Crystallization and X-ray Diffraction Studies of a 434 Cro–DNA Complex

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Crystals have been obtained of the bacteriophage 434 Cro protein bound to a synthetic DNA operator. An analysis of the packing shows that the complexes stack end to end along crystallographic axis, forming long rods with non-crystallographic 113 screw symmetry. The average number of DNA base-pairs per turn is 10-27, which is somewhat more overwound as compared with the 434 repressor–DNA crystals of Anderson et al. Diffraction extends to 3 Å along the rod direction and to 5 Å in perpendicular directions.

Insight into how gene regulatory proteins recognize specific sequences of DNA has been gained by the crystal structure determination of several prokaryotic repressors (McKay & Steitz, 1981; Anderson et al., 1981; Pabo & Lewis, 1982; Schevitz et al., 1985). Drawing on genetic and biochemical data, models of how CAP, λ Cro and λ repressor bind to their operator sites have been proposed (Ohiendorf et al., 1982; Lewis et al., 1983; Weber & Steitz, 1984). The common feature among these models, namely that the recognition helix of the repressor interacts specifically with DNA by inserting into the major groove, was confirmed by the recent structure of a 434 repressor–DNA complex at 7 Å resolution (H L = 0.1 nm) (Anderson et al., 1985). An understanding of how DNA-binding proteins with homologous recognition helices can discriminate among potential binding sites will require the comparison of this with the structures of other complexes.

The bacteriophage 434 Cro protein and repressor act to regulate tightly the developmental switch between the lytic and lysogenic states of the phage. This regulation is dependent upon the fact that the two proteins recognize the same six operator sites but bind to them with differential affinities. The six phage 434 operator sites bear a high degree of homology to one another and are identical in the outermost four base-pairs in 11 of the 12 operator half-sites. The amino acid sequence of 434 Cro (Grosschedl & Schwartz, 1979) contains a region homologous to the conserved helix-turn-helix motif and is > 45% identical in sequence with the DNA-binding domain of 434 repressor (Sauer et al., 1982). Despite the similarity between 434 Cro and repressor, these proteins can discriminate among binding sites that also bear a high degree of homology to one another.

We sought to obtain crystals of a complex of 434 Cro bound to the identical operator site as in the 434–R1 complex. Since 434 Cro and repressor recognize the same operator sites but discriminate among them differently, a comparison of the two complexes will clarify the determinants of DNA sequence-specific recognition. We report here the crystallization of 434 Cro complexed with a 14 base-pair synthetic operator and present an analysis of the crystal packing.

The 434 Cro repressor is a highly basic protein of 71 amino acids (Grosschedl & Schwartz, 1979; our unpublished results) with a molecular weight of 7900. It can be isolated to 85% homogeneity from bacterial strains engineered to produce Cro as 1 to 2% of total cellular protein (R. P. Wharton, C. W. & M. Ptashne, unpublished results). The 14 base-pair synthetic operator 5′-HO-d(A-C-A-A-T-A-T-A- T-A-T-G-T)-OH 3′ was purchased from P-L Biochem. and used without further purification. It was chosen as a consensus sequence among the six 434 operators and differs at one base from the naturally occurring operator, 5′-O2, in which A replaces T at position 7 (Wharton, 1985). The DNA was mixed with protein in a molar ratio of two Cro monomers per double-stranded DNA operator in 10 mM-Tris·HCl (pH 8), 1 mM-EDTA, 0.1 mM-mercaptoethanol, 0.7 mM-NaCl. The final concentration of complex used successfully in crystallization attempts was 1 to 2 mM.

Crystals were grown at room temperature by the method of hanging drop vapor diffusion (McPherson, 1982), in which the complex solution was combined with well solution at a ratio of 2:1 or 3:1. The largest crystals grow in 36% (w/v) ammonium sulfate, 10 mM-cobalt hexamine, 1 mM-MgCl₂, reaching their final size in one to three weeks. They grow as needles 1 to 2 mm in length and up to 0.2 mm thick. Crystal growth is nucleated by introducing a deformation into the hanging drop. It is at this deformation only that crystals nucleate and grow towards the center of the drop. Suitable crystals can be grown under other conditions without nucleation but not as reproducibly. Analysis of dissolved crystals on polyacrylamide gels indicates a stoichiometry of one 434 Cro dimer per 14 base-pair double-stranded operator (data not shown).

Precession photographs show that the complex crystallizes in the face-centered, monoclinic space group C2, with unit cell dimensions a = 351.8 Å, b = 78.2 Å, c = 129.1 Å and β = 129.4°. Diffraction from these crystals is quite anisotropic, extending to 3.0 Å roughly along the c* direction and to 5.0 Å
in perpendicular directions. We account for this anisotropy, as well as other striking features of the diffraction pattern, in the packing model presented below. The analysis is similar to that described by Anderson et al. (1985).

The Cro–DNA complexes, each consisting of a 434 Cro dimer bound to a double-stranded 14 base-pair operator with a center of 2-fold symmetry, stack end-to-end in the crystal to form long rods with local 113 symmetry. (Thus, the symmetry operations describing the relationship between complexes is a rotation of 360/11 degrees and a translation along the helix axis of 3/11 a (helical repeat distance).) These rods are normal to the crystallographic b axis and lie parallel to the (5 0 -1) planes (Fig. 1). The base-pair interactions at the junctions between complexes are assumed to preserve the continuity of the path of the DNA double helix. Along the (1 0 5) axis, a distance of 502 Å, there are precisely 11 stacked complexes which comprise a single repeat of the non-crystallographic screw. Each turn of the screw contains 2.75 complexes; thus, there is a true repeat of the screw only after four turns containing 11 complexes. Each repeat of the 113 screw contains 154 base-pairs, which form 15 Turns of the pseudocontinuous DNA helix, with 10-27 base-pairs per turn and an average rise per base-pair of 3.96 Å. There is half a repeat of the 113 screw, consisting of five 1/2 complexes, in the (0 2) asymmetric unit. The rods of 434 Cro–DNA complexes extend unbroken over many unit cells. Adjacent rods are packed in a pseudohexagonal array. At the origin of each unit cell, a complex lies with its 2-fold axis superimposed upon the crystallographic dyad. We outline below the observations from which we have deduced this packing model.

Diffraction photographs reveal the presence of a region of diffuse diffraction at about 3-4 Å in the direction of the highest resolution diffraction. When the crystal is tilted to bring this region into reflecting position, a series of diffuse meridional and off-meridional layer-lines is observed in a pattern characteristic of fiber diffraction from a helix. Strong crystalline reflections in this region show that the fiber repeat is incorporated into the crystal lattice. The most intense layer-line occurs at 2.26 Å, and the rise per helical repeat, measured from the spacing between meridional layer-lines, is 45-6 Å. As in the crystals of the R -60 DNA complex (Anderson et al., 1984), the diffuse diffraction arises from the presence of Cro–DNA complexes stacked end-to-end, with the base-pair stacking between adjacent complexes constituting an important packing interaction. A repeat unit consisting of a complex of a Cro dimer with a 14 base-pair operator having an average rise per base-pair of 3.26 Å (as determined from the most intense meridional layer line) will have the observed helical repeat of 45-6 Å. The positions of the off-meridional layer-lines indicate an approximate 3, screw symmetry of the rods, but the actual symmetry deviates somewhat from this.

The crystallographic direction along which the rods lie can be determined from still and oscillation photographs to be approximately 7° from c*. The direction of the rod axis was determined more precisely from the position of spikes of intensity in zero layer precession photographs. The (0 k l) zone shows strong spikes perpendicular to the (5 0 -1) planes (Fig 2) and the (0 k l) zone shows intense spikes perpendicular to the (0 0 0) planes (not shown). From these features of the diffraction pattern we deduce that the helical rods of Cro–DNA complexes lie in the a, c plane along the crystallographic (1 0 5) direction. Sets of planes formed by adjacent rods would be expected to give rise to intense reflections normal to these planes.

The crystallographic repeat along the (1 0 5) axis is 502 Å. Given the rise per helical repeat of 45-6 Å deduced from the fiber-like diffraction, precisely 11 Cro–DNA complexes can lie stacked end-to-end along this direction. Assuming that the oligomers in the complexes stack such that the path of the DNA helix is continuous over many repeats, an 113 non-crystallographic screw axis generates a helix containing 15 turns of DNA at 10-27 base-pairs per turn. This value is consistent with those
observed for \( \text{B-DNA} \) (Dickerson et al., 1982). Other screw symmetries which repeat after 11 complexes are not ruled out on grounds of crystallographic symmetry, but give values for the average twist per base-pair which deviate significantly from \( \text{B-DNA} \).

Since crystallographic studies of the homologous \( \text{434} \) repressor complex indicate that the DNA remains essentially \( \text{B-form} \) when bound by repressor (Anderson et al., 1985), we have not considered models that yielded very different twists in the DNA.

The appearance in the diffuse, fiber-like diffraction of a pattern characteristic of a \( 3_1 \) screw can be accounted for by the fact that \( 11_3 \) symmetry represents a moderate deviation from \( 3_1 \) symmetry. The angular spacing between adjacent complexes in the \( 434 \) Cro-DNA helix is 130°, compared with the 120° spacing expected in a 3, helix. The rise per complex is the same in the two cases, and so is the predicted spacing between meridional layer-lines. An \( 11_3 \) helix is predicted to give rise to ten off-meridional layer-lines, but since only those arising from lower-order Bessel terms would appear in the fiber diffraction, observable diffraction is expected only on four layer-lines: numbers 3, 4, 7 and 8. Layer-lines 3 and 4 are separated by 1/502 Å\(^{-1}\) and hence are not expected to be resolved; the same holds true for layer-lines 7 and 8. The positions of the unresolved pairs of off-meridional layer-lines are nearly coincident with those expected for a \( 3_1 \) screw with an identical rise per base-pair, and hence the overall pattern of fiber diffraction mimics that of a \( 3_1 \) helix.

Our packing model also accounts for the third along \( c^* \) seen in projection pictures of the \( 0k1 \) zone to a resolution of about 11 Å (not shown). A single repeat of the \( 11_3 \) screw, which spans five unit cells along the (1 0 5) direction, contains 15 turns of DNA. Viewed in projection along the crystallographic \( a \) axis, there are three turns of DNA per unit cell along the \( c^* \) direction. At low resolution, the stacked DNA oligomers should appear continuous and the three turns of DNA will form identical repeats. It appears that the DNA diffraction dominates at low resolution, hence giving rise to the thirding seen along this axis.

Each \( 434 \) Cro dimer is assumed to bind the operator with its dyad coincident with the operator dyad. The \( 11_3 \) helix then has a dyad located at the center of every complex, perpendicular to the helix axis, and hence has overall 2-fold symmetry. The rods of Cro-DNA complexes can thus be packed with a rod dyad coincident with the crystallographic dyad axis, \( b \). This gives rise to the arrangement shown in Figure 1, in which the \( a \) axis in each unit cell is intersected by five rods with a center-to-center spacing of 70 Å along \( a \). Symmetry considerations also permit an alternative packing in which the crystallographic dyad is not coincident with a rod dyad, giving rise to twice the number of complexes in the unit cell. In this arrangement, ten rods intersect the \( a \) axis in each unit cell with an average spacing along \( a \) of 35 Å and a spacing along \( b \) of 39 Å. From the structure of the homologous \( R_{1769} \)-DNA complex, the dimension of a complex along its dyad is expected to be at least 42 Å. Complexes, therefore, may not lie with their dyads nearly along \( a \) and must be interdigitated in such a way that overlap with neighboring complexes does not occur. Given the rod symmetry described, no such arrangement of complexes with these dimensions can be accommodated in the unit cell. These packing considerations therefore rule out the alternative model.

In addition to the dyad of the complex, there is also a dyad at the junction of between complexes that is normal to the rod axis. In the packing model shown, the dyad of the complex is coincident with the crystallographic dyad. The packing obtained when the inter-complex dyad is instead coincident with the crystallographic dyad is identical except for a shift in origin. There is only one ambiguity: the rods could be packed as shown, or with a 180° rotation about the rod axis.

The anisotropic diffraction from the crystals is explained by our packing scheme. Along the rods, strong interactions due to base-pair stacking result in a high degree of order in this direction and hence high resolution diffraction. The packing interactions provided by protein-protein contacts perpendicular to the rods may be weaker, permitting rotational disorder of the rods and limiting diffraction in those directions. The packing is also consistent with the observed needle-like habit of the crystals, the preferred direction of growth coinciding with the direction along which the DNA stacking occurs.

We have obtained crystals of \( 434 \) Cro bound to a 14 base-pair operator and described the packing in which adjacent complexes stack end-to-end to form pseudocontinuous helices in the crystal. A similar
end-to-end stacking of complexes was found in the crystals of a 434 repressor fragment bound to the identical DNA operator, but the overall angular separation between adjacent complexes is different in the two cases. The average number of base-pairs per turn in the R1-69-DNA crystals is 10.5, whereas in the Cro-DNA crystals described here it is 10.27 base-pairs per turn. It is possible that upon binding Cro the DNA adopts a conformation that differs slightly from the one assumed when it binds the repressor fragment. The details of DNA conformation have indeed been shown to be significant for specificity of Cro and R1-69 binding (Konidelka & Ptashne, unpublished results; Anderson, Harrison & Ptashne, unpublished results). We expect that determination of the crystal structure of 434 Cro bound to its operator will reveal the details of the interaction and its effect upon DNA conformation.

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