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Crystals of the ribonucleotide reductase R2 protein from *Chlamydia trachomatis* obtained by heavy-atom co-crystallization

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Ribonucleotide reductases (RNRs) catalyse the conversion of ribonucleotides to deoxyribonucleotides, utilizing radical chemistry to carry out the reaction. Class I RNRs consist of R1 and R2 subunits: R1 contains the active site and R2 generates and stores a stable tyrosyl radical. The conserved tyrosine where the radical is stored until needed in R1 has previously been believed to be an absolute requirement for R2 activity. The *Chlamydia trachomatis* R2 lacks this tyrosine and a phenylalanine is present in its place, but the protein is still active. Here, the crystallization of *C. trachomatis* R2 is described. A heavy-atom co-crystallization approach was used to obtain crystals. Hopefully, the *C. trachomatis* R2 structure will provide key clues as to how this enzyme is able to function while lacking the features that have previously been believed to be essential for activity.

1. Introduction

Ribonucleotide reductases (RNRs) catalyse the formation of deoxyribonucleotides from ribonucleotides. This reaction is essential to all cellular organisms. All RNRs utilize radical chemistry to catalyse the ribonucleotide reduction. There are three classes of RNRs classified by the source of the radical used in the reaction. Class I, found in eukaryotes, bacteria and viruses, is an $\alpha_2\beta_2$ protein in which the α subunit is denoted R1 and the β subunit R2. The active site and the sites for allosteric regulation are located on the R1 subunit and the radical is generated *via* the reductive cleavage of dioxygen at a diiron carboxylate site located in the R2 subunit. For a recent review of RNRs, see Eklund *et al.* (2001).

Human chlamydial infections are a leading cause of sexually transmitted disease, blindness and respiratory disease. *Chlamydia trachomatis* is the agent causing blindness and sexually transmitted disease. *C. trachomatis* cannot import deoxyribonucleotides and therefore has to perform ribonucleotide reduction (McClarty & Tipples, 1991; Roshick *et al.*, 2000; Tipples & McClarty, 1993). The genome of human *C. trachomatis* serovar D has been sequenced (Stephens *et al.*, 1998) and the only RNR present belongs to class I. The R2 subunit of chlamydial RNRs has two sequence abnormalities that are very interesting; these are present in all of the seven chlamydial R2 genes that have been sequenced. Firstly, the most N-terminal iron ligand is normally an aspartate in R2 but the chlamydia family has a glutamate in this position, which is more similar to methane monooxygenase, for example, than to other R2s. Secondly, normal R2s have a conserved tyrosine were the radical is stored until it is used at the active site in the R1 subunit; this residue is a phenylalanine (F127) in chlamydia. A phenylalanine residue cannot function as a radical storage position, but this enzyme is still active both *in vivo* and *in vitro* (Roshick *et al.*, 2000). It has been suggested that the tyrosine (Tyr129) positioned two residues from the phenylalanine has taken over the function of the normal tyrosine (Roshick *et al.*, 2000); however, this would imply a major structural rearrangement. If, on the other hand, the phenylalanine (Phe127) is positioned at the 'normal' tyrosine position, the reaction has to be carried out in a novel way and the radical has to be stored in an as yet unknown fashion. Here, we describe the crystallization of the R2 subunit of RNR from *C. trachomatis* using a heavy-atom co-crystallization approach.

2. Materials and methods

2.1. Cloning, expression and purification

C. trachomatis R2 was cloned, expressed and purified as described previously (Roshick *et al.*, 2000). To remove some remaining impurities, the fraction eluted by the KCl gradient from the DEAE column containing R2 was dialyzed and then reloaded onto the DEAE column and eluted in a single step with 125 m*M* KCl. The protein is 346 amino acids long and is a homodimer with a total molecular weight of 81 kDa.

2.2. Crystallization

Crystallization experiments were performed using the hanging-drop vapour-diffusion technique in 24-well tissue-culture plates (Ducruix

& Giegé, 1999; McPherson, 1982). Screening with Hampton Research Crystal Screen kits I and II (Jancarik & Kim, 1991) at room temperature and 277 K produced no promising crystal leads. However, some conditions produced phase separation in the drop. A few of these conditions were subjected to further crystallization trials with the addition of various heavy-metal ions to the protein drop. Experiments were set up as follows: 1 µl drops of protein solution were applied to the lid of a standard Petri dish, 1 µl of heavy-metal solution was added to each of the drops (in one case water was added as a control) and finally 1 µl of reservoir solution was added to the drops and the Petri dish was sealed with Parafilm to allow equilibration against the mother liquor (about 5 ml in the bottom of the dish). The following heavy-metal solutions were used in the screen (Me, methyl; Ac, acetate; 20% sat. means that a saturated solution was diluted five times before use): K_2PtCl_4 (1 m*M*), MeHgAc (20% sat.), $SmAc_3$ (1 m*M*), UO_2Ac_2 (1 m*M*), $Pt(NH_3)_2Cl_2$ (1 m*M*), $KAu(CN)_2$ (1 m*M*), $PbAc_2$ (1 m*M*), KI (1 m*M*), Me_3PbAc (1 m*M*), EMTS (1 m*M*), HgAc (20% sat.), $Ba(NO_3)_2$ (1 m*M*), $SrCl_2$ (1 m*M*), $YbCl_3$ (1 m*M*), PHMBA (20% sat.), H_2PtCl_6 (1 m*M*), $IrCl_3$ (20% sat.), $CdSO_4$ (1 m*M*). 2 d after setup the effect of the different heavy atoms was already obvious as the drops displayed various degrees of precipitation, ranging from clear drops to heavy precipitation. Two weeks after setup, small crystals with poor morphology were observed in the drop composed of condition 22 from Hampton Crystal Screen II (12% PEG 20K, 0.1 *M* MES pH 6.5) with addition of $Pb(CH_3COO)_2$. Exposure of a crystal at the MAX-lab I711 beamline in Lund, Sweden verified the macromolecular content but the diffraction was very poor. Subsequent optimization of the crystallization condition resulted in beautiful tetragonal bipyramidal crystals ranging from 0.05 × 0.05 × 0.1 to 0.2 × 0.2 × 0.5 mm (Fig. 1). The best crystals were obtained by mixing 6 µl of 2.7 mg ml^{-1} protein solution containing 0.33 m*M* $Pb(CH_3COO)_2$ in 8 m*M* Tris at pH 7.5 with 1 µl of mother liquor consisting of 5–7% PEG 20K, 0.1 *M* MES pH 6.5–6.2. We were able to crystallize both wild-type and the F127Y mutant R2 proteins using these conditions.

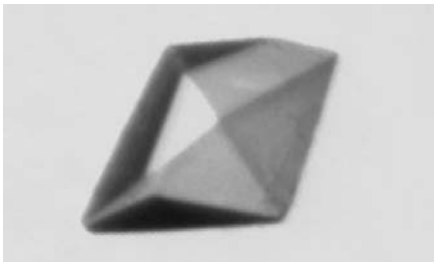

Figure 1
Typical crystal of *C. trachomatis* R2, approximate size 0.1 × 0.1 × 0.2 mm. This is the actual crystal from which the data was collected.

3. Data collection and X-ray analysis

Crystals were cryoprotected by soaking for a few minutes in mother liquor where some of the water had been replaced with PEG 400 to a total concentration of 35%(*v*/*v*); the crystals were subsequently flash-frozen in liquid nitrogen. Diffraction data were collected on a MAR 345 image plate at the I711 beamline at MAX-lab, Lund, Sweden. The data were processed and scaled using *DENZO* and *SCALEPACK* (Otwinowski, 1993). The crystals belong to the tetragonal crystal system, scaled well in *P*422 and could be assigned to space group $P4_12_12$ or $P4_32_12$ based on the systematic absences. One monomer (346 amino-acid residues) per asymmetric unit gives a calculated solvent content of 36% (see Table 1 for data statistics).

The closest homology to any solved R2 structure was to the mouse protein (Kauppi *et al.*, 1996), with 23% identity. However, since the structures of previously solved R2 proteins are very similar, especially in their iron-coordinating helical core, despite the low sequence identity (Högbom *et al.*, 2002), we assumed that molecular replacement would be a viable option for obtaining initial phases for the data.

For molecular replacement, a polyserine model of the core seven-helix bundle of the mouse R2 protein was produced. Molecular-replacement searches in space groups $P4_12_12$ and $P4_32_12$ with the *CNS* software (Brünger *et al.*, 1998) resulted in no clear solution peaks. Because of the advantageous situation with only one monomer per asymmetric unit, we were able to pick out the correct solution by analysing the packing; one of the solutions in space group $P4_32_12$ seemed to produce the correct dimer interface by crystallographic symmetry. This model, which turned out to have the correct orientation and space group, had an initial *R* value of 56.5%. The model has been built and refined and will be presented elsewhere.

4. Results and discussion

In our hands, the R2 protein from *C. trachomatis* produced no crystal leads in standard crystallization trials. For this reason, we had to resort to other trials. The rationale behind choosing conditions producing phase separation for further trials was that these conditions were at least able to concentrate the protein significantly without inducing non-productive precipitation. The use of heavy-metal ions that bind to the protein is widely used in post-crystal stages of protein structure-determination projects to obtain phase information by MIR or MAD methods. In this case, we rationalized that binding of a metal ion to the protein surface prior to crystallization would change its properties and thus enable crystal contacts that otherwise cannot form. Hence, different heavy-metal ions were added to the protein solution prior to crystallization experiments. The experiments were set up in standard Petri dishes sealed with Parafilm. 18 different metal compounds were tested simultaneously by adding the metal solutions to protein solution drops on a single lid. Reservoir solution was also added and the drops were allowed to equilibrate over the original mother liquor that previously produced phase separation. The effect of the heavy-metal additives was obvious as they resulted in different degrees of protein precipitation and in one case in crystals.

Table 1
Data-collection statistics for the *C. trachomatis* R2 structures.

Values in parentheses are for the outer resolution shell.

	Wild type	F127Y mutant
Unit-cell parameters		
$a = b$ (Å)	62.7	62.6
c (Å)	171.8	171.0
Space group	$P4_32_12$	$P4_32_12$
Resolution (Å)	20–1.70 (1.76–1.70)	30–2.00 (2.07–2.00)
No. observations	147173	117520
Unique reflections	38503	23651
R_{sym}†	0.082 (0.284)	0.085 (0.308)
Completeness (%)	99.5 (99.9)	99.3 (100.0)

† $R_{sym} = \sum_j \sum_h |\langle I_{hj} - I_h \rangle| / \sum_j \sum_h I_{hj}$, where I_{hj} is the jth observation of reflection h.

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