Acta Crystallographica Section F
Structural Biology and Crystallization Communications
ISSN 1744-3091

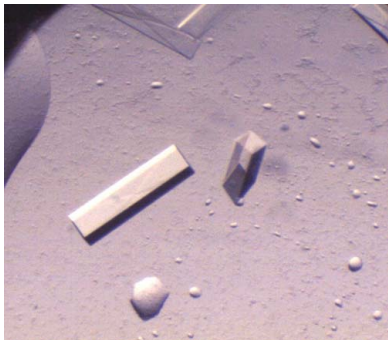
Crystallization and preliminary X-ray studies of a galactose-specific lectin from the seeds of bitter gourd (*Momordica charantia*)

Thyageshwar Chandran, Alok Sharma and M. Vijayan*

Molecular Biophysics Unit, Indian Institute of Science, Bangalore 560 012, India

Correspondence e-mail: mv@mbu.iisc.ernet.in

Received 18 May 2010
Accepted 5 July 2010



A galactose-specific lectin from the seeds of bitter gourd (*Momordica charantia*) is a four-chain type II ribosome-inactivating protein (RIP) resulting from covalent association through a disulfide bridge between two identical copies of a two-chain unit. The available structural information on such four-chain RIPs is meagre. The bitter gourd lectin was therefore crystallized for structural investigation and the crystals have been characterized. It is anticipated that the structure of the orthorhombic crystals will be analysed using molecular replacement by taking advantage of its sequence, and presumably structural, homology to normal two-chain type II RIPs.

1. Introduction

Lectins are a group of proteins that recognize and bind to carbohydrates without modifying them; they specifically recognize diverse sugar structures (Vijayan & Chandra, 1999; Loris, 2002; Sharon, 2007) and mediate a variety of biological processes, including antifungal (Ngai & Ng, 2007), antiproliferative (Zhang *et al.*, 2009) and mitogenic (Wong *et al.*, 2006) activites. They are found in all forms of life and display a variety of folds and quaternary structures (Chandra *et al.*, 2006; Sinha *et al.*, 2007). Those from plants have been the most extensively studied and can be structurally classified into six distinct groups. One of them involves the β-trefoil fold (3D Lectin Database; http://www.cermav.cnrs.fr/lectines).

Type II ribosome-inactivating proteins (RIPs) inhibit translation in eukaryotes by irreversibly inactivating the 28S rRNA and preventing the binding of elongation factor II to the ribosome (Olsnes & Pihl, 1973; Olsnes & Kozlov, 2001; Nielsen & Boston, 2001; Stripe, 2004). The molecules of these proteins are made up of a catalytic chain (chain A) which has rRNA N-glycosidase activity and a lectin chain (chain B) containing two β-trefoil domains. The lectin chain facilitates the internalization of the catalytic chain on binding to the cell surface (Hartley *et al.*, 1996). The chains, both of which have a molecular weight of approximately 30 kDa, are connected by a disulfide bridge. The two-chain type II RIPs of known crystal structure from plants are ricin (Rutenber *et al.*, 1991), abrin (Tahirov *et al.*, 1995), European mistletoe lectin (Eu-ML; Sweeney *et al.*, 1998; Niwa *et al.*, 2003; Jiménez *et al.*, 2005), ebulin (Pascal *et al.*, 2001), Himalayan mistletoe lectin (Hm-RIP; Mishra *et al.*, 2004), *Abrus precatorius* agglutinin (APA; Bagaria *et al.*, 2006) and cinnamomin (Azzi *et al.*, 2009). Of these, Eu-ML and APA can form dimers through the noncovalent association of two typical two-chain type II RIP molecules. In *Ricinus communis* agglutinin (RCA), dimeric association takes place through a disulfide bridge (Sweeney *et al.*, 1997; Hegde & Podder, 1998; Sharma *et al.*, 1998). The only structural information available for such covalently linked four-chain type II RIPs is from the X-ray analysis of RCA at 2.63 Å resolution (PDB code 1rzo; A. G. Gabdoulkhakov, Y. Savochkina, N. Konareva, R. Krauspenhaar, S. Stoeva, S. V. Nikonov, W. Voelter, C. Betzel & A. M. Mikhailov, unpublished work). Therefore, the structure analysis of a lectin of this type from bitter gourd (*Momordica charantia*) was initiated. The isolation, purification and physiochemical studies of the lectin have been reported previously (Mazumder *et al.*, 1981; Das *et al.*, 1981; Khan *et al.*, 1981; Sultan & Swamy, 2003, 2005; Toyama *et al.*, 2008; Tanaka *et al.*, 2009; Kavitha *et al.*, 2010). The lectin is galactose-

specific and is glycosylated (Mazumder *et al.*, 1981) and has a molecular weight of ∼120 kDa. The sequence of the lectin is already known (BAH05018; Tanaka *et al.*, 2009). As in RCA, the two lectin chains in bitter gourd lectin (*M. charantia* lectin; MCL) have identical sequences and the same is true of the two catalytic chains. The lectin chains of RCA and bitter gourd lectin share a sequence identity of 27%, while the sequence identity between the catalytic chains is 39%. The sequence identity of the lectin chains to other type II RIPs of known structure varies between 26 and 35%. The corresponding range for the catalytic chain is 36–47%.

2. Materials and methods

2.1. Protein purification and preliminary studies

Dried ripe bitter gourd (*M. charantia*) seeds were procured from a local supplier. The seeds were ground to a fine powder using a mixer and were defatted by continuous stirring with petroleum ether for 10 h. The slurry was air-dried and dissolved in phosphate-buffered saline (PBS; 20 m*M* anhydrous Na_2HPO_4, 4 m*M* $NaH_2PO_4{\cdot}2H_2O$, 150 m*M* NaCl pH 7.5) and stirred for 10 h at 277 K; the slurry was then passed through double-layered muslin cloth and the filtrate was centrifuged at ∼9000*g* and 277 K for 20 min. The proteins in the supernatant were precipitated by 80% saturated ammonium sulfate, which was followed by centrifugation at ∼9000*g* and 277 K for 30 min. The pellet was redissolved in PBS and dialyzed against the same at 277 K for 10 h. Dialyzed samples were further centrifuged at ∼9000*g* and 277 K for 30 min. The supernatant was applied onto a galactosyl Sepharose 4B column previously equilibrated with PBS. After loading, the column was extensively washed with PBS until the absorbance of the flowthrough at 280 nm was below 0.01. The bound proteins were eluted using 0.4 *M* galactose dissolved in the same buffer. Fractions showing a positive absorbance at 280 nm were pooled together and concentrated using a Centricon with a 10 kDa cutoff at 277 K. The protein was analyzed using 12% SDS–PAGE. The concentrated protein was extensively dialyzed against Tris–HCl buffer (20 m*M* Tris–HCl, 20 m*M* NaCl pH 7.5) and was used for further studies including crystallization.

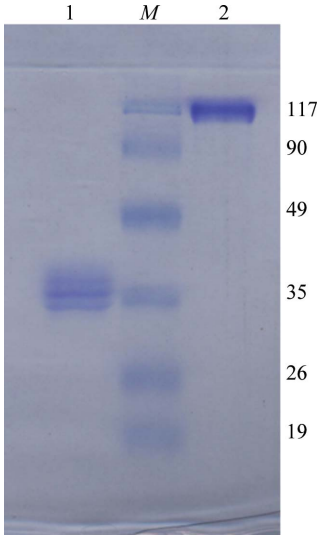


Figure 1
Analysis of *M. charantia* lectin using 12% SDS–PAGE. The lectin was electrophoresed in the presence (lane 1) and in the absence (lane 2) of β-mercaptoethanol. Protein molecular-mass standards (labelled in kDa) were loaded in lane *M*.

Table 1
Unit-cell parameters and X-ray data-collection statistics.

Values in parentheses are for the last resolution shell.

No. of crystals	1
X-ray generator	Bruker Microstar Ultra II rotating anode
Wavelength (Å)	1.5418
Detector	MAR 345
Crystal-to-detector distance (mm)	200
Rotation range per image (°)	1
Total rotation range (°)	170
Exposure time per image (s)	300
Resolution range (Å)	30–2.36 (2.49–2.36)
Space group	$P2_12_12$
Unit-cell parameters (Å)	a = 144.97, b = 134.21, c = 44.67
Mosaicity (°)	0.54
Total No. of measured intensities	219398 (31270)
Unique reflections	36839 (5289)
Multiplicity	6 (5.9)
Mean $I/\sigma(I)$	12.2 (3.7)
Completeness (%)	99.9 (100)
R_{merge}† (%)	11.8 (47.7)
R_{meas}‡ (%)	12.9 (52.5)
Overall B factor from Wilson plot (Å^2)	30.5

† $R_{merge} = \sum_{hkl}\sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$. ‡ $R_{meas} = \sum_{hkl}[N/(N-1)]^{1/2} \times \sum_i |I_i(hkl) - \langle I(hkl)\rangle| / \sum_{hkl}\sum_i I_i(hkl)$.

To test for haemagglutination activity, the purified protein was incubated for 1 h with varying concentrations of different sugars (0.2–50 m*M*) and then mixed with 50 µl native rabbit erythrocytes. The sugars used were β-lactose, D-galactose, D-glucose, D-mannose and sucrose. Mannose-specific banana lectin was used as a positive control, while PBS was used as a negative control.

Circular-dichroism (CD) spectra were recorded at 298 K on a Jasco J-715 spectropolarimeter. Spectra were recorded at a scan speed of 20 nm min^{-1} with a response time of 4 s and a slit width of 1.5 nm. A cylindrical quartz cell of 1 mm path length was used. The spectrum used was the mean of three successive scans. Buffer scans recorded under the same conditions were subtracted from the protein spectra.

2.2. Protein crystallization

An 8 mg ml^{-1} lectin solution was used for crystallization trials. Initial crystallization conditions were explored with Crystal Screens and Crystal Screen 2 from Hampton Research using the microbatch

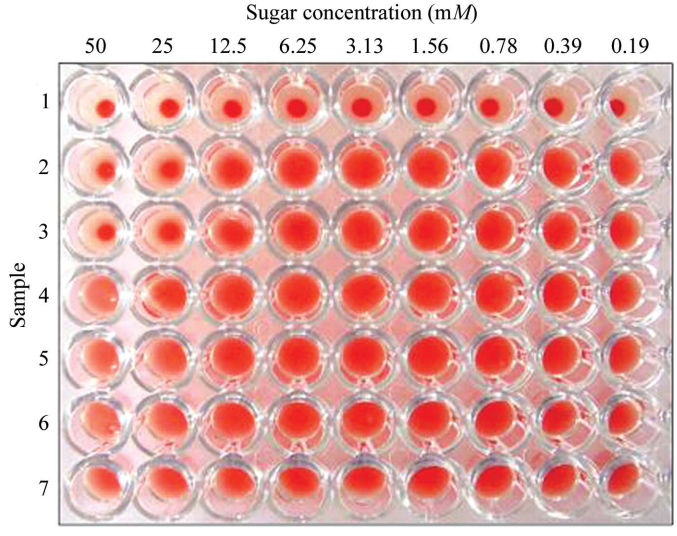


Figure 2
Haemagglutination-inhibition test. Row 1, PBS. Rows 2–6, *M. charantia* lectin incubated with β-lactose, D-galactose, D-glucose, D-mannose and sucrose, respectively. Row 7, banana lectin (positive control).

crystallization method. Typically, for each condition, 1 µl protein solution was mixed with an equal volume of precipitant solution in 96-well microbatch plates containing paraffin oil and silicone oil in equal proportions. Positive conditions obtained were further optimized using both the hanging-drop and the microbatch crystallization methods. Rectangular rod-shaped crystals were obtained using the microbatch crystallization method with 20%(*w*/*v*) polyethylene glycol 10 000 as the precipitant and 0.1 *M* HEPES pH 7.5 as the buffer.

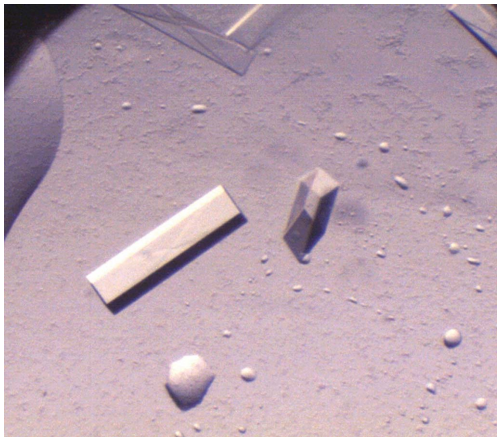


Figure 3
Crystals of *M. charantia* lectin grown by the sitting-drop vapour-diffusion method. Crystals grew to maximum dimensions of approximately 0.7 × 0.16 × 1.6 mm.

```
MCL          --MRMRVLAVYIVVALSLTINGIECNLSLSQSNFSADTYKSFIKNLRKQLTIGASYGSAG
RCA          ------IFPK-----------QYPIINFTTADATV-ESYTNFIRAVRSHLTTGA-DVRHE
APA          ----------------------QDPIKFTTGSATP-ASYNQFIDALRERLTGG---LIYG
Eu-ML        ----------------------YERLRLRVTHQTTGDEYFRFITLLRDYVSSGS--FSNE
Hm-RIP       ----------------------YERLDLDVTSQTTGEEYFRFITLLRDYVSSGS--FSNE
Ebulin       ---------------------IDYPSVSFNLAGAKS-TTYRDFLKNLRDRVATGT-YEVNG
Ricin        ------IFPK-----------QYPIINFTTAGATV-QSYTNFIRAVRGRLTTGA-DVRHE
Abrin        ----------------------EDRPIKFSTEGATS-QSYKQFIEALRERLRGG---LIHD
Cinnamomin   YLQLLILLPSSPFMVVEPHTLNYQTVTFTTKNATK-TSYTQFIEALRAQLASGE-EP-HG
                                                  *  *:  :*  :  *

MCL          IPILKHS---VPICERFLLVDLTNGDNET-ITLAINVEDAGFAAYRAADRSYFFQNAPPI
RCA          IPVLPNR-VGLPISQRFILVELSNHAELS-VTLALDVTNAYVVGCRAGNSAYFFHPDNQE
APA          IPVLRDP-STVEKPNQYVTVELSYSDTVS-IQLGIDLTNAYVVAYRAGSESFFFRNAPAS
Eu-ML        IPLLRQSTIPVSDAQRFVLVELTNQGGDS-ITAAIDVTNLYVVAYQAGDQSYFLRDAPRG
Hm-RIP       IPLLRQSGGGV-EAARFVLVELTNEGGDS-ITAAIDVTNLYVVAYQAGSQSYFLS----G
Ebulin       LPVLRRE-SEVQVKNRFVLVRLTNYNGDT-VTSAVDVTNLYLVAFSANGNSYFFKDATEL
Ricin        IPVLPNR-VGLPINQRFILVELSNHAELS-VTLALDVTNAYVVGYRAGNSAYFFHPDNQE
Abrin        IPVLPDP-TTLQERNRYITVELSNSDTES-IEVGIDVTNAYVVAYRAGTQSYFLRDAPSS
Cinnamomin   IPVMRER-STVPDSKRFILVELSNWAADSPVTLAVDVTNAYVVAYRTGSQSFFLREDNPD
             :*::      :    ::: * *:     : :  .::: :  ...  :   ::*:

MCL          ---ASYVIFTDTN-QNIMNFNNTFESIEIVGGTTRSETPLGIMHFEASIF-----HLFVH
RCA          DAEAITHLFTDVQNSFTFAFGGNYDRLEQLGG-LRENIELGTGPLEDAISALYYYSTCGT
APA          ---ASTYLFTGTQ-QYSLPFDGNYDDLEKWAHQSRQRISLGLEALRQGIK---FLRSGAS
Eu-ML        ---AETHLFTGTT-RSSLPFNGSYPDLERYAG-HRDQIPLGIDQLIQSVT---ALRFPGG
Hm-RIP       ---PGTHLFTGTT-RSSLPFNGSYPDLEQYAG-HRKQIPLGIDQLIQSVT---ALRFPG-
Ebulin       ---QKSNLFLGTT-QHTLSFTGNYDNLETAAGTRRESIELGPNPLDGAIT---SLWYDG-
Ricin        DAEAITHLFTDVQNRYTFAFGGNYDRLEQLAGNLRENIELGNGPLEEAISALYYYSTGGT
Abrin        ---ASDYLFTGTD-QHSLPFYGTYGDLERWAHQSRQQIPLGLQALTHGIS---FFRSGGN
Cinnamomin   --PAIENLLPDTK-RYTFPFSGSYTDLERVAGERREEILLGMDPLENAISALWISNLN--
                    :: ..     : * ..:  :*  .   *.   **   :  .:

MCL          DENYVPTSFLVLIQMVLEAAKFKFIEQKVIHSIMDMEDFTPGLAMLSLEENWTQLSLQLQ
RCA          QIPTLARSFMVCIQMISEAARFQYIEGEMRTRIRYNRRSAPDPSVITLENSWGRLSTAIQ
APA          DDEEIARTLIVIIQMVAEAARFRYVSKLVVISLSNRAAFQPDPSMLSLENTWEPLSRAVQ
Eu-ML        STRTQARSILILIQMISEAARFNPILWRARQYINSGASFLPDVYMLELETSWGQQSTQVQ
Hm-RIP       NTRTQARSILILIQMISEAARFNPILWRARQYINSGASFLPDVYMLELETSWGQQSTQVQ
Ebulin       ---GVARSLLVLIQMVPEAARFRYIEQEVRRSLQQLTSFTPNALMLSMENNWSSMSLEVQ
Ricin        QLPTLARSFIICIQMISEAARFQYIEGEMRTRIRYNRRSAPDPSVITLENSWGRLSTAIQ
Abrin        DNEEKARTLIVIIQMVAEAARFRYISNRVRVSIQTGTAFQPDAAMISLENNWDNLSRGVQ
Cinnamomin   QQRALARSLIVVIQMVAEAVRFRFIEYRVRESISRAEMFRPDPAMLSLENKWSALSNAVQ
                   . :::: ***: **.:*. :       :       *.  :: :* .*   *  :*

MCL          ASESLNGVFGDSVSLYNSMDEPIGVDSMYY--------------------------
RCA          ESNQG--AFASPIQLQRRNGSKFNVYDVS--ILIPIIALMVYRCAPPP--------
APA          HTVQD--TFPQNVTLINVRQERVVVSSLSH-PSVSALALMLFVCNPLNATQSPLLI
Eu-ML        HSTDG--VFNNPIRLAIPPGNFVTLTNVR--DVIASLAIMLFVCGE----------
Hm-RIP       QSTEG--VFNNPIRLAIP-GNFVTLTNVR--DVIASLAIMLFVC------------
Ebulin       LSGDNVSPFSGTVQLQNYDHTPRLVDNFEELYKITGIAILLFRCVATKT-------
Ricin        ESNQG--AFASPIQLQRRNGSKFSVYDVS--ILIPIIALMVYRCAPPPSSQF----
Abrin        ESVQD--TFPNQVTLTNIRNEPVIVDSLSH-PTVAVLALMLFVCNPPN--------
Cinnamomin   QSNQGG-VFSSPVELRSISNKPVYVGSVSD-RVISGLAIMLFICRSTD--------
              : .    *   : *         : ..
```

(*a*)

```
MCL          PILTANMAFQLYQCPYGVIRMMPTITMPNQNNEQCSP-QQRTTRISGRDGLCVDVYGALT
RCA          ------------------------------ADVCMD-PEPIVRIVGRNGLCVDVTGEEF
APA          ----------------------------VVEQSKICSSHYEPTVRIGGRDGLCVDVSDNAY
Eu-ML        ----------------------------DAVTCTA-SEPIVRIVGRNGMTVDVRDDDF
Hm-RIP       ----------------------------CSA-SEPTVRIVGRNGMNVDVRDDDF
Ebulin       ----------------------------DGETCAIPAFFTRRIVGRDGLCVDVRNGYD
Ricin        ------------------------------ADVCMD-PEPIVRIVGRNGLCVDVRDGRF
Abrin        ----------------------IVEKSKICSSRYEPTVRIGGRDGMCVDVYDNGY
Cinnamomin   -RASSDQFIDHLLMIRPILADVADVATDADNDDTCAD-PEPTVRISGRNGLCVRVRDGKY
                                                *       ** **:*: * * .

MCL          ADGSRVILYPCGQQQ--NQQWTFYPDNTIRSLGKCLATSALSSGSNVVITNCDYLRYDDG
RCA          FDGNPIQLWPCKSNTDWNQLWTLRKDSTIRSNGKCLTISKSSPRQQVVIYNCSTATVGAT
APA          NNGNPIILWKCKDQLEVNQLWTLKSDKTIRSKGKCLTTYGYAPGNYVMIYDCSSAVAEAT
Eu-ML        HDGNQIQLWPSKSNNDPNQLWTIKKDGTIRSNGSCLTTYGYTAGVYVMIFDCNTAVREAT
Hm-RIP       HDGNQIQLWPSKSNNDPNQLWTIKRDGTIRSNGSCLTTYGYTAGVYVMIFDCNTAVREAT
Ebulin       TDGTPIQLWPCGTQR--NQQWTFYNDKTIRSMGKCMTANGLNSGSYIMITDCSTAAEDAT
Ricin        HNGNAIQLWPCKSNTDANQLWTLKRDNTIRSNGKCLTTYGYSPGVYVMIYDCNTAATDAT
Abrin        HNGNRIIMWKCKDRLEENQLWTLKSDKTIRSNGKCLTTYGYAPGSYVMIYDCTSAVAEAT
Cinnamomin   NNGNPIQLWPCKQNSDVNQLWTLRRDGTIRSNGKCLTTNGYSAGDYVMIYDCRTPVTAAS
              :*. : :: .  .   ** **:  * **** *.*::      .   ::* :*

MCL          -WMVSSSGTMMNKSSHLVLTANAATSRTNLTGENNVFAAKQAWRIGNYVEPIVTTIIGLR
RCA          RWQIWDNRTIINPRSGLVLAATSGNSGTKLTVQTNIYAVSQGWLPTNNTQPFVTTIVGLY
APA          YWDIWDNGTIINPKSGLVLSAESSSMGGTLTVQKNDYRMRQGWRTGNDTSPFVTSIAGFF
Eu-ML        IWQIWGNGTIINPRSNLVLAASSGIKGTTLTVQTLDYTLGQGWLAGNDTAPRETTIYGFR
Hm-RIP       IWQIWGNGTIINPRSNLALAASSGIKGTTLTVQTLDYTLGQGWLAGNDTAPREVTIYGFN
Ebulin       KWEVLIDGSIINPSSGLVMTAPSGASRTTLLLENNIHAASQGWTVSNDVQPIATLIVGYN
Ricin        RWQIWDNGTIINPRSSLVLAATSGNSGTTLTVQTNIYAVSQGWLPTNNTQPFVTTIVGLY
Abrin        YWEIWDNGTIINPKSALVLSAESSSMGGTLTVQTNEYLMRQGWRTGNNTSPFVTSISGYS
Cinnamomin   IWQFWANGTIINPQSALVLSAESGNPRTTLTVQADIYASRQGWLAGNNTEPFVTSIVGFN
              * .  . :::*  * *.::* :.    .*  :   .   *.*   * . *  . * *

MCL          HMCLEATDNDTNVWLESCVKNKTKQYWALYSDDTIRVNNNRNLCVSS-STDSSSKLIVIR
RCA          GMCLQANS--GKVWLEDCTSEKAEQQWALYADGSIRPQQNRDNCLTT-DANIKGTVVKIL
APA          KLCMEAHG--NSMWLDVCDITKEEQQWAVYPDGSIRPVQNTNNCLTC-EEHKQGATIVMM
Eu-ML        DLCMESAG--GSVYVETCTAGQENQRWALYGDGSIRPKQLQSQCLTN-GRDSISTVINIV
Hm-RIP       DLCMESNG--GSVWVETCVS-QQNDRWALYGDGSIRPEQNQDQCLTS-GRDSVAG-INIV
Ebulin       EMCLQANGENNNVWMEDCDVTSVQQQWALFDDRTIRVNNSRGLCVTS-NGYVSKDLIVIR
Ricin        GLCLQANS--GQVWIEDCSSEKAEQQWALYADGSIRPQQNRDNCLTS-DSNIRETVVKIL
Abrin        DLCMQAQG--SNVWMADCDSNKKEQQWALYTDGSIRSVQNTNNCLTS-KDHKQGSTILLM
Cinnamomin   DLCMQANG--DAMWVVECESSKAEQKWALYPDGSIRPHQDRDRCLTSTDNHSQGSIIIIS
              :*::: .    :::  *   . :: **:: * :**  :  . *::          : :

MCL          RCDG-SINQRWVFTPQGTISNPGYEAVMDVAQNDVYLKKIVLSSATDKGNGQQWTVFY--
RCA          SCGPASSGQRWMFKNDGTILNLYNGLVLDVRRSDPSLKQIIVHPFHGNLN-QIWLPLF--
APA          GCSNAWASQRWVFKSDGTIYNLYDDMVMDVKSSDPSLKQIILWPYTGNAN-QMWATLF--
Eu-ML        SCSAGSSGQRWVFTNEGAILNLKNGLAMDVAQANPSLQRIIIYPATGNPN-QMWLPVP--
Hm-RIP       SCSGGSSGQRWVFTNEGAILNLKNGLAMDVA--NPGLGQIIIYPATGKPN-QMWLPVP--
Ebulin       KCQG-LATQRWFFNSDGSVVNLKSTRVMDVKESDVSLQEVIIFPATGNPN-QQWRTQVPQ
Ricin        SCGPASSGQRWMFKNDGTILNLYSGLVLDVRASDPSLKQIILYPLHGDPN-QIWLPLF--
Abrin        GCSNGWASQRWVFKNDGSIYSLYDDMVMDVKGSDPSLKQIILWPYTGKPN-QIWLTLF--
Cinnamomin   SCSPGSEGQRWVFMNDGTILNLKNGLVMDVKGSDPSLHQIIIWPATGKPN-QKWLPLL--
              *      ***.*  :*:: .     .:**   :  * .::: .  .. * * *
```

(*b*)

Figure 4
Sequence alignment of (*a*) chain A and (*b*) chain B of type II RIPs of known structure together with that of bitter gourd lectin. The alignment was performed using *ClustalW* (Larkin *et al.*, 2007).

2.3. Data collection, processing and preliminary X-ray analysis

Cryoprotection of the crystals prior to data collection was achieved by soaking individual crystals for ∼1 min in crystallization solution containing 25%(*v*/*v*) glycerol. The diffraction data were collected at 100 K. The data were processed using *MOSFLM* (Leslie, 1992) and scaled using *SCALA* from the *CCP*4 program suite (Collaborative Computational Project, Number 4, 1994). The crystal parameters and data-processing statistics are summarized in Table 1.

3. Results and discussion

In the presence of 10 m*M* β-mercaptoethanol (β-Me) three closely spaced bands of ∼35 kDa were observed on 12% SDS–PAGE, while in the absence of β-Me a single band of ∼120 kDa was observed (Fig. 1). The matrix-assisted laser desorption/ionization–time-of-flight (MALDI–TOF) mass spectrum of the protein showed a prominent peak at 127 kDa and a less prominent peak at 64 kDa. The CD spectrum, as for other type II RIPs and as also reported by Kavitha *et al.* (2010) in the case of bitter gourd lectin, indicated that the structure contains helices as well as sheets. The haemagglutination inhibition test with various sugars confirmed that the protein has affinity towards lactose as well as galactose, which is in agreement with previous studies (Mazumder *et al.*, 1981). Agglutination did not take place until the sugar concentration was increased to 12.5 m*M* (Fig. 2). Glucose, mannose and sucrose did not inhibit agglutination. Since glucose did not inhibit the agglutination, it can be presumed

that the protein interacts with lactose *via* the galactose end. Crystals of the protein initially appeared after 3 d and grew to maximum dimensions in about one month (Fig. 3). The crystals were orthorhombic and diffracted to a resolution of 2.36 Å at the home source (Table 1). The Matthews coefficient (3.96 $Å^3\ Da^{-1}$) indicates the possibility of half of a twofold-symmetric molecule, with a molecular weight of ∼60 000, in the asymmetric unit, with a solvent content of ∼64% (Matthews, 1968). In terms of amino-acid sequence (Fig. 4), the lectin is closest to ebulin among type II RIPs of known structure, with sequence identities of 35 and 47% for the lectin and catalytic chains, respectively. Therefore, the ebulin molecule is the obvious choice as a search model for the structure solution of bitter gourd lectin using molecular replacement.

The data sets used in the present work were collected at the X-ray Facility for Structural Biology at the Institute, supported by the Department of Science and Technology (DST). MV was successively a Distinguished Biotechnology Research Professor of the DBT and a DAE Homi Bhabha Professor during the period of this investigation. Financial support from DST is acknowledged.

References

Azzi, A., Wang, T., Zhu, D.-W., Zou, Y.-S., Liu, W.-Y. & Lin, S.-X. (2009). *Proteins*, **74**, 250–255.
Bagaria, A., Surendranath, K., Ramagopal, U. A., Ramkumar, S. & Karande, A. A. (2006). *J. Biol. Chem.* **281**, 34465–34474.
Chandra, N. R., Kumar, N., Jeyakani, J., Singh, D. D., Gowda, S. B. & Prathima, M. N. (2006). *Glycobiology*, **16**, 938–946.
Collaborative Computational Project, Number 4 (1994). *Acta Cryst.* D**50**, 760–763.
Das, M. K., Khan, M. I. & Surolia, A. (1981). *Biochem. J.* **195**, 341–343.
Hartley, M. R., Chaddock, J. A. & Bonness, M. S. (1996). *Trends Plant Sci.* **1**, 254–260.
Hegde, R. & Podder, S. K. (1998). *Eur. J. Biochem.* **254**, 596–601.
Jiménez, M., Saiz, J. L., André, S., Gabius, H.-J. & Solís, D. (2005). *Glycobiology*, **15**, 1386–1395.
Kavitha, M., Bobbili, K. B. & Swamy, M. J. (2010). *Biochimie*, **92**, 58–64.
Khan, M. I., Mazumder, T., Pain, D., Gaur, N. & Surolia, A. (1981). *Eur. J. Biochem.* **113**, 471–476.
Larkin, M. A., Blackshields, G., Brown, N. P., Chenna, R., McGettigan, P. A., McWilliam, H., Valentin, F., Wallace, I. M., Wilm, A., Lopez, R., Thompson, J. D., Gibson, T. J. & Higgins, D. G. (2007). *Bioinformatics*, **23**, 2947–2948.
Leslie, A. G. W. (1992). *Jnt CCP4/ESF–EACBM Newsl. Protein Crystallogr.* **26**.
Loris, R. (2002). *Biochim. Biophys. Acta*, **1572**, 198–208.
Matthews, B. W. (1968). *J. Mol. Biol.* **33**, 491–497.
Mazumder, T., Gaur, N. & Surolia, A. (1981). *Eur. J. Biochem.* **113**, 463–470.
Mishra, V., Ethayathulla, A. S., Sharma, R. S., Yadav, S., Krauspenhaar, R., Betzel, C., Babu, C. R. & Singh, T. P. (2004). *Acta Cryst.* D**60**, 2295–2304.
Ngai, P. H. K. & Ng, T. B. (2007). *Appl. Microbiol. Biotechnol.* **74**, 366–371.
Nielsen, K. & Boston, R. S. (2001). *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **52**, 785–816.
Niwa, H., Tonevitsky, A. G., Agapov, I. I., Saward, S., Pfüller, U. & Palmer, R. A. (2003). *Eur. J. Biochem.* **270**, 2739–2749.
Olsnes, S. & Kozlov, J. V. (2001). *Toxicon*, **39**, 1723–1728.
Olsnes, S. & Pihl, A. (1973). *Eur. J. Biochem.* **35**, 179–185.
Pascal, J. M., Day, P. J., Monzingo, A. F., Ernst, S. R., Robertus, J. D., Iglesias, R., Pérez, Y., Férreras, J. M., Citores, L. & Girbés, T. (2001). *Proteins*, **43**, 319–326.
Rutenber, E., Katzin, B. J., Ernst, S., Collins, E. J., Mlsna, D., Ready, M. P. & Robertus, J. D. (1991). *Proteins*, **10**, 240–250.
Sharma, S., Bharadwaj, S., Surolia, A. & Podder, S. K. (1998). *Biochem. J.* **333**, 539–542.
Sharon, N. (2007). *J. Biol. Chem.* **282**, 2753–2764.
Sinha, S., Gupta, G., Vijayan, M. & Surolia, A. (2007). *Curr. Opin. Struct. Biol.* **17**, 498–505.
Stripe, F. (2004). *Toxicon*, **44**, 371–383.
Sultan, N. A. M. & Swamy, M. J. (2003). *Curr. Sci.* **84**, 200–203.
Sultan, N. A. M. & Swamy, M. J. (2005). *Arch. Biochem. Biophys.* **437**, 115–125.
Sweeney, E. C., Tonevitsky, A. G., Palmer, R. A., Niwa, H., Pfueller, U., Eck, J., Lentzen, H., Agapov, I. I. & Kirpichnikov, M. P. (1998). *FEBS Lett.* **431**, 367–370.
Sweeney, E. C., Tonevitsky, A. G., Temiakov, D. E., Agapov, I. I., Saward, S. & Palmer, R. A. (1997). *Proteins*, **28**, 586–589.
Tahirov, T. H., Lu, T.-H., Liaw, Y.-C., Chen, Y.-L. & Lin, J.-Y. (1995). *J. Mol. Biol.* **250**, 354–367.
Tanaka, H., Toyama, J. & Akashi, R. (2009). *Asian. J. Plant Sci.* **8**, 544–550.
Toyama, J., Tanaka, H., Horie, A., Uchiyama, T. & Akashi, R. (2008). *Asian J. Plant Sci.* **7**, 647–653.
Vijayan, M. & Chandra, N. (1999). *Curr. Opin. Struct. Biol.* **9**, 707–714.
Wong, J. H., Wong, C. C. & Ng, T. B. (2006). *Biochim. Biophys. Acta*, **1760**, 808–813.
Zhang, G. Q., Sun, J., Wang, H. X. & Ng, T. B. (2009). *Acta Biochim. Pol.* **56**, 415–421.