Cry crystallization and preliminary X-ray crystallographic studies of trichosanthin delta C7

Li X.1,4, Ding Y.1, Too H.2, Wang Z.1, Liu Yi.1, Dong Y.1,3, Shaw P.2 & Rao Z.1*

1 Laboratory of Structural Biology and the MOE Laboratory of Protein Science, School of Life Science & Engineering, Tsinghua University, Beijing 100084, China.
2 Department of Biochemistry, Chinese University of Hong Kong, Hong Kong, China
3 Institute of Biophysics, Chinese Academy of Science, Beijing, 100101, China.
4 Medical College, Xi’an Jiaotong University, Xi’an 710061, China.

Correspondence To: Zihe Rao, Phone: 86-10-6277-1493; Fax: 86-10-6277-3145; E-mail: raozh@xtal.tsinghua.edu.cn

Abstract: Trichosanthin (TCS) is a type I ribosome-inactivating protein (RIP) which possesses rRNA N-glycosidase activity. TCS has various pharmacological properties. It is possible to reduce the antigenicity of TCS by deleting up to seven C-terminal residues of TCS (TCS-C7) with minimal effect on its activity [1]. TCS-C7 has been crystallized and the crystal diffracted to 1.8 Å. It belongs to space group P2₁, with unit-cell parameters a=71.6 Å, b=74.4 Å, c=87.6 Å, β=97.0°. It is given that there are four molecules per asymmetric unit.

Keywords: Trichosanthin delta C7, ribosome-inactivating proteins, antigenicity, crystallization

Introduction

Ribosome-inactivating proteins (RIPS) are a group of cytotoxins, which possess a unique rRNA N-glycosidic activity by hydrolyzing a single N-glycoside bond between adenine and ribose at A4324 in the 28S rRNA of rat liver ribosomes. This damages the ribosomes irreversibly with the consequent arrest of protein synthesis [2-4]. They are abundant and widely distributed in higher plants. RIPS can be classified into type I and
Type II. Type I RIPs are single-chain, basic proteins of molecular weights about 30000 daltons. They are potent inhibitors of protein synthesis in the cell-free system, but are relatively non-toxic to intact of the molecule. Type II RIPs consists of a catalytically active A chain linked to a carbohydrate-binding B chain by disulfide linkage. The A chain is homologous to type I RIP and is responsible for the toxicity of the molecule. The B chain possesses lectin properties which facilitates entry of the A chain into the cytoplasm of the cell [5].

Trichosanthin (TCS) is a plant protein extracted from root tubers of *Trichosanthes kirlowii* Maxim, Cucurbitaceae [6-8]. It has been identified as the active component in the Chinese herbal medicine “Tian Hua Fen”. TCS is a type I ribosome-inactivating protein (RIP) with 247 amino acid and inactivates eukaryotic ribosomes via its N-glycosidase activity. It possesses a large domain (N-terminal domain, from 1 to 184) and a small domain (C-terminal domain, from 185 to 247) [9]. The active pocket responsible for N-glycosidase activity is located in the cleft between the two domains [10]. TCS has been used for inducing mid-term abortion and treating ectopic pregnancies, hydatidiform and trophoblastic moles in China [11, 12]. In recent years, TCS had also been found to posses various pharmacological properties including immunomudulatory, anti-tumor and anti-HIV activities [13]. Clinical trials have been performed. The result showed an increase of CD4+ and CD8+ T cells in patients [14, 15].

Although TCS is effective in killing tumor cells in vivo and in vitro, its side effects on patients have not been systematically evaluated. In the long run, it would be useful if the antigenicity of TCS can be reduced and the halftime in vivo increased. It is possible to reduce the antigenicity of TCS by site-specific coupling of PEG modification (PEGylation) [16] or deleting the C-terminal residues [1]. Reducing molecular weight could reduce its antigenicity [1]. In the latter case, it is interesting to find that the mutants deleting more than seven residues in the C-terminal are expressed as inclusion body and failed to re-fold from urea or guanidine hydrochloride [1]. In order to explore the role of the C-terminal residues, X-ray crystallographic analysis of TCS-C7 is in progress.

**Experimental**

**Construction of expression vectors and protein purification.** The expression vectors were constructed by Chan et al. [1]. Protein expression and purification were performed as described [17].

**Crystallization.** Initial screening for crystallization conditions was performed using commercially purchased sparse-matrix screens from Hampton Research (Crystal Screens I and II). After refinement to the crystallization conditions, the crystallization condition for good crystal was obtained. All crystals were grown using the hanging-drop vapour-diffusion technique by mixing 2µl protein (20mg/ml) with 2µl of precipitating solution (15% PEG8000, 0.2M CaAc2, 0.1M Na CaCodylate, pH 6.5) on a siliconized cover slide and equilibrating against 500µl of the same precipitant solution. Crystals were grown in a crystallization room maintained at 291K. Crystals grew in 3 days at 291K and reached their maximum size (0.4x0.1x0.05mm) in about a week (Figure 1).

**Data collection and processing.** Diffraction data of the crystals were collected in house Mar345 image plate
with a Rigaku rotating Cu anode X-ray generator at 48kV and 98mA (λ=1.5418 Å). The crystal was mounted in a nylon-fiber loop and flash-frozen in a nitrogen-gas stream at 100K. The crystal-to-detector distance was 140mm. Each frame was exposed for 300 seconds and oscillated 1.5 degrees. Indexing and integration of all images was performed in DENZO and scaling of the intensity data was performed in SCALEPACK; both are from the HKL program package [18].

Figure 1 Crystal of TCS-C7 grown from 15% PEG8000, 0.2M CaAc₂, 0.1M Na CaCodylate, pH 6.5 (0.4×0.1×0.05mm).

A 1.8Å data set was obtained by measuring the intensities of 61794 reflections (Figure 2). The final merged data set of 8268 unique reflections with I/σ(I) > 0.0 is 98.2% complete (95.8% in the last resolution shell) and is characterized by Rmerge=0.071. The crystals of TCS-C7 belong to space group P2₁ with four protein molecules in the asymmetric unit. The crystal parameters and data collection statistics are listed in Table 1.

Table 1. Data collection and processing statistics

<table>
<thead>
<tr>
<th>Space group</th>
<th>P2₁</th>
</tr>
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<tbody>
<tr>
<td>Unit-cell parameters</td>
<td></td>
</tr>
<tr>
<td>a=71.6Å</td>
<td></td>
</tr>
<tr>
<td>b=74.4Å</td>
<td></td>
</tr>
<tr>
<td>c=87.6Å</td>
<td></td>
</tr>
<tr>
<td>β=96.9°</td>
<td></td>
</tr>
<tr>
<td>Resolution</td>
<td>40-1.8Å</td>
</tr>
<tr>
<td>Total number reflections</td>
<td>61794</td>
</tr>
<tr>
<td>Number of unique reflections</td>
<td>19310 (1950⁵)</td>
</tr>
<tr>
<td>I/σ(I)</td>
<td>10.4 (5.1⁵)</td>
</tr>
<tr>
<td>Redundancy</td>
<td>3.2 (3.1⁵)</td>
</tr>
<tr>
<td>Completeness</td>
<td>98.2% (95.8%⁵)</td>
</tr>
<tr>
<td>Rmerge</td>
<td>7.1% (24.8%⁵)</td>
</tr>
</tbody>
</table>

⁵Numbers in parentheses are the corresponding numbers for the highest resolution shell (1.87-1.8 Å)
Results and Discussion

The structure of native TCS and many mutants have been studied, the crystallization conditions are almost same as well as the space group and unit cell of these crystals [5, 10, 19]. The same condition has been tried for TCS-C7 crystallization at first, but no crystal appeared. Another different crystallization condition was developed for TCS-C7, moreover the space group and unit cell are also different from before. The comparison result of the secondary structure of C7 and wild-type trichosanthin by circular dichroism (CD) spectroscopy has indicated the decreasing of alpha helix and increasing of beta sheet [1]. This rather different crystallization condition may also be affected by the deletion of seven C-terminal residues of TCS and moreover the three-dimensional structure may be changed.

With the assumption of four molecules of TCS-C7 in the asymmetric unit, a value of the Matthews parameter of 2.14 Å³Da⁻¹ is obtained, with a corresponding solvent content of approximately 42%. The Vₘ value and solvent content lie within the range usually found for protein crystals [20].

The structure has been solved and will be published elsewhere. Compared with the native structure, there are three main differences: (i) The deletion of the last 7 residues caused remarkable conformational changes in main chain of helix H7 between wild type TCS and TCS-C7. (ii) The hydrogen bond system around the
C-terminal is rather different. (iii) Trp192, which locates at the active pocket of TCS and is one of the crucial amino acids of TCS, is much solvent exposed in TCS-C7.

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REFERENCES


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