Acta Crystallographica Section D
Biological Crystallography
ISSN 0907-4449

Crystallization and preliminary X-ray diffraction analysis of the mitochondrial transcription factor sc-mtTFB from *Saccharomyces cerevisiae*

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Received 23 November 1999
Accepted 3 April 2000

Eukaryotic mitochondria contain a distinct mini-chromosome. In yeast, transcription of the mitochondrial genome is mediated by a nuclear-encoded RNA polymerase consisting of a single polypeptide core enzyme and a specificity factor termed sc-mtTFB which bears some similarity to bacterial σ-factors. sc-mtTFB from *Saccharomyces cerevisiae* has been cloned, expressed, purified and crystallized. The crystals belong to the monoclinic space group $C2$, with unit-cell parameters a = 89.7, b = 44.6, c = 98.9 Å, β = 110°. Based on one molecule per asymmetric unit, the solvent content is estimated to be 48%. Small crystals of dimensions 0.01 × 0.05 × 0.13 mm diffract to at least 2.7 Å resolution on a rotating-anode X-ray source.

1. Introduction

In addition to their nuclear genome, eukaryotic cells contain a distinct mini-chromosome within their mitochondria. In *S. cerevisiae*, this genome is 80 kbp in size and encodes for 35 genes. The expression of the mitochondrial genome in *S. cerevisiae* and other eukaryotic cells is distinct from the nuclear-transcription machinery. Transcription is mediated by nuclear-encoded RNA polymerases consisting of a single polypeptide core enzyme and a specificity factor. In *S. cerevisiae*, the core enzyme has been characterized as the 153 kDa protein sc-mtRNAP, while sc-mtTFB (43 kDa) constitutes the specificity factor required for promoter recognition (Schinkel *et al.*, 1987). The core enzyme of the yeast mitochondrial RNA polymerase (RNAP) shows significant homology with the RNAPs of T7, T3 and SP6 bacteriophages, which are significantly smaller at about 100 kDa each (Masters *et al.*, 1987). The co-factor sc-mtTFB, on the other hand, displays some similarities with bacterial σ-factors such as σ70 from *Escherichia coli* (Jang & Jaehning, 1991). In σ70, these conserved regions have been suggested to function in promoter recognition and melting and in the stabilization of the *E. coli* RNAP (Helmann & Chamberlin, 1988; Siegele *et al.*, 1989). A recent study showed that sc-mtTFB binds to sc-mtRNAP prior to DNA binding and is released from the core enzyme after transcription initiation (Mangus *et al.*, 1994). Interestingly, both sc-mtRNAP as well as sc-mtTFB alone reportedly bind DNA in a non-specific manner, but only the assembled RNAP is able to recognize the promoter (Schinkel *et al.*, 1987).

The structural nature of the sc-mtTFB–sc-mtRNAP interaction as well as the interaction of this complex with the promoter is unknown. As part of our ongoing studies of transcriptional machinery, the co-factor sc-mtTFB was overexpressed in *E. coli*, purified to homogeneity and crystallized. Here, we report the preparation, crystallization and preliminary X-ray characterization of the mitochondrial transcription factor sc-mtTFB.

2. Methods and results

2.1. Sample preparation

The gene for the specificity factor sc-mtTFB was obtained by PCR (polymerase chain reaction) from yeast nuclear DNA. The identity of the gene was confirmed through sequencing of the entire gene. After suitable restriction sites had been added to the ends of the *sc-mtTFB* gene through a second round of PCR, the gene was cloned into the pTrcHisC plasmid (Invitrogen). For ease of purification, a His_6 tag was fused to the N-terminus of the protein. For expression, *E. coli* JM109 transformed with the pTrcHisC plasmid containing the *sc-mtTFB* gene was inoculated into 1 l of Circlegrow media (BIO101) containing 100 mg of ampicillin. No induction step was required. The culture was grown at 310 K overnight (20 h) and the cells were harvested by centrifugation. The cell pellets were resuspended in 60 ml 50 m*M* sodium phosphate buffer pH 8.0 containing 100 m*M* NaCl and 0.1 m*M* PMSF (phenylmethylsulfonyl fluoride), sonicated and then centrifuged to remove the cell debris. The supernatant was run through a Talon cobalt-affinity column (Clontech). The column was

Table 1
Data-collection parameters.

Crystal dimensions (mm)	0.01 × 0.05 × 0.13
Crystal-to-detector distance (mm)	200
Maximum data resolution (Å)	2.7
Oscillation range for each exposure (°)	1
Temperature (K)	90
Exposure time (min)	20
Total crystal rotation (°)	172
Average data redundancy	3
Unit-cell parameters (Å, °)	a = 89.7, b = 44.6, c = 98.9, β = 110
Space group	$C2$
Molecules per asymmetric unit	1

Table 2
Data-processing statistics.

Resolution range (Å)	$\langle I/\sigma(I)\rangle$	Completeness	χ^2	R_{sym}
20.00–5.37	21.15	99.6	0.956	0.045
5.37–4.28	21.4	99.7	1.001	0.049
4.28–3.74	19.18	99.3	1.061	0.058
3.74–3.40	13.4	98.2	1.029	0.076
3.40–3.16	9.3	97.7	1.024	0.102
3.16–2.97	6.7	96.7	1.062	0.146
2.97–2.82	5.3	93.8	1.031	0.174
2.82–2.70	4.7	87.8	0.979	0.203
All	14.0	96.6†	1.017	0.071

† Completeness in the high-resolution shell (2.82–2.70 Å) is 80%.

washed with the suspension buffer described above and the protein was eluted with 50 m*M* sodium phosphate pH 8.0, 50 m*M* imidazole, 100 m*M* NaCl and 0.1 m*M* PMSF. The eluted protein was then dialyzed against a solution containing 300 m*M* NaCl, 10% glycerol and 50 m*M* Tris–HCl pH 8.0 (buffer *A*) and passed over a Superdex75 (Pharmacia Biotech) column to assure homogeneity. The purity of the protein was assessed by SDS–PAGE analysis. Using this procedure, approximately 10 mg of pure sc-mtTFB protein are produced per liter of culture.

Figure 1
Crystal of the mitochondrial transcription factor sc-mtTFB. The crystal shown has approximate dimensions 0.05 × 0.01 × 0.15 mm. The crystals belong to space group $C2$, with unit-cell parameters a = 89.7, b = 44.6, c = 98.9 Å, β = 110°.

2.2. Crystallization

Hampton Crystal Screens (Hampton Research) were used for initial screening of crystallization conditions at 291 K. Crystals were grown by hanging-drop vapor diffusion employing 2 µl drops containing equal volumes of protein (10 mg ml^{-1} in buffer *A*) and precipitant solution. The reservoir contained 0.5 ml of precipitant solution. Crystals suitable for X-ray diffraction analysis could be grown over a period of 2–4 weeks from setups containing 30% PEG 8000, 0.2 *M* calcium acetate, 0.1 *M* sodium cacodylate pH 6.5 and 5%(*v*/*v*) xylitol as an additive.

2.3. X-ray diffraction analysis

For X-ray analysis, crystals were mounted in a 0.2 mm diameter rayon loops (Teng, 1990) containing a minimal amount of mother liquor and flash-cooled (Hope, 1988) to 90 K in a nitrogen-gas cold stream (Molecular Structure Corporation). No additional cryoprotectant was required to stabilize the crystal. Data were collected using focused (MSC Blue Confocal Optics) 5 kW Cu $K\alpha$ X-rays generated by a Rigaku RU-200 rotating-anode generator. A Rigaku R-AXIS IV imaging-plate scanner was used to record the diffraction pattern. The data were indexed, integrated and scaled using *HKL* 1.9.1 (Otwinowski & Minor, 1997). The data-collection parameters and data-processing results are summarized in Tables 1 and 2.

3. Results and discussion

Crystals of sc-mtTFB (Fig. 1) diffract to at least 2.7 Å resolution on a rotating-anode X-ray source. The data indexed in a *C*-centered monoclinic lattice with unit-cell parameters a = 89.7, b = 44.6, c = 98.9 Å, β = 110°. Analysis of the three-dimensional diffraction data (*XPREP*; Sheldrick, 1991) indicated that the space group was $C2$. Assuming one molecule per asymmetric unit, the Matthews coefficient (Matthews, 1968) was calculated to be 2.41 Å^3 Da^{-1}, which corresponds to a solvent content of 48%, which is well within the range for most protein crystals. The search for suitable heavy-atom derivatives is under way.

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