Purification, crystallization and preliminary X-ray diffraction analysis of the seryl-tRNA synthetase from *Candida albicans*

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Purification, crystallization and preliminary X-ray diffraction analysis of the seryl-tRNA synthetase from *Candida albicans*

The seryl-tRNA synthetase (SerRS) from *Candida albicans* exists naturally as two isoforms resulting from ambiguity in the natural genetic code. Both enzymes were crystallized by the sitting-drop vapour-diffusion method using 3.2–3.4 M ammonium sulfate as precipitant. The crystals belonged to the hexagonal space group *P*6122 and contained one monomer per asymmetric unit, despite the synthetase existing as a homodimer (with a molecular weight of ~116 kDa) in solution. Diffraction data were collected to 2.0 Å resolution at a synchrotron source and the crystal structures of unliganded SerRS and of its complexes with ATP and with a seryl-adenylate analogue were solved by molecular replacement. The structure of *C. albicans* SerRS represents the first reported structure of a eukaryotic cytoplasmic SerRS.

1. Introduction

Aminoacyl-tRNA synthetases (aaRSs) produce the aminoacyl-tRNA substrates for protein synthesis. In order to translate the genome into proteome with high fidelity, aaRSs exhibit strict substrate specificity and are able to differentiate tRNAs and amino acids efficiently by following conserved genetic code rules (Ibba & Söll, 1999).

A unique genetic code is used in several species of the fungal CTG clade, where leucine CUG codons are ambiguously decoded as serine and leucine (Santos & Tuite, 1995; Santos et al., 1996; Gomes et al., 2007; Butler et al., 2009). This genetic code ambiguity is introduced by a mutant serine-tRNA (tRNA<sub>CAU</sub>Ser) which is recognized by both the leucyl-tRNA and seryl-tRNA synthetases (LeuRS and SerRS, respectively; Suzuki et al., 1997). In the cellular context, tRNA<sub>CAU</sub>Ser is charged with 95–97% serine and 0.6–5% leucine (Gomes et al., 2007). Hence, SerRS is the main operating aaRS for the translation of CUG codons. However, the SerRS gene contains a single CUG codon whose mistranslation results in the synthesis of two SerRS isoforms, SerRS<sub>Ser197</sub> (major isoform) and SerRS<sub>Leu197</sub> (minor isoform), which are constitutively expressed. The evolutionary pathway of CUG codons in the CTG-clade is poorly understood and led us to hypothesize that structural characterization of both SerRS variants should unveil novel features of the fungal tRNA-serylation system and may provide new clues about this novel codon-reprogramming event with implications for understanding the evolution of the genetic code of *Candida albicans* and, eventually, new features of its biology and pathogenesis.

2. Material and methods

2.1. Cloning of two *C. albicans* SerRS variants

The *C. albicans* SerRS coding sequence (GenBank accession No. AF290915) was amplified from strain CAI4 genomic DNA by PCR using the primers SerRS1 (5'-GGA ATT CCA TAT GTT AGA CAT TAA TGC ATT TCT CG-3') and SerRS2 (5'-GGA TCC CGC TTT TCT TAC CTT TAG CTT TTT TAA C-3'). The PCR fragment was digested with *Ndel* and *BamHI* endonucleases and ligated to *Ndel*/*Sali*-digested pT7-7 expression plasmid (USB). The resulting expression vector encodes the full-length *C. albicans* SerRS followed
by a 17-residue linker and a C-terminal hexahistidine (His$_6$) tag (the linker and tag sequence is PKNTTSVKAKGKNGSRRHHHHHH).

The two natural C. albicans SerRS isoforms were generated by site-directed mutagenesis using primers 5'-GCT TTA ATC AAC TAC GGT TTA TCG TTT TTG AGT AGC AAA GGA TAC G-3' and 5'-CGT ATC CTT TGC TAC TCA AAA ACG ATA AAC CGT AGT TGA TTA AAG C-3' for the SerRS_Ser197 variant and primers 5'-CTT TAA TCA ACT ACG GTT TAT TGT TTG TTA GTA GCA AAG GAT ACG-3 for the SerRS_Leu197 isoform.

2.2. Overexpression and purification of recombinant SerRS

Large-scale production of C. albicans SerRS was achieved in Escherichia coli BL21 (DE3) CodonPlus RIL (Stratagene) with culture inoculation by the plating method (Suter-Crazzolara & Unsicker, 1995). Expression cultures [LB medium with 100 µg ml$^{-1}$ ampicillin, 34 µg ml$^{-1}$ chloramphenicol and 1% (w/v) glucose] were incubated at 310 K until the OD$_{600}$ reached ~0.3. At this point, the incubation temperature was lowered to 303 K and the bacterial cells were allowed to grow until mid-exponential phase (0.5 < OD$_{600}$ < 0.7). SerRS expression was induced by the addition of 0.5 mM IPTG (Biosynth) and continued for 3 h. Cells were harvested by centrifugation (8100 g, 20 min, 277 K), resuspended in lysis buffer [50 mM Na HEPES pH 7.6, 100 mM KCl, 10 mM MgCl$_2$, 20 mM imidazole and 10% (v/v) glycerol supplemented with 1 mg ml$^{-1}$ lysozyme (Sigma)] and stored at 253 K until further use.

For SerRS purification, the cell suspensions were thawed and supplemented with protease inhibitors (Complete EDTA-free, Roche) and DNase I (AppliChem) to 5 µg ml$^{-1}$ final concentration and the cells were disrupted by sonication. The homogenate was centrifuged (13 800 g, 20 min, 277 K) and the supernatant was filtered (0.22 µm low-protein-binding PVDF membrane) and loaded onto a 5 ml HisTrap HP column (GE Healthcare) pre-equilibrated in lysis buffer. The bound protein was eluted with a linear 10–500 mM imidazole gradient in lysis buffer. EDTA (500 µM final concentration) was added to the SerRS-containing fractions (which eluted at ~250 mM imidazole).

The SerRS-containing fractions were pooled, diluted 20-fold in buffer A [20 mM Na HEPES pH 7.6, 10 mM KCl, 5 mM MgCl$_2$, 0.1 mM Na$_2$EDTA and 5% (v/v) glycerol] and loaded onto a 5 ml Econo-Pac High Q cartridge (Bio-Rad) pre-equilibrated in buffer A. The bound SerRS was eluted with a linear 10–500 mM NaCl gradient (in buffer A) over 15 column volumes. The fractions corresponding to the SerRS peak were pooled, dialyzed against buffer B [50 mM Na HEPES pH 7.6, 150 mM KCl, 10 mM MgCl$_2$ and 8% (v/v) glycerol] and concentrated (to ~20 mg ml$^{-1}$) using a 10 kDa molecular-weight cutoff ultracentrifugal concentration device (Millipore).

For analysis of the recombinant proteins by gel-filtration chromatography, 50 µl concentrated SerRS sample (4.0 mg ml$^{-1}$) was applied onto a Superose 12 10/300 column (GE Healthcare) pre-equilibrated in buffer B at room temperature.

2.3. Crystallization

Initial crystallization conditions were obtained in sitting drops using a commercial ammonium-sulfate-based sparse-matrix screen (JScreen Classic 6, Jena Bioscience). After refinement, reproducible growth of crystals of native recombinant SerRS (both isoforms) was obtained in drops composed of identical volumes of protein solution (8–14 mg ml$^{-1}$) and reservoir solution [100 mM Na MES pH 5.6–5.8, 3.2–3.4 M ammonium sulfate and 0–2% (v/v) glycerol] equilibrated against a 500 µl reservoir. For preparation of the binary complexes SerRS--SerSA and SerRS--ATP, SerRS_Ser197 was pre-incubated on ice for 2–3 h with 15 mM 5’-O-[N-(3-aminopropyl)]adenosine (RNA-TEC) or 10 mM ATP (freshly prepared in buffer B; Sigma), respectively, and crystallized using 100 mM Na MES pH 5.8–6.2, 3.3–3.4 M ammonium sulfate and 0–5% (v/v) glycerol as precipitant.

Figure 1

Purification of C. albicans SerRS isoforms. (a) Analytical gel-filtration chromatography profile of the recombinant proteins showing that they both behave as dimers in solution with an apparent molecular weight of 116 kDa; vertical dotted lines highlight the elution volumes of the molecular-weight markers shown in Table 1 (black, blue dextran; blue, aldolase; red, albumin; grey, ovalbumin; yellow, chymotrypsin). (b) Coomassie Blue-stained SDS–PAGE (12.5%) analysis of recombinant SerRS isoforms (left, SerRS_Leu197; right, SerRS_Ser197). Both samples contained identical minor impurities that did not interfere with the crystallization process.

Table 1

<table>
<thead>
<tr>
<th>Sample elution volume on a Superose 12 10/300 column (GE Healthcare).</th>
<th>Elution volume (ml)</th>
<th>Molecular weight (kDa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue dextran</td>
<td>7.43</td>
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<tr>
<td>Aldolase</td>
<td>11.33</td>
<td>158</td>
</tr>
<tr>
<td>Albumin</td>
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<td>67</td>
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<td>Ovalbumin</td>
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<tr>
<td>Chymotrypsin</td>
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<td>25</td>
</tr>
<tr>
<td>SerRS_Ser197</td>
<td>11.79</td>
<td>116</td>
</tr>
<tr>
<td>SerRS_Leu197</td>
<td>11.73</td>
<td>118</td>
</tr>
</tbody>
</table>
2.4. SerRS thermal stability characterization

To characterize protein stability, the melting temperature ($T_m$) of SerRS was determined by following its behaviour in solution by DLS (Zetasizer Nano, Malvern). SerRS (50 µl 1.0 mg ml$^{-1}$) was heated from 298 to 326 K in a 45 µl quartz cuvette (Hellma Inc.). In order to reduce evaporation, the protein solution was overlaid with paraffin oil (20 µl). Protein aggregation was monitored by measuring Z-average diameter (nm) and scattering intensity (kilodcounts per second) as a function of temperature, using 1 K temperature increments and 3 min equilibration before each measurement. The resulting inflection point of the particle-size curve was defined as $T_m$.

2.5. Data collection and processing

All diffraction data were collected at 100 K from single crystals at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). All data sets were collected using an ADSC Q4R detector on beamline ID14-EH1. The SerRS_Ser197 and SerRS-Leu197 and SerRS–SerSA† data sets were collected to 2.0 Å resolution in 0.5° oscillation steps (270 frames). Data for the SerRS–ATP complex were collected to 2.55 Å resolution in 1° oscillation steps over a range of 120°. The SerRS_Leu197 diffraction images were collected at 2.3 Å resolution in 0.6° oscillation steps (200 frames). Diffraction images were integrated with MOSFLM (Leslie, 1999) and scaled with SCALA (Collaborative Computational Project, Number 4, 1994).

2.6. Structure solution

The three-dimensional structure of C. albicans SerRS was solved by molecular replacement (MR) with Phaser (McCoy, 2007) using the catalytic domain of Pyrococcus horkoshii SerRS as the search model (PDB entry 2dq0; the model contained residues 107–447 of chain A with all non-identical non-glycine residues truncated to Ala; Itoh et al., 2008). The SerRS model was rebuilt with ARP/wARP (Perrakis et al., 2001) on electron-density maps calculated using the phases from the MR solution and the C. albicans SerRS sequence information. Refinement (energy-gradient minimization, simulated-annealing and restrained individual B-factor refinement) with CNS (Brünger et al., 1998) was alternated with manual model building using Coot (Emsley & Cowtan, 2004). The coordinates of SerRS_Ser197 were used as an MR search model to solve the three-dimensional structures of SerRS_Leu197, SerRS–SerSA and SerRS–ATP.

3. Results and discussion

Recombinant C. albicans SerRS was purified in two chromatographic steps, yielding essentially pure material as judged by size-exclusion chromatography and SDS–PAGE analysis (Fig. 1). The protein yields were 10 and 4 mg of purified SerRS_Ser197 and SerRS_Leu197, respectively, per litre of culture. The apparent molecular weights of the proteins, as determined by gel-filtration chromatography (Fig. 1a) and dynamic light scattering (data not shown), suggest that they exist as homodimers in solution, as observed for other class II synthetases (First, 2005). In order to further understand the impact of Ser to Leu replacement on the dimer structure, the thermal stability of each C. albicans SerRS isoform was evaluated by dynamic light scattering (DLS; Fig. 2). The melting curve demonstrated that the presence of Leu at position 197 slightly decreases protein stability, presumably owing to the loss of polar interactions in SerRS_Leu197.

For both SerRS isoforms, crystals appeared after 2 or 3 d (Fig. 3). The SerRS_Ser197 and SerRS–SerSA crystals and cocrystals were successfully cryoprotected in sodium malonate pH 6.0 solution. However, better diffraction data were obtained for the SerRS_Leu197 crystals when the pH of the cryoprotecting solution was lowered to 5.0. All SerRS

![Figure 2](image-url)

**Figure 2**

Differential incorporation of serine or leucine at the CUG position 197 of SerRS alters the thermal stability. Comparison of the thermal denaturation profiles of SerRS_Ser197 and SerRS_Leu197 shows that the incorporation of Leu into both monomers of SerRS induces a decrease in thermal stability.
crystallization communications

Figure 3
Recombinant C. albicans SerRS_Ser197 crystal belonging to space group P6_22.

crystals analyzed belonged to the hexagonal space group P6_22, with unit-cell parameters \( a = b = 90.1, c = 276.8 \) Å (crystallographic statistics are reported in Table 2). Assuming the presence of one C. albicans SerRS molecule in the asymmetric unit, the calculated Matthews coefficient is 3.22 Å\(^3\) Da\(^{-1}\), which corresponds to a solvent content of 61.8% (Matthews, 1968).

The molecular coordinates of the catalytic domain of P. horikoshii SerRS (PDB entry 2dq0; Itoh et al., 2008) were used to solve the structure of the C. albicans enzyme by the molecular-replacement method. The program Phaser (McCoy, 2007) located one monomer of C. albicans SerRS_Ser197 in the asymmetric unit (rotation-function \( Z \) score of 6.8, translation-function \( Z \) score of 25.1). The electron-density map obtained was of good quality and a significant portion of the model (403 out of 471 residues) could be traced automatically with ARP/wARP (Perrakis et al., 2001). The difference electron-density maps obtained for the SerRS–SerSA and SerRS–ATP complexes showed unambiguous density for the ligands at the SerRS active site. The structures of both C. albicans SerRS isoforms are currently in the final stages of refinement and will be compared in order to evaluate the structural impact of serine/leucine exchange at the CUG codon position.

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References