The bacteriophage D108 Ner repressor binds a conformationally distinct operator

Abstract  The Ner protein encoded by the transposable coliphage D108, an 8.6 kDa λ Cro-like repressor, binds to an operator spanning 50 bp of DNA. The distinguishing features of this operator are two perfect 11-bp inverted repeats (5′-CCGTGAGCTAC-3′) that are separated by an 8-bp AT-rich spacer. Hyperreactivity of the ner operator to potassium permanganate and the hydroxyl radical indicate that the AT-rich spacer assumes a variant conformation consistent with a bend. Using an electrophoretic mobility shift assay, we demonstrated that Ner does not display significant affinity for a single 11-bp site. Furthermore, DNase I protection analysis and circular-permutation binding assays reveal that alterations in the length and sequence of the AT-rich spacer that separates the 11-bp inverted repeats significantly alter Ner-operator interactions, and demonstrate that the intrinsically bent ner operator is conformationally altered upon protein binding.

Key words  DNA binding protein · DNA bending · Transposable bacteriophage · Mu

Introduction

The monomeric Ner repressor encoded by the transposable Mu-like bacteriophage D108 binds to an operator that spans five turns of the DNA-helix and overlaps the transcriptional start sites for the converging Pe (early) and Pc [lysogenic (c) repressor] promoters (Kukolj et al. 1989; Levin and DuBow 1989). Bound Ner inhibits expression of the lysogenic (c) repressor from the Pc promoter and, in conjunction with the Escherichia coli integration host factor (IHF), regulates transcription from the early lytic promoter (Pe) to ensure stable, yet low-level, expression of genes whose products catalyse transposition of the 37-kb double stranded DNA phage genome (Goosen et al. 1984; Goosen and van de Putte 1986). How the 8.6-kDa Ner protein contacts this operator over such a large region is unknown. Ner homologues which can complement other DNA-binding functions have been found in bacteria (Autexier and DuBow 1992) and humans (Garcia et al. 1992). Raman spectral studies of D108 Ner (Benevides et al. 1994) and NMR analysis of Mu Ner (Gronenborn et al. 1989) reveal that the Ner proteins are highly basic, predominantly alpha-helical (Benevides et al. 1994) and use a helix-turn-helix motif for DNA binding (Strzelecka et al. 1995a). Binding of D108 Ner to its operator induces significant changes in helical pitch and twist, yet the operator remains in the B-form (Benevides et al. 1994).

Small, basic proteins in prokaryotes have been identified as DNA “architectural elements” with the capability of altering and compacting DNA superhelical structure (Nash 1996). These histone-like proteins are proposed to contact multiple turns of the double helix by binding and bending the DNA (Nash 1996; Rice et al. 1996). Enzymatic and chemical probing of structural alterations in the DNA in the Mu and D108 regulatory regions reveal that IHF, a histone-like protein, destabilizes repression of the early (Pe) promoter (Kukolj and DuBow 1992; van Ulsen et al. 1996). IHF alleviates Ner-dependent repression of the D108 early promoter (Kukolj and DuBow 1992), and also disrupts a nucleo-protein complex formed by the H-NS repressor in the Mu regulatory region (van Ulsen et al. 1996). Two identical 11-bp sequences (5′-CCGTGAGCTAC-3′), which are inverted with respect to one another and are separated by an 8-bp AT-rich spacer, comprise the central portion of the large D108 ner operator (Fig. 1).
 Ner binds to this bipartite operator through base-specific chemical contacts that are restricted to the two 11-bp inverted repeats (Kukolj et al. 1989). How the Ner protein can contact bases spanning one turn of the DNA helix, and how it can cover (and protect from cleavage by DNase I) five helical turns of DNA is not currently known. Laser Raman spectroscopy has suggested that both the Ner protein and its operator undergo structural distortions upon binding (Benevides et al. 1994). We report here that a single 11-bp site is not sufficient for Ner binding, and that the two 11-bp segments must assume a proper and distinct spatial configuration, with respect to one another, in order to serve as high-affinity binding substrates. The unbound ner operator displays an intrinsic distortion that maps to the AT-rich spacer. We present evidence that this distortion in operator structure is altered upon binding of Ner.

Materials and methods

Binding sites, plasmids, DNA fragments and end-labeling

DNA manipulations were performed as described previously (Kukolj et al. 1989; Sambrook et al. 1989). The single 11-bp ner operator, flanked by EcoRI and NdeI restriction sites, was constructed by annealing the chemically synthesized oligonucleotides 5'-AATTCCTGGTAGCTACCA-3' and 5'-TATGGTAGCTCAA-3'. Plasmid pNB1, containing this synthetic 11-bp sequence, was constructed by ligating the annealed oligonucleotides to EcoRI+ NdeI-cleaved pBR322 (thereby replacing the entire tet gene), and used to transform the E. coli strain DH1, screening for Amp' and Tet' colonies. An 89-bp Sau96-II cleavage restriction fragment, containing this single 11-bp site and used in operator binding studies, was isolated by the "crush and soak" procedure and radioactively labeled at the 3' Sau96 end with [\(^{32}\)P]dATP (5000 Ci/mmol, Amersham Canada) using T4 polynucleotide kinase, and then cleaved with DraI to generate the 93-bp DraI-NdeI fragment uniquely labeled at the 5' NdeI end.

Operator-binding assay

Binding of Ner to \(^{32}\)P-labeled operator fragments was assayed using a modification of the gel mobility shift assay as previously described (Kukolj and DuBow 1991). Briefly, 10-50 fmol of a specified restriction fragment was incubated with various concentrations of Ner protein in 20 \(\mu\)l of binding buffer [25 mM TRIS-HCl pH 7.5, 100 mM NaCl, 1 mM EDTA, 5% (v/v) glycerol, 100 \(\mu\)g/ml bovine serum albumin] for 25 min at room temperature. One microlitre of 0.25% (v/v) bromophenol blue in binding buffer was added to the reactions, which were then loaded onto 4% or 5% polyacrylamide gels (29:1 acrylamide to bisacrylamide in 89 mM TRIS, 89 mM borate, 1 mM EDTA, pH 8.3) and subjected to electrophoresis at 10 V/cm. Gels were transferred onto filter paper, dried and autoradiographed on Agfa Curix RP1 film under DuPont Cronex intensifying screens at \(-70^\circ\)C. Scatchard analysis of densitometer scans of binding assays was performed, as previously described (Kukolj and DuBow 1991), to determine the apparent \(K_d\) values for the different operators.

Construction of the general-purpose vector pBRND

The pBRND vector, a pUC119 derivative containing tandem copies of pBR322 segments flanking a central EcoRI site, resembles a previously described plasmid (Shuey and Parker 1986) and was constructed by a multistep procedure. The EcoRI site in the host plasmid pUC119 (Vieira and Messing 1987) was first removed by digesting with EcoRI, backfilling the cohesive ends, and ligating the blunt-ended products to produce pUC119-RI. The two inserts for this vector were obtained from pBR322 which had been separately cleaved with BamHI and Clal. These linearized plasmids were backfilled using dNTPs and Klenow fragment, and EcoRI linkers (5'-CCGAATTCGG-3'), radioactively labeled at their 5' ends, were ligated to the blunt-ended BamHI and Clal sites. The BamHI-linearized pBR322, containing EcoRI linkers, was then digested with HindIII and EcoRI to produce a 355-bp DNA fragment.