(dC-dA-dA), plus IPTG.

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to inhibition by actinomycin (Figure 3).

IPTG is known to greatly reduce the affinity of repressor for operator (3,16). We have shown (10) that IPTG does not inhibit repressor binding to BrdU-λh80 DNA. Repressor has a relatively high affinity for poly(dA-dT) ($K = 1 \times 10^{8} M^{-1}$, ref. 4) but as shown in figure 4, IPTG does not affect binding to this polymer. Poly(dT-dT-dG)·poly(dC-dA-dA) was found to bind repressor extremely well ($K \simeq 3 \times 10^{9} M^{-1}$) (9). Later we noticed that this polymer provides in correct sequence four out of the six bases in the symmetrical regions at each end of the lac operator. Therefore, it was important to ascertain whether repressor binding to this polymer is affected by IPTG. As shown in figure 4, even this polymer is not close enough to the lac operator to elicit an IPTG response.

DISCUSSION. An attractive model for repressor-operator interaction is that one region of the lac repressor has a moderate general affinity for DNA (e.g., electrostatic interaction), whereas another region has specific affinity (e.g., H-bonding) mainly for the sequences unique to the lac operator. Together they would generate the high affinity for operator. This model is not supported by the present evidence. We report here that operator binding and nonoperator binding activities are inactivated at similar rates by heat and trypsin. Trypsin treatment is known to remove 59 amino acids from the amino terminal portion of the lac repressor without attacking the middle regions of the peptide chain. The trypsin-treated repressor still retains inducer binding activity and a tetrameric structure, but does not bind to operator (11). Genetic studies implicate only the amino terminal regions in operator binding (17). Since removal of the amino terminal regions of the lac repressor by trypsin also abolishes nonoperator DNA binding activity, it appears that the amino terminal portion of the repressor is the site critical for both operator and nonoperator DNA binding.

Operator and nonoperator DNA binding activities are equally sensitive to inhibition by actinomycin, and from previous work (4,5) we know that both activities respond similarly to salt, temperature, and pH. BrdU substitution for thymidine in DNA also increases both operator and nonoperator binding affinities tenfold (ref. 18 and our unpublished data). The simplest interpretation of these results is that similar binding mechanisms are used for both operator and nonoperator DNA.

Therefore, it is of interest that the binding of repressor to nonoperator DNA, even repeating poly(dT-dT-dG)·poly(dC-dA-dA), is not affected by IPTG. The lac repressor is known to change conformation in the presence of IPTG (19-21). Apparently these conforma-

tional changes can be tolerated for nonoperator binding but not for operator binding.

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