02.X-11 STRUCTURAL STUDIES OF THE CRO REPRESSOR PROTEIN FROM BACTERIOPHAGE λ.

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The Cro protein from bacteriophage λ is a repressor molecule which recognizes and tightly binds certain sequence-specific operator regions within the DNA of the bacteriophage. Cro is the smallest repressor protein that has been characterized (MW 7351 daltons), and is of interest both because of its role in the delicately balanced regulatory system of bacteriophage λ, and also as a general model for protein-DNA interactions.

Crystallographic analysis of a rhombohedral crystal form has shown that each asymmetric unit contains four Cro monomers arranged with approximate 222 point symmetry. The structure of the protein was determined from an electron density map phased to a nominal resolution of 2.8 Å with five heavy-atom derivatives and averaged over the four monomers.

In the crystal the four monomers cluster together around the local intersecting two-fold symmetry axes, suggesting that the protein may retain this tetrameric arrangement in solution. Pairs of dyad-related α-helices protrude from the surfaces of adjacent cro monomers in such a manner that they can be accommodated within two successive major grooves of the DNA, suggesting that this is the probable mode of interaction between the protein and DNA. This model for the binding of Cro to its operator DNA, in which the nucleic acid retains its normal right-handed B conformation, is supported by both chemical and genetic evidence. Efforts are underway to improve the accuracy of the structure by using the local symmetry of the four monomers, and by crystallographic refinement, in order to better understand the detailed interactions between protein and nucleic acid.

02.X-12 STRUCTURE OF CATABOLITE GENE ACTIVATOR PROTEIN AT 2.9Å RESOLUTION SUGGESTS BINDING TO LEFT HANDED B-DNA.

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From a 2.9Å resolution crystal structure of E. coli catabolite gene activator protein (CAP) complexed with cyclic AMP (cAMP) and from model building studies we conclude that CAP may bind to left-handed B-type DNA, contacting its major groove via two α-helices. Furthermore, we suggest that the conversion of right- to left-handed DNA in a closed supercoil resulting from CAP binding facilitates in some way the opening of the promotor and thus activates transcription by RNA polymerase. The CAP subunit has two distinct structural domains separated by a cleft. The smaller carboxy-terminal domain is presumed to bind DNA while the amino-terminal domain is seen to bind cAMP inside an eight-strand antiparallel "β-roll" structure. The CAP dimer is notably asymmetric; in one subunit, the cleft between domains is "open", while in the other subunit it is "closed". That is, the relative orientation of the two domains is different in the two subunits with the result that there is not a unique two-fold axis relating the two subunits.

The crystals of CAP are orthorhombic, space group $P2_12_12_1$, a=46.5Å, b=97.1Å, c=105.4Å, with one dimeric CAP molecule per asymmetric unit. Phases were computed for the 75% of the most intense reflections and had an average figure of merit of 0.74.