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# Crystallization and preliminary X-ray analysis of the Tet-repressor/operator complex

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**Abstract**

Three crystal forms of the repressor protein TetR class D in complex with the palindromic 17 bp operator sequence containing T overhangs on both sides were obtained by hanging-drop vapor-diffusion methods using PEG 4000 and PEG monomethylether 5000 as precipitants. Although the crystallization conditions were very similar, up to three different crystal forms were observed in the same drop. The space groups are monoclinic $C2$, $P2_1$ and hexagonal $P6_122$. The asymmetric units of the latter two crystal forms contain one repressor–operator complex. The crystal structures of these forms were solved by molecular replacement using the Tet-repressor molecule of the complex with tetracycline as a search model.

## 1. Introduction

The most common mechanism of tetracycline (Tc) resistance in Gram-negative bacteria is associated with the protein TetA located in the cytoplasmic membrane which actively exports the antibiotic before it can harm the bacterium. This protein is encoded on mobile genetic elements, its expression being regulated by the inducible repressor TetR. Two antiparallel oriented operators O1 and O2 precede the genes encoding for TetA and TetR, respectively (Hillen, Gatz, Altschmied, Schollmeier & Meier, 1983). TetR is found in seven forms characterized thus far called classes A to E, G and H with 45–78% amino-acid sequence identity (Schnappinger & Hillen, 1996). TetR binds to the operators and inhibits expression of the two genes. If Tc enters the cell, it forms a complex with TetR and induces a conformational change, so that the operators are released. Expression is now turned on, leading to production of TetA and resistance of the cell against Tc.

X-ray crystal structures of TetR (Orth *et al.*, in preparation) and of the TetR–Tc complex (Hinrichs *et al.*, 1994; Kisker, Hinrichs, Tovar, Hillen & Saenger, 1995) showed TetR to occur as a homodimer with helix–turn–helix (HTH) motifs. In the induced form of TetR (in complex with Tc), the DNA binding $\alpha$-helices of the HTH motifs are separated by 39 Å, too far to be able to bind to the operator DNA with a separation of 34 Å

| DNA length | Code | Sequence |
|---|---|---|
| 22 | TA | T C A C T C T A T C A T T G A T A G G G A C<br>G T G A G A T A G T A A C T A T C C C T G A |
| 21 | AT | T T A C T C T A T C A T T G A T A G G G A<br>A T G A G A T A G T A A C T A T C C C T A |
| 21 | TT | T A C T C T A T C A T T G A T A G G G A T<br>T A T G A G A T A G T A A C T A T C C C T |
| 21 | blunt | C A C T C T A T C A T T G A T A G G G A G<br>G T G A G A T A G T A A C T A T C C C T C |
| 18 | TT | C T C T A T C A T T G A T A G G G T<br>T G A G A T A G T A A C T A T C C C |
| 17 | TA | C T C T A T C A T T G A T A G G A<br>T G A G A T A G T A A C T A T C C |
| 17 | AT | A C T C T A T C A T T G A T A G G<br>G A G A T A G T A A C T A T C C T |
| 17 | blunt | C T C T A T C A T T G A T A G G G G<br>G A G A T A G T A A C T A T C C C |
| Operator | O1 | A C A C T C T A T C A T T G A T A G G G A T A<br>T G T G A G A T A G T A A C T A T C C C T A T |
| Operator | O2 | C A A C T C T A T C A A T G A T A G G G A A T<br>G T T G A G A T A G T T A C T A T C C C T T A |

Fig. 1. Operator sequences used for successful crystallization with $TetR^D$. The boxed sequences correspond to the palindromic operator sequences equal for both repressor binding sites O1 and O2 (Hillen *et al.*, 1983).

Table 1. *Conditions used for the crysallization at 291 K of $TetR^D$ with operator sequence 18 TT (Fig. 1)*

| | Form 1 (C2) | Form 2 ($P6_122$) | Form 3 ($P2_1$) |
|---|---|---|---|
| Buffer, pH 8 | 100 m*M* imidazole | 100 m*M* imidazole | 150 m*M* imidazole |
| Precipitant | 12.5% PEG MME 5K | 12.5% PEG 4K | 12.5% PEG MME 5K |
| Additives | 100 m*M* NaCl 100 m*M* $CaCl_2$ | 100 m*M* NaCl 100 m*M* $CaCl_2$ | 150 m*M* NaCl 150 m*M* $Ca(CH_3COO)_2$ |

between two successive major grooves in which the HTH $\alpha$-helices insert. Since there is a only small structural difference between uninduced TetR and the induced TetR–Tc complex, it was not possible to derive structural changes associated with induction and to explain the properties of a large number of TetR variants (Müller *et al.*, 1995). To obtain information on the tertiary structure of the DNA bound form of TetR, we cocrystallized the repressor with a 17 bp operator DNA.

## 2. Crystallization

TetR class D ($TetR^D$) was expressed, purified to homogeneity (Ettner *et al.*, 1996) and concentrated with a Centricon $M_r$ 30 kDa (AMICON) in 20 m*M* Tris–HCl pH 8 to 0.8 m*M* dimer. The concentration was measured spectrophotometrically using an extinction coefficient at 280 nm of 38 000 $M^{-1}$ $cm^{-1}$ (Kisker *et al.*, 1995). Complementary oligonucleotides with 16–22 bases per single strand and covering the 13 bp palindromic centre of operators O1 and O2 were obtained from TIB-Molbiol/Berlin, separately purified (Heinemann & Alings, 1991) and hybridized by cooling from 368 to 293 K. The DNA duplexes containing blunt ends or ends with a single overhanging base at each side were concentrated with a centricon $M_r$ = 3 kDa (AMICON) in 100 m*M* NaCl, 20 m*M* Tris–HCl pH 7.5 to 1.4 m*M*, the concentration being determined using the calculated extinction coefficient (Cantor, Warshaw & Shapiro, 1970) of 275 000 $M^{-1}$ $cm^{-1}$, for 18 TT (Fig. 1). The repressor–operator complex was formed by mixing the two components with 1:1 to 2:1 molar ratios of the oligonucleotide to the protein and incubated for 1 h at 277 K. Since the binding of TetR to operator DNA is tight, the binding constant is $5 \times 10^{11}$ $M^{-1}$ (Hansen & Hillen, 1987), complex formation could be shown by band shifts in native gel electrophoresis (data not shown).

The solutions of the $TetR^D$–DNA complexes were screened for crystallization conditions using a protein–DNA complex crystal screen (Scott *et al.*, 1995) and the hanging-drop vapour-diffusion method. 3 µl of the $TetR^D$–DNA solutions and 3 µl of the reservoirs were mixed and allowed to equilibrate with 1 ml reservoir. Crystals were obtained in 12.5% PEG 4000 or 12.5% PEG monomethylether 5000 in the presence of 200–300 m*M* salt and 100–150 m*M* imidazole pH 8. Operator sequences resulting in successful crystallization experiments of the corresponding $TetR^D$–operator complexes are listed in Fig. 1, and exact crystallization conditions are listed in Table 1. Single crystals suitable for X-ray diffraction data collection were obtained only with the 17 bp duplex with two T overhangs (18 TT).

Slight variations of the contents of the reservoir solution led to different crystal forms or to successive occurrence of these forms. In the same crystallization assay, the $TetR^D$–18 TT complex (Fig. 1) yielded triangular or parallelogram like plates after 2 weeks (form 1), hexagonal pins after 4 weeks (form 2), and rectangular plates after 9 weeks (form 3), Fig. 2. At present, it is not clear why this polymorphism is observed, but it might be the reason why complexes with other DNA sequences given in Fig. 1 could not form single crystals because of mixed crystal packing. X-ray patterns of such misgrown crystals typically show reflections only in one crystal orientation. On images with the crystal rotated 90°, no diffraction or only poor diffraction is observed, indicating that these specimens consist of stacks of thin plates. In almost all of these crystals, diffuse streaks at a distance of 3.4 Å indicate the presence and orientation of B-type DNA.

Table 2. *Crystallographic characteristics and summary of data collection*

| | Form 1 | Form 2 | Form 3 |
|---|---|---|---|
| Lattice type | Monoclinic | Hexagonal | Monoclinic |
| Space group | *C*2 | $P6_122$ | $P2_1$ |
| Cryotemperature (K) | 100 | 100 | 148 |
| Cell constants (Å, °) *a* | 297.4 | 88.34 | 50.13 |
| *b* | 153.3 | 88.34 | 49.82 |
| *c* | 362.2 | 320.46 | 96.20 |
| $\beta$ | 98.9 | | 97.00 |
| Unit-cell volume ($10^6$ $Å^3$) | 16.510 | 2.378 | 0.252 |
| Volume per asymmetric unit ($10^6$ $Å^3$) | 4.127 | 0.198 | 0.126 |
| Complex per asymmetric unit | ~22 | 1 | 1 |
| Solvent content (%) | ~60 | 61.4 | 44.3 |
| $V_m$† ($Å^3$ $Da^{-1}$) | ~3.2 | 3.19 | 2.2 |
| Resolution (Å) | 7.0 | 3.9 | 3.0 |
| No. of measured reflections | 56116 | 52364 | 37994 |
| No. of unique reflections | 25656 | 6965 | 9302 |
| $\langle I \rangle / \sigma(I)$ overall | 14.45 | 13.4 | 7.9 |
| Completeness (%) overall | 75.8 | 96.4 | 96.1 |
| $R_{merge}$ overall | 6.9 | 9.8 | 10.6 |
| Resolution range of last shell | 7.25–7.20 | 4.04–3.90 | 3.11–3.00 |
| $\langle I \rangle / \sigma(I)$ last shell | 3.16 | 2.88 | 2.92 |
| Completeness last shell | 72.5 | 76.8 | 88.2 |
| $R_{merge}$ last shell | 39.1 | 29.5 | 21.3 |

† $V_m$ crystal packing parameter (Matthews, 1968).

## 3. X-ray diffraction analysis

The three crystal forms (Table 2) belong to space groups *C*2, $P6_122$ and $P2_1$, respectively. The crystals are extremely

Fig. 2. Crystals of $TetR^D$–DNA (18 TT, Fig. 1), form 2 (hexagonal pins). The largest dimension is 0.5 mm.