fragments, 800 to 1300 base pairs in length, were isolated after agarose gel electrophoresis. Fragments were ligated into the plasmid pUC9. Recombinant plasmids were transfected into the *Escherichia coli* strain JM83. Colonies were transferred to nitrocellulose and probed with radiolabeled oligonucleotide (*9*). The filters were hybridized with the probe at 42°C for 18 hours in 4× Denhardt's (0.08 percent polyvinyl pyrolidone, 0.08 percent Ficoll and 0.08 percent bovine serum albumin), 2× standard saline citrate (SSC), and 0.1 percent sodium dodecyl sulfate (SDS). The filters were washed with 0.5× SSC and 0.2 percent SDS at 37°C. A positive colony (pPv1) was detected by autoradiography.

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15. Monoclonal antibodies 219c and 427 were derived from Balb/c mice immunized with sporozoites of the ONG strain of *P. vivax* from Vietnam and react with *P. vivax* sporozoites from North Korea, Thailand, and Colombia. Antibody 219c gave a circumsporozoite precipitin test with *P. vivax* sporozoites and identified proteins of 46 to 57 kD in an immunoblot assay with SDS-solubilized *P. vivax* sporozoites. Immulon II plates (Dynatech) were coated with purified 219c. Serial tenfold dilutions of synthetic peptide (*14*) from 100 μg/ml to 10 ng/ml were incubated with the antibodies. After overnight incubation, the plates were washed and incubated with horseradish peroxidase-labeled antibody 219c for 2 hours. Substrate was then added to determine the amount of enzyme-linked antibody bound. There was a strongly positive reaction down to 100 ng/ml of peptide with the monoclonal antibody to *P. vivax*, 219c. As a control for specificity, a monoclonal antibody to the *P. falciparum* sporozoite was coated on the plate. No *P. vivax* synthetic peptide bound to the *P. falciparum* antibody.
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Systematic Variation in DNA Length Yields Highly Ordered Repressor-Operator Cocrystals

Abstract. *Crystals have been grown that contain the operator-binding domain of the λ repressor and the λ operator site O_L1. Crystallization conditions were tested with a set of DNA fragments, ranging in length from 17 to 23 base pairs. The best crystals were grown with a 20–base pair DNA fragment. These crystals have space-group symmetry $P2_1$, with unit cell dimensions* a = *37.1 Å*, b = *68.8 Å*, c = *56.8 Å*, *and a* β *angle of 91.5°. They diffracted to at least 2.5 Å resolution. High resolution data from these crystals should allow the direct determination of how a repressor recognizes its operator site.*

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The crystal structures of four gene-regulatory proteins—the lambda cro protein, the NH_2-terminal domain of the λ repressor, and the CAP protein and the *trp* repressor of *Escherichia coli*—have been reported (*1–4*). Each of these proteins binds to its operator site(s) as a dimer and forms a complex that is approximately twofold symmetric. Each protein has a conserved helix-turn-helix motif (*5, 6*). Model-building studies suggest that the second helix of this bihelical unit fits into the major groove of right-handed B-form DNA and makes site-specific contacts with the base pairs (*7–9*). Genetic and biochemical studies are generally consistent with the proposed models (*10*). In the case of λ repressor, mutations that disrupt binding to DNA have been identified at each of the residues that is presumed to make contacts in the major groove (*11, 12*). These models are also supported by analysis, at 7-Å resolution, of cocrystals that contain the NH_2-terminal domain of a related phage repressor, the 434 repressor, and its 14–base pair operator site (*13*). Further work on these crystals is in progress (*13*), but the crystals do not appear to be well ordered at high resolution (*14*).

It is necessary to have cocrystals that diffract to high resolution in order to understand the detailed molecular basis of protein-DNA recognition and to detect any conformational changes that may occur as the complexes form. Such cocrystals have been reported for the Eco RI restriction enzyme and its recognition site, and preliminary analysis has revealed conformational changes in the DNA (*15*). We attempted to crystallize a λ operator site with the NH_2-terminal fragment (residues 1–92) of λ repressor. The protein fragment was produced by cleaving the repressor with papain and was purified by methods similar to those reported previously (*16, 17*). The first DNA used for cocrystallization was a 17–base pair duplex containing the sequence found in the operator site O_L1 (Fig. 1). In spite of a thorough search of crystallization conditions, we were unable to grow useful cocrystals with this DNA fragment, and we began to systematically test the effect of DNA length on crystallization.

DNA fragments often stack when DNA (*18*) or protein-DNA complexes (*14, 15*) are crystallized, and, consequently, we expected that the length of the DNA would be a critical variable in the search for cocrystals. When DNA fragments stack to form a continuous helix, changing the length of the DNA alters the distance between neighboring complexes along the helix and also changes the azimuthal relationship of neighboring protein molecules around the double-helical axis. Such changes should have major effects on the crystallization. To continue our search for suitable cocrystals, we synthesized the DNA fragments shown in Fig. 1. Each contains the 17–base pair λ operator site O_L1, which is the highest affinity site for repressor binding (*19*). However, the length of the fragments and, concomitantly, the nature of the ends were varied. Most of the fragments had one or two unpaired bases on each end, and these were chosen to allow base-pairing with a neighboring fragment in the cocrystals. We expected that base-pairing between the ends might accentuate the natural tendency of the DNA to stack and might provide very stable contacts between the ends of the helices.

Crystallization conditions were screened by the hanging drop vapor diffusion method (*20*), and we used DNA that had been purified by gel electrophoresis (*21*). Each duplex was tested with polyethylene glycol (PEG) 400 and PEG 4000, with organic solvents, with several polycations, and with concentrated salt solutions. Crystals were obtained under widely different conditions and with a number of the DNA fragments. Three fragments gave crystals large enough for us to take diffraction photographs: cocrystals with the 18-nucleotide-long fragment (18-mer) were grown by adding 10 m*M* $CaCl_2$, cocrystals with the 20-mer were grown from 20 percent PEG 400, and cocrystals with the 22-mer were grown by adding 15 m*M* $Co(NH_3)_6Cl_3$.

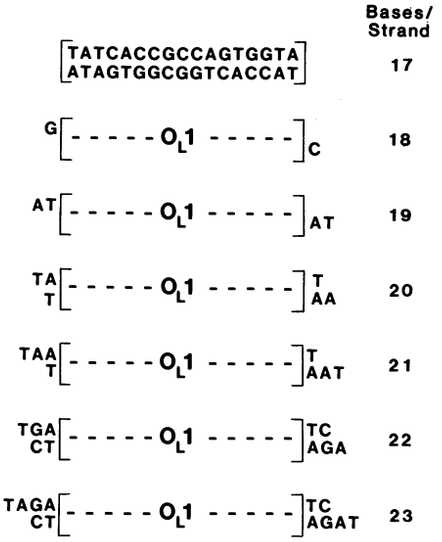


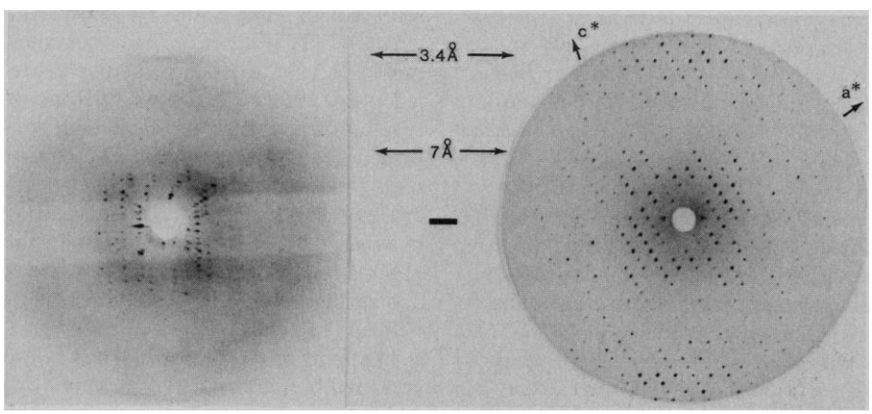
Fig. 1. DNA duplexes used for cocrystallization. The 17–base pair duplex (*26*) has the same sequence as the operator site O_L1, which is the best repressor-binding site in bacteriophage λ (*19*). This sequence is included in every one of the larger fragments. All other DNA fragments were synthesized on an Applied Biosystems 380A synthesizer, by means of the phosphoramidite method (*27*), starting with approximately 10 μmol of the 3′ base. The purity of the DNA is critical for cocrystallization. Each strand was purified once on reversed phase HPLC with the dimethoxytrityl group attached, the protecting group was removed, and the strand was purified by gel electrophoresis and again by reversed phase HPLC.

These first crystals were too disordered to be useful, but the 20-mer and 22-mer were synthesized again. This time each strand was carefully purified by both reversed phase high-performance liquid chromatography (HPLC) and by gel electrophoresis. Co-crystals containing the 22-mer grew readily, but diffraction photographs revealed that these crystals were still disordered. At high resolution, they only gave a fiber diffraction pattern (Fig. 2).

Excellent cocrystals were obtained with the 20–base pair operator site. The crystals have symmetry of the monoclinic space group $P2_1$, with unit cell dimensions of a = 37.1 Å, b = 68.8 Å, and c = 56.8 Å and a β angle of 91.5°; the crystals diffract to at least 2.5 Å resolution. An x-ray diffraction photograph is shown in Fig. 2. Crystals grow most readily from solutions containing a molar excess of DNA. However, analysis of carefully washed crystals on polyacrylamide gels (*21*) demonstrates that we have specifically crystallized the repressor-operator complex (Fig. 3), which contains one NH_2-terminal dimer per duplex. The dimensions of the protein dimer (*2*) and of the 20–base pair duplex imply that the asymmetric unit could accommodate one repressor-operator complex. This would give a V_M of 2.17 $Å^3$ per dalton, which is near the mean value (2.37 $Å^3$ per dalton) observed in a survey of protein crystals (*22*).

X-ray diffraction photographs showed that the DNA is in the ac plane, with the double-helical axis lying along the shorter diagonal through this plane. This diagonal had a length of 67 Å, indicating that the rise per base pair is 3.35 Å. The strong diffraction, corresponding to a spacing of approximately 3.4 Å, indicates that the operator site maintains a B-form structure in the complex. Also, the absence of diffuse scattering in this region showed that the DNA is very well-ordered within the crystal. If the single-stranded ends of the DNA form a base pair with the next fragment as expected, there is an average of 10.0 base pairs per turn for this DNA fragment.

Structural analysis of this cocrystal should answer a number of important questions about repressor-operator interactions, including many questions



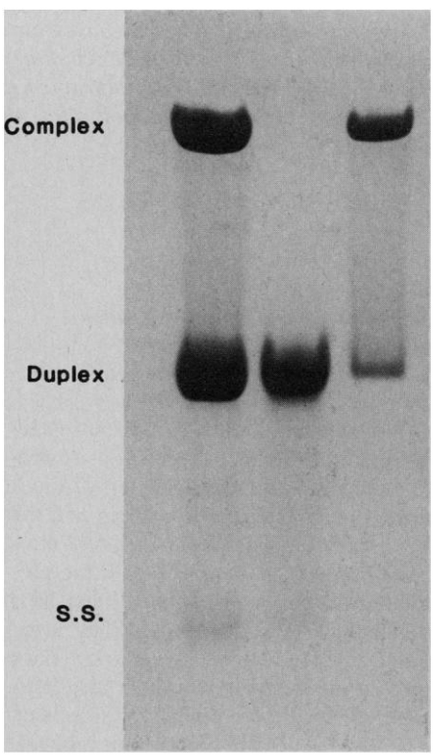


Fig. 2 (above). (Left) Diffraction from cocrystals containing the NH_2-terminal fragment of repressor and the 22-mer. No discrete reflections were present beyond 7-Å resolution, but an underlying fiber diffraction pattern suggests that the DNA was partially aligned. (Right) Diffraction from crystals containing the NH_2-terminal fragment of repressor and the 20–base pair operator site. This 15° precession photograph shows the h01 zone. The strong set of reflections near 3.4 Å is due to scattering from the double-helical B-form DNA. Fig. 3 (right). Analysis of crystallization sample and crystals by gel electrophoresis. The electrophoresis buffer consisted of 89 m*M* tris, 89 m*M* boric acid, and 2 m*M* EDTA (*p*H 8.0); the DNA was stained with methylene blue. The left lane shows the protein-DNA mixture used for crystallization, which contained the duplex (12.0 mg/ml; 0.91 m*M*), the NH_2-terminal fragment (9.2 mg/ml; 0.91 m*M* in protein monomer), 15 m*M* *bis*-tris propane-HCl (*p*H 7.0), and 1 m*M* NaN_3. Since this crystallization sample contained two duplexes for each NH_2-terminal dimer, half of the DNA was present as a complex and half as the duplex. [In this lane and the next lane, a trace of single-stranded (S.S.) DNA can also be seen at the bottom of the gel.] Crystals were grown at 20°C with the hanging-drop vapor diffusion method (*20*). Typically, 2 μl of the protein-DNA sample were mixed with 2 μl of 20 percent (v/v) of PEG 400 and set over a well containing 20 percent PEG 400. Crystals grew in 3 to 4 weeks. Seeding drops with small crystals allowed large crystals to be grown very rapidly and reproducibly. The middle lane shows the material remaining in solution after a crystal had grown. The drop had been depleted of complex, and only the free DNA remained. The right lane has a carefully washed crystal, which consisted primarily of the repressor-operator complex.

that cannot be answered with less-ordered cocrystals. It should be possible to determine: (i) the precise contacts made by the repressor, including contacts made by the flexible NH_2-terminal arm and contacts that might be made via bridging water molecules; (ii) whether the conformation of the protein changes as it binds to the operator; (iii) whether the DNA bends or twists significantly; and (iv) how the local structure of the DNA differs from that of a uniform B-DNA fiber (*23, 24*). Because the protein structure was determined previously and a detailed model for the complex was proposed (*8*), this study should also help evaluate the reliability of model-building and the importance of cocrystals for future studies of protein-DNA interactions.

Although it is tedious, systematic screening of crystallization conditions with helices of different lengths or with different ends may be a useful approach for cocrystallization of other proteins. Our results suggest that crystallization conditions and crystal quality can vary drastically as the length of the duplex and the nature of the ends are changed. Thus the 20-mer gave excellent cocrystals when precipitated with 20 percent PEG 400, but the best conditions with the 21-mer (100 m*M* $MgCl_2$ and 9 percent PEG 400) only gave microcrystals. Cocrystals containing the 22-mer grew quite readily from 15 m*M* $Co(NH_3)_6Cl_3$, but these were poorly ordered.

Is it possible to predict which length of DNA will be optimal for cocrystallization? Anderson, Ptashne, and Harrison predicted that a 21-mer would be particularly useful for cocrystallization (*14*). They assumed (i) that duplexes will stack to form a pseudocontinuous helix, (ii) that DNA fragments, like DNA fragments in solution, will have 10.5 base pairs per helical turn (*25*), and (iii) that a small repeating unit is desirable. Presumably our use of self-complementary ends increases the chances of forming a pseudocontinuous helix in the crystal. If this occurs, there will be exactly two helical turns as the DNA traverses our unit cell, but a 20-mer, rather than a 21-mer, yields the best crystals. Our experience shows that it may be necessary to try several DNA fragments with any particular protein.

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Brain-Derived Acidic Fibroblast Growth Factor: Complete Amino Acid Sequence and Homologies

Abstract. *Bovine brain-derived acidic fibroblast growth factor (aFGF) is a protein mitogen originally identified in partially purified preparations of whole brain. The protein was purified to homogeneity and shown to be a potent vascular endothelial cell mitogen in culture and angiogenic substance in vivo. The homology of aFGF to human interleukin-1β was inferred from partial sequence data. The complete amino acid sequence of aFGF has now been determined and observed to be similar to both basic FGF and interleukin-1's. A neuropeptide-like sequence, flanked by basic dipeptides, was observed within the aFGF sequence.*

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Brain has been recognized as a plentiful source of mitogenic activity for primary fibroblasts for almost 50 years (*1*). Two unique fibroblast growth factors (FGF's), one with an acidic (*2*) and the other having a basic (*3*) *p*I, have been purified to homogeneity from brain. Both hypothalamus-derived endothelial cell growth factor (ECGF) and eye-derived growth factor II (EDGF II) appear to be similar, or identical, to the acidic mitogen (*4*). Brain-derived acidic fibroblast growth factor (aFGF) is purified as a pair of microheterogeneous forms, aFGF-1 and aFGF-2, differing in the presence or absence of an amino terminal hexapeptide (*5*). Their masses, as estimated from sodium dodecyl sulfate polyacrylamide gel electrophoresis, are 16,000 to 17,000 daltons and their isoelectric points are from *p*H 5 to *p*H 7 (*2*). We reported earlier the identification, purification, amino acid composition, and partial amino acid sequence of bovine aFGF and its apparent homology to human interleukin-1β (IL-1β) (*2, 5, 6*). The acidic and basic FGF's have about the same mass, bind avidly to heparin affinity columns (*3, 7*), and are active on a wide variety of types of cells (*5, 8, 9*), including vascular endothelial cells. The acidic, and probably the basic, FGF's are potent angiogenic substances in vivo