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Crystallization and preliminary X-ray diffraction studies of *Streptococcus pyogenes* plasmid pSM19035-encoded ω transcriptional repressor

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The transcriptional repressor, ω protein, from the *Streptococcus pyogenes* broad-host-range plasmid pSM19035 was crystallized at pH 7.5 and 8.5 by the vapour-diffusion method using PEG 4000 as precipitant. Two crystal forms were obtained; the first belongs to the tetragonal space group $P4_12_12$ or $P4_32_12$ and the second to the hexagonal space group $P6_1$ or $P6_5$. The crystals are most likely to contain one ω protein in the asymmetric unit, with V_m values of 3.2 and 3.5 Å^3 Da^{-1}, respectively. The crystals diffract X-rays to 2.4 and 2.9 Å resolution for the tetragonal and hexagonal systems, respectively.

1. Introduction

The 71-amino-acid long ω protein has no apparent sequence identity to any other protein with known activity. It is encoded on plasmid pSM19035 from *Streptococcus pyogenes* (Ceglowski *et al.*, 1993) and forms tetramers in solution which dissociate into monomers at low protein concentration. The ω protein binds to the promoter region of genes involved in plasmid copy-number control and better than random segregation at cell division and was shown to repress transcription of three genes: (i) *copS*, which inhibits transcription of the replication-initiation protein (*rep*S; Brantl, 1994), (ii) gene δ, whose product shares homology with proteins involved in active partitioning of diverse plasmid and bacterial chromosomes (Williams & Thomas, 1992; Ceglowski *et al.*, 1993) and (iii) gene ω itself (de la Hoz *et al.*, unpublished results). The target sites of the ω protein, which overlap with the promoter region of the above genes, share a set of repeated heptameric motifs 5′-ATCACAA-3′ or 5′-ATCACTT-3′, to which ω protein binds cooperatively (de la Hoz & Alonso, unpublished). These heptamers (7–10 copies) are arranged either in a contiguous 'head-to-tail' array or in repeats of three heptameric motifs, *i.e.* two heptamers in a direct orientation and one heptamer in an inverse orientation including two overlapping base pairs (Ceglowski *et al.*, 1993). Although the binding sites of ω protein and RNA polymerase (RNAP) overlap, it was demonstrated that ω protein does not inhibit binding of RNAP to the promoter region (de la Hoz & Fernández, unpublished results). Indeed, ω protein does not interfere with the formation of RNAP closed complex, but either hinders the formation of open complex or RNAP promoter clearance (protein–protein interaction) or influences the DNA structure immediately adjacent to the promoter. At present, very little structural knowledge is available about the mechanism by which a transcriptional repressor promotes formation of a closed complex.

To understand the mechanism of cooperative DNA binding and the regulation of transcription by ω protein, we initiated the analysis of its three-dimensional structure by X-ray crystallography. Here, we describe the crystallization and preliminary X-ray diffraction analysis of the ω gene product from plasmid pSM19035.

2. Experimental and results

2.1. Crystallization

The ω protein was purified by a procedure to be published elsewhere (S. Ayora & J. C. Alonso, unpublished work). Crystallization

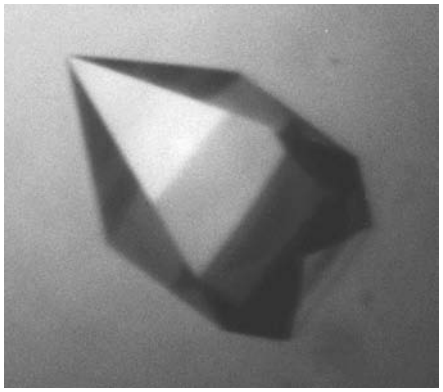

Figure 1
A tetragonal crystal of ω product from plasmid pSM19035. The crystal dimensions are approximately 0.7 × 0.4 × 0.4 mm.

Table 1
Data-collection statistics.

Values in parentheses refer to the highest resolution shell.

	Crystal 1	Crystal 2
Space group	Tetragonal $P4_12_12$ or $P4_32_12$	Hexagonal $P6_1$ or $P6_5$
Unit-cell parameters (Å)	a = 41.96; c = 118.44	a = 47.06; c = 88.60
V_m† (Å^3 Da^{-1})	3.2	3.5
Resolution limits (Å)	26.3–2.4 (2.49–2.40)	24.0–2.9 (3.0–2.9)
Measured reflections	19668 (1870)	17951 (1496)
Independent reflections	4371	2445
Completeness (%)	96.6 (93.2)	95.9 (96.5)
Mean $I/\sigma(I)$	14.8 (4.0)	6.7 (2.8)
R_{sym}‡	0.063 (0.428)	0.118 (0.365)

† Calculated assuming one molecule per asymmetric unit (Matthews, 1968). ‡ $R_{sym} = \sum_{hkl} |I_i - I_{avg}| / \sum_{hkl} \sum |I_i|$.

experiments on the ω protein were performed using the hanging-drop vapour-diffusion method at 291 K. In a typical screen experiment, 2 µl of the protein solution, containing about 20 mg ml^{-1} protein in 50 m*M* Tris–HCl buffer pH 7.5, 5% glycerol, 100 m*M* NaCl, was mixed with the same volume of the reservoir and equilibrated against 1 ml of reservoir solution. Screening was carried out with the sparse-matrix conditions described by Jancarik & Kim (1991) using a Crystal Screen kit (Hampton Research, Laguna Hills, CA, USA). When PEG 4000 was used as precipitant at pH 7–8, rod-shaped crystals appeared. Depending on the additives, the rods have tetragonal or hexagonal forms. Tetragonal crystals were obtained using 20% PEG 4000, 65 m*M* sodium acetate, 65 m*M* Tris–HCl pH 7.5, while hexagonal crystals grew from 30% PEG 4000, 200 m*M* sodium acetate, 100 m*M* Tris–HCl pH 8.5.

2.2. X-ray diffraction studies

Crystals were sealed in glass capillary tubes with small amounts of mother liquor. Diffraction data were collected on a 345 mm MAR Research imaging plate at room temperature using monochromated Cu $K\alpha$ radiation from an Enraf–Nonius rotating-anode GX21 generator. Diffraction data were integrated using *DENZO* and scaled using *SCALEPACK* (Otwinowski & Minor, 1997). The crystals belong either to the tetragonal (Fig. 1) or to the hexagonal system. The symmetry and the observed systematic extinction of the diffraction data are compatible with space group $P4_12_12$ or $P4_32_12$ (tetragonal system) or $P6_1$ or $P6_5$ (hexagonal system). Assuming that one ω-protein molecule is present in the asymmetric unit, the solvent content is 57 or 61%, respectively. The ω-protein crystals diffract X-rays to about 2.4 and 2.9 Å with a conventional X-ray source for the tetragonal and hexagonal systems, respectively. The crystals are stable at room temperature; cryogenic conditions were not necessary.

Table 1 shows that the present crystals yield diffraction data to 2.4 Å resolution and are suitable for X-ray analysis. A search for heavy-atom derivatives and the preparation of selenomethionyl ω protein are under way.

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References

Brantl, S. (1994). *Mol. Microbiol.* **14**, 473–483.
Ceglowski, P., Boitsov, A., Chai, S. & Alonso, J. C. (1993). *Gene*, **136**, 1–12.
Jancarik, J. & Kim, S.-H. (1991). *J. Appl Cryst.* **24**, 409–410.
Otwinowski, Z. & Minor, W. (1997). *Methods Enzymol.* **276**, 307–326.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Williams, D. R. & Thomas, C. M. (1992). *J. Gen. Microbiol.* **138**, 1–16.