Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization of the N-terminal domain of the *Escherichia coli* regulatory protein TyrR

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Received 3 May 1999
Accepted 16 August 1999

The N-terminal domain of the regulatory protein TyrR from *Escherichia coli* forms a dimer in solution and has been purified and crystallized. The crystals belong to space group $C2$ with unit-cell parameters a = 134.5, b = 72.1, c = 96.7 Å, β = 98.5°. The crystals diffract to 2.8 Å. Assuming a molecular weight of 23219 Da, a V_m of 2.5 Å^3 Da^{-1} is obtained for two dimers in the asymmetric unit.

1. Introduction

The TyrR protein plays a major role in the genetic regulation of the biosynthesis and uptake of aromatic amino acids in *Escherichia coli*. It regulates the initiation of transcription of eight operons, acting in some cases as a repressor and in others as an activator. Its regulatory activity occurs in response to intracellular levels of tyrosine, phenylalanine and tryptophan (Pittard & Davidson, 1991; Pittard, 1997). The activation function involves a specific interaction between DNA-bound TyrR and the α-subunit of the initiating RNA polymerase. Amino acids in TyrR involved in this interaction have been identified by mutagenesis experiments (Yang *et al.*, 1996, 1997).

TyrR is a 114 kDa homodimer with two binding sites for aromatic amino acids (Argaet *et al.*, 1994; Wilson *et al.*, 1995). One site is ATP-dependent and binds tyrosine with high affinity; the other is ATP-independent and binds either phenylalanine (K_d = 300 mM) or tryptophan (K_d = 1 mM), but not tyrosine.

The protein appears to contain three structural domains (Cui & Somerville, 1993; Kwok, 1998). The C-terminal domain contains operator-recognition elements, while the central domain is known to bind ATP. The N-terminal domain (amino-acid residues 1–190), which is the subject of this communication, contains the following structural features: (i) sites for interaction with the α subunit of RNA polymerase (Yang *et al.*, 1996, 1997), (ii) the ATP-independent aromatic amino-acid binding site (Kwok, 1998) and (iii) determinants which cause the domain to dimerize in solution (Kwok, 1998). The binding of phenylalanine or tryptophan to the ATP-independent aromatic amino-acid binding site is thought to be essential for TyrR-mediated activation of transcription initiation at high intracellular concentrations of these two amino acids.

Our aim is to determine the three-dimensional structure of TyrR in order to provide a basis for understanding its function at the molecular level. As part of this program, we have expressed and characterized each of the TyrR domains (Kwok, 1998; M. Dixon & B. E. Davidson, unpublished results). The N-terminal domain was expressed with a C-terminal His_6 tag and purified with a metal-affinity column (Kwok *et al.*, in preparation). Previous studies (Kwok, 1998) have established that this domain is a dimer in solution. We describe below the crystallization of the N-terminal domain and the results of a preliminary X-ray analysis of the crystals which yielded the space group and the unit-cell parameters.

2. Materials and methods

2.1. Expression and purification of TyrR(1–190)::His

A culture of *E. coli* BL21(DE3) cells containing the plasmid pMU5803 was used to express a protein containing amino-acid residues 1–190 of TyrR with a C-terminal extension KLAAALGHHHHHH. The expressed protein, which we refer to as TyrR(1–190)::His, was purified to homogeneity and used for crystallization experiments. Details of pMU5803 together with the expression and purification of TyrR(1–190)::His are described elsewhere (Kwok *et al.*, in preparation).

2.2. Crystallization

Crystals were initially grown by vapour-diffusion techniques using the Hampton Crystal Screen II at both 277 and 291 K. Crystallization solutions were prepared by mixing 2 µl of protein solution with 2 µl of reservoir solution in Crystal Clear Strips (Hampton Research). The crystals produced at the lower temperature were superior to the others and all subsequent work was performed at the lower temperature. Optimal conditions for crystallization were obtained from a solution produced by mixing 4 µl of 14 mg ml^{-1} TyrR(1–190)::His with 4 µl of a reservoir solution consisting of 0.31 M ammonium

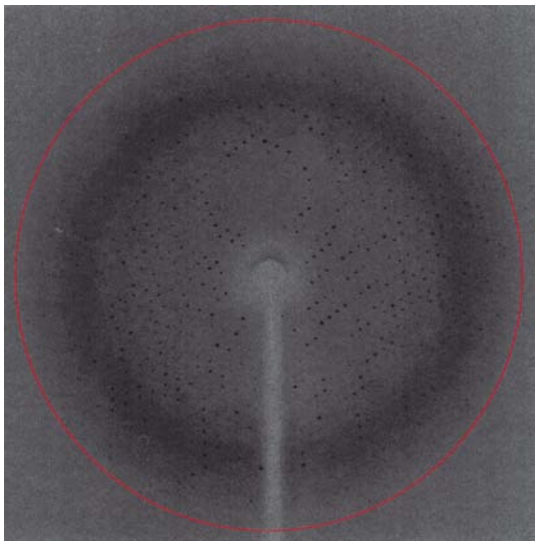

Figure 1
X-ray diffraction image from a 2° oscillation of the crystal. The red line indicates the 2.8 Å diffraction limit.

sulfate, 33% PEG–MME (polyethylene glycol monomethyl ether) 5000 and 0.1 *M* MES pH 6.3. This solution was suspended from a cover slip placed over the well of a Linbro multiwell tissue-culture plate which contained 500 µl of reservoir solution. The crystals showed extreme sensitivity to temperature and movement while growing. Crystals took three weeks to grow to full size. Small intergrown crystals resulted when trays were disturbed before this time.

2.3. Data collection and analysis

X-ray data were collected with an R-AXIS IIC detector mounted on a Rigaku generator producing Cu $K\alpha$ radiation at a power of 5 kW (50 kV, 100 mA). The data were processed using the program *HKL* (Otwinowski & Minor, 1997). Data were collected from two crystals. The first, at a temperature of 277 K, was mounted directly from its mother liquor into a standard quartz glass capillary. The second was soaked in a cryobuffer containing 20% PEG–MME 5000, 17.5% DMSO, 0.2 *M* ammonium sulfate and 0.1 *M* MES pH 6.3. This second crystal was flash-cooled in a nitrogen-gas stream at 100 K using the modifications to a Rigaku R-AXIS IIC detector described by Carr *et al.* (1996). Fig. 1 shows a 2° oscillation image obtained from the second crystal.

3. Results and discussion

Crystals could be reproducibly grown to dimensions of 0.3 × 0.2 × 0.08 mm and used for data collection. The crystal maintained at 277 K suffered substantial radiation damage during the course of X-ray exposure. A data set of 12459 observations with 6898 unique reflections was 57.2% complete to 3.5 Å with $R_{\rm merge} = 0.129$ and $I/\sigma(I) = 6.4$ in space group *C*2. Post-refined unit-cell parameters were $a = 135.3$, $b = 73.5$, $c = 98.6$ Å, $\beta = 99.12^\circ$. The low-temperature data set comprised of 56376 observations of 20324 unique reflections and was 91.9% complete to 2.8 Å with $R_{\rm merge} = 0.053$ and $I/\sigma(I) = 20.5$. Post-refined unit-cell parameters were $a = 134.5$, $b = 72.1$, $c = 96.7$ Å, $\beta = 98.5^\circ$. A value for the Matthews coefficient (Matthews, 1968) of 2.5 Å^3 Da^{-1} was obtained assuming two dimers in the asymmetric unit and a molecular weight of 23219 Da for each subunit. An attempt to solve the structure of this protein using the multiple isomorphous replacement technique is under way. An initial good mercurial derivative has been obtained.

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