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# Molecular-replacement studies of *Trichosanthes kirilowii* lectin 1: a structure belonging to the family of type 2 ribosome-inactivating proteins

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*Trichosanthes kirilowii* lectin 1 (TKL-1) isolated from the tuber of *T. kirilowii* consists of two chains, each with a molecular weight of about 30 kDa. It has immunological properties which are similar to some ribosome-inactivating proteins (RIPs). TKL-1 was crystallized in space group $P2_12_12_1$ and diffraction data were collected to 2.7 Å resolution. The molecular-replacement method was applied to solve the structure, using different chains of ricin, abrin-a and trichosanthin as search models. A set of consistent solutions was further verified by $R_{\rm omit}$ profile analysis. In addition, the spatial arrangement of the two chains of TKL-1 is identical to that of type 2 RIPs.

## 1. Introduction

Lectins belong to a heterogeneous group of proteins that have the ability to bind carbohydrates specifically and reversibly; the plant lectins have been the most widely investigated. Three isolectins (*T. kirilowii* lectins; TKLs), were isolated from a type of Chinese medicinal herb, tianhuafen, the tuber of *T. kirilowii maxim*, the same plant tissue from which trichosanthin (TCS) was obtained (Wang *et al.*, 1983). All three isolectins are composed of two polypeptide chains, both with a molecular weight of roughly 30 kDa, and have an acidic pI point in the range 5.2–5.7. They can agglutinate erythrocytes and bind specifically to galactose and GalNAc. Moreover, TKLs possess toxicity towards melanocytoma cancer cells (Sun & Wang, 1994).

Double immunodiffusion experiments were performed in order to investigate the relationship between TKLs and ribosome-inactivating proteins (RIPs; Wang *et al.*, 1990). The rabbit antiserum raised against TKLs could react with the type 2 RIP ricin but not with the type 1 RIP TCS. Meanwhile, the antiserum against TCS could react with TKLs but not with ricin. These results indicate that some similar neutralizing epitopes with a structural resemblance exist in both TKLs and RIPs. However, a major difference between them is that TKLs could not inhibit protein synthesis as efficiently as ricin in the rabbit reticulocyte lysate cell-free system. Therefore, it is of great interest to determine the molecular structures of the TKLs in order to reveal the complicated structure–function relationship among these proteins.

Here, we report crystallographic studies on TKL-1, which has molecular weights of 29 and 33 kDa for chains A and B, respectively. Its amino-acid composition is similar to that of TCS (Xiong *et al.*, 1994), ricin (Rutenber *et al.*, 1991) and abrin-a (Tahirov *et al.*, 1995). The complete amino-acid sequence has not yet been determined, but the partial N-terminal sequences of its two chains have been determined (Sun & Wang, 1995).

## 2. Data and solution

TKL-1 has been crystallized under three different sets of conditions (Wang *et al.*, 1998); the one producing the best crystals used the hanging-drop method, in which the droplets consisted of equal volumes of protein solution (4.2 mg ml$^{-1}$) and reservoir solution containing 15% PEG 8000, 0.5 *M* $Li_2SO_4$ and 0.1 *M* sodium cacodylate buffer pH 6.5. Intensity data were collected from these crystals at room temperature on a MAR Research image-plate detector system using Cu $K\alpha$ radiation generated by a Rigaku RU-200 rotating-anode generator operated at 50 kV and 50 mA. The data were processed with the programs *DENZO* and *SCALEPACK* (Otwinowski & Minor, 1997). The data-collection statistics are given in Table 1.

Since there are biochemical similarities between TKL-1 and RIPs of known structure, such as TCS, ricin and abrin-a, the molecular-replacement (MR) method was applied using the program *AMoRe* (Navaza, 1994) in order to solve the structure. The structures of the corresponding chains of ricin (PDB entry 2aai; Rutenber *et al.*, 1991) and abrin-a (PDB entry 1abr; Tahirov *et al.*, 1995) and of the single chain of TCS (PDB entry 1tcs; Xiong *et al.*, 1994) were used as search models for MR studies with the 10–4 Å resolution data. All three probes, the ricin and abrin-a A chains

**Table 1**
Data statistics for TKL-1 crystals.

| | |
|---|---|
| Space group | $P2_12_12_1$ |
| Unit-cell parameters (Å) | $a$ = 44.7 (2), $b$ = 69.5 (2), $c$ = 180.9 (5) |
| Resolution range (Å) | 30–2.7 |
| Number of observations | 64456 |
| Number of unique reflections | 12184 |
| Completeness (%)† | 74.0 (68.1) |
| $R_{sym}$ (%) †‡ | 8.8 (37.1) |
| $\langle I\rangle/\langle\sigma\rangle$† | 7.4 (2.0) |

† Values in parentheses are for the highest resolution shell (2.8–2.7 Å). ‡ $R_{sym} = \sum|I(k) - \langle I\rangle|/\sum I(k)$, where $I(k)$ and $\langle I\rangle$ represent the diffraction intensity values of the individual measurements and the corresponding mean value, respectively.

and TCS, could define a consistent solution for the A chain of TKL-1. However, the solution of the B chain of TKL-1 could only be derived from the B chain of abrin-a.

Inspecting the chain packing in the unit cell, the C$\alpha$ tracing of the three probes, namely, the ricin and abrin-a A chains and TCS, could be superimposed on each other in the TKL-1 crystal unit cell and had no serious clashes with the B chain of abrin-a. In addition, the spatial arrangement of the A and B chains are identical to those of abrin-a and ricin. In fact, the MR solution could also be obtained using the entire abrin-a molecule as a search model (see Fig. 1).

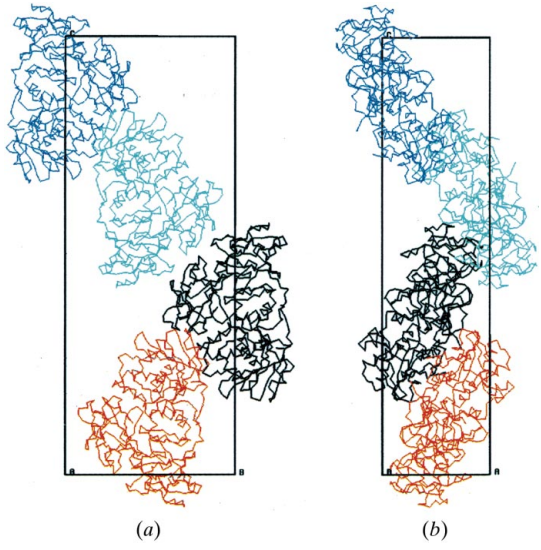


**Figure 1**
The molecular packing derived from abrin-a in the unit cell of TKL-1 crystals. (*a*) and (*b*) are views along the *a* and *b* axes, respectively.

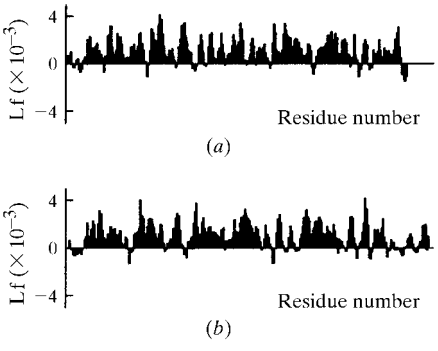


**Figure 2**
$R_{omit}$ profiles of the MR solution of TKL-1 obtained with (*a*) the A chain and (*b*) the B chain of abrin-a as search models.

$R_{omit}$ profile analysis (Tsuchiya & Takenaka, 1998) was used to verify the MR results. $R_{omit}$ values were calculated using the program *X-PLOR* (Brünger, 1992) by omitting in turn every three residues of the model using 15–3.5 Å resolution data. The scale factor $F_{calc}$ was adjusted to $F_{obs}$ for each omission. To investigate the role of the conserved structural part, the probe residues were not modified in the calculation of $F_{calc}$. Lf, the value of $R_{omit} - R_{all}$, was then plotted against the residue number. The resultant profiles are consistent with the $R_{omit}$ profile rule, *i.e.* the Lf values for the correct solution are mostly above zero, whereas the Lf values for an incorrect solution are distributed randomly around zero. The profiles of the MR solution of TKL-1 obtained from the A and B chains of abrin-a are shown in Fig. 2.

Based on these $R_{omit}$ profiles and the sequences of some type 2 RIPs, the initial model derived from abrin-a was modified and underwent a rigid-body refinement with the program *X-PLOR* using 10–3.5 Å data, resulting in an $R$ factor of 45.1% and an $R_{free}$ of 44.5% (with 5% omitted data).

## 3. Concluding remarks

Because of the unknown sequence of TKL-1, an MR solution has been found using several target models. In addition to the normal statistics of the MR studies, there are some indications to show that the MR solution is the correct one and that TKL-1 has a structure similar to abrin-a. For instance, exactly the same spatial arrangement of the two chains as in abrin-a was obtained from the present MR studies with single chains as independent targets. Furthermore, results obtained from $R_{omit}$ profile analysis obey the general law of structural homology, *i.e.* most residues with Lf > 0 are in the structure's core and are conserved, whereas the residues with Lf < 0 are either in the loops or on the surface of the structure and are poorly conserved. The $R_{omit}$ profile analysis is a quite powerful tool in the verification of an MR solution, even in the case of a structure of unknown sequence.

Type 2 RIPs are one of four groups of plant lectins (Wood *et al.*, 1999). They consist of heterodimers linked by a disulfide bond. The A chain inactivates ribosome through its N-glycosidase activity and the B chain, which possesses lectin properties, binds to galactosyl-terminated receptors on the cell surface, facilitating penetration of the A chain into the cytosol (Barbieri *et al.*, 1993). Molecular-replacement studies indicate that TKL-1 belongs to this group of lectins and that the structure of the A chain is more conservative; however, our previous studies also showed that TKL-1 might not possess the same ribosome-inactivating activity as ricin and abrin-a (Sun & Wang, 1994). Its ribosome-inactivating activity has not yet been observed. The appearance of this biological property may be related to the test system used. However, our preliminary structure refinement results indicate that residue Tyr74, which is important for the biological activity of abrin-a, may be replaced with a non-aromatic residue in TKL-1, although other structural details of the proposed active site in RIPs are present in the TKL-1 model. Therefore, it cannot terminate protein synthesis in same way as type 2 RIPs. Kaku *et al.* (1996) have reported a similar case of a sialylated oligosaccharide-specific lectin isolated from *Sambucus sieboldiana* which has a sequence highly homologous to type 2 RIPs, but has no ribosome-inactivating activity.

All the facts above imply that these RIPs and RIP-like proteins, which have similar structures, may play different biological roles in organisms. A question is raised about the evolutionary relationship between such proteins. In order to understand this better, it will be very useful to determine the sequence of TKL-1 and improve the resolution of the structure.

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