Amino Acids (2012) 43:973–981
DOI 10.1007/s00726-011-1162-1

ORIGINAL ARTICLE

Balsamin, a novel ribosome-inactivating protein from the seeds of Balsam apple *Momordica balsamina*

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Received: 10 July 2011 / Accepted: 14 November 2011 / Published online: 26 November 2011

Abstract Plant seeds, a rich source of proteins, are considered important for their application as functional ingredients in a food system. A novel ribosome-inactivating protein (RIP), balsamin was purified from the seeds of Balsam apple, *Momordica balsamina*. Balsamin was purified by ion exchange chromatography on CM Sepharose and gel filtration on superdex-75. It has a molecular weight of 28 kDa as shown by SDS-PAGE analysis. Balsamin inhibits protein synthesis in a rabbit reticulocyte lysate-based cell free translation assay with an IC_{50} of 90.6 ng ml^{-1}. It has RNA *N*-glycosidase activity and releases a 400-base long fragment termed the Endo fragment from 28S rRNA in the same manner as does saporin-6 from *Saponaria officinalis*. The N-terminal sequence analysis of the first 12 amino acids of balsamin revealed that it shares 83% similarity with type I RIP α-MMC from *Momordica charantia* and 50% similarity with β-MMC (from *Momordica charantia*), bryodin I (from *Bryonia dioica*) and luffin a (from *Luffa cylindrica*). Balsamin was further characterized by mass spectrometry. CD spectroscopic studies indicate that secondary structure of balsamin contains helix (23.5%), β-strand (24.6%), turn (20%) and random coil (31.9%). Thus RIPs activity expressed in vegetables like *Momordica* sp. advocates its usage in diet.

Keywords Ribosome inactivating protein (RIP) · *Momordica balsamina* · RNA *N*-glycosidase · Balsamin · Cucurbitaceae

Introduction

Ribosome-inactivating proteins (RIPs) are a unique family of proteins isolated from the seeds of many plants and are abundant in angiosperms (Stripe and Barbieri 1986). RIPs possess RNA *N*-glycosidase activity (EC 3.2.2.22) that is involved in specific cleavage of a single N–C glycosidic bond in universally conserved region of large ribosomal ribonucleic acid (rRNA). This depurination of rRNA irreversibly inactivates the ribosomes by restricting their binding to elongation factors, thereby blocking their participation in protein synthesis (Endo et al. 1987). RIPs are classified into two groups based on the difference in their primary structure. Type I RIPs consist of a single polypeptide chain of approximately 26–35 kDa that possesses RNA *N*-glycosidase activity. Type II RIPs consist of two subunits, an A-chain similar to type I and a lectin subunit B-chain that recognizes receptors on the membranes and facilitates endocytosis. Though all RIPs share the identical enzymatic activity, they differ in their biological activities. Some RIPs display a variety of antimicrobial activities in

Electronic supplementary material The online version of this article (doi:10.1007/s00726-011-1162-1) contains supplementary material, which is available to authorized users.

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vitro, such as antifungal, antibacterial and broad spectrum antiviral activities against human and animal viruses (Stripe et al. 1992). RIPs are of great interest because of their potential application in medical biotechnology.

Majority of RIPs are found in plant families such as Euphorbiaceae, Phytolaccaceae, Cucurbitaceae and Caryophyllaceae. In the Cucurbitaceae family, several RIPs such as bryodin from *Bryonia dioica*, luffin from *Luffa cylindrica*, trichosanthin from *Trichosanthes kirilowii*, momorcharin and MAP30 from *Momordica charantia* have been reported and investigated for their potential medicinal usage. Plant RIPs have been shown to possess multiple biological activities such as anti-tumor, anti-human immunodeficiency virus and insecticidal properties (Ng et al. 2010). RIPs are also used in crop biotechnology with the aim of increasing resistance to insect, fungal and viral pathogens (Hong et al. 1996). The A-chains of type-II and type-I RIP have been linked to antibodies to construct immunotoxins that are specifically toxic to the target cell. Immunotoxins are potentially useful in cancer and AIDS therapy (Ramakrishan et al. 1992). The type-II RIP ricin has been used as potential anti-cancer agent (Vitetta et al. 1987). Recently, interest in RIPs has been growing on account of their anti-viral activities (Au et al. 2000).

Momordica balsamina (aka Balsam apple, bitter cucumber or bitter melon) a high-climbing vine from family Cucurbitaceae is native to the tropical regions of Africa, Asia, Arabia and Caribbean. This plant is a monoecious climber and found in India up to an altitude of 300 m. It has been widely used traditionally in Africa to treat various diseases such as diabetes and malaria (Ramalhete et al. 2011). A nucleic acid sequence submitted from this plant indicating its homology to other proteins with *Momordica* sp. remained inconclusive (Ortigao and Better 1992). Leaves and fruit extracts of this plant have displayed hypoglycemic, anti-inflammatory and analgesic effect in rats (Karumi and Bobboi 1999; Karumi et al. 2003). The solvent extract of *M. balsamina* has shown anti-malarial activity in vitro and in vivo without any toxicity in healthy mice (Benoit-Vical et al. 2006). Fruit pulp extract of this plant has given valuable information on anti-HIV property (Bot et al. 2007); however, such claims require validation by sensitive techniques (Puri 2010). Here we report the purification and characterization of balsamin, a novel type-I RIP, from the seeds of *M. balsamina*.

Materials and methods

Materials

Momordica balsamina seeds were procured from National seed stock and cultivated in Botanical garden, Punjabi University, India. Plant specimens were identified and confirmed by senior taxonomist. Gels for ion exchange and gel filtration chromatography were obtained from Sigma (St. Louis, US). All other chemicals were standard commercial products of analytical grade.

Purification of balsamin

Seeds (20 g) of *M. balsamina* were decorticated and ground by mortar and pestle to a powder form. The powder (20 g) was homogenized in 50 ml 150 mM NaCl. The mixture was stirred gently at 4°C. The slurry was filtered through muslin cloth and centrifuged at 10,000×*g* for 20 min at 4°C. The clear supernatant was collected and the proteins were precipitated by slow addition of solid ammonium sulfate (0–60%) with constant stirring using a magnetic stirrer at 4°C. After 8 h, the crude extract was centrifuged at 10,000×*g* for 10 min at 4°C. The supernatant was removed and resulting precipitate was dissolved in 15 ml of 10 mM phosphate buffer, pH 6.5 (Buffer A) and then dialyzed against same buffer. The protein solution was loaded onto a CM-Sepharose fast flow column (20 cm × 1.5 cm), which was equilibrated with five column volumes of buffer A. The column was then washed with the same buffer at a flow rate of 1 ml min^{-1} until no protein eluted. Bound proteins were eluted with a linear gradient of 0–0.4 M NaCl in 10 mM phosphate buffer, pH 6.5 (Buffer B). The fractions that contained low-molecular mass proteins (based on SDS-PAGE analysis) were pooled. The fractions were concentrated by ultrafiltration using Amicon Ultra-15 10 kDa membrane. Concentrated fractions were loaded onto a superdex 75 column (10/300 GL, Amersham Biosciences Co., Piscataway, USA), prior equilibrated with (10 mM phosphate buffer, pH 6.5). The protein was eluted with 10 mM phosphate buffer, pH 6.5 at a flow rate of 0.5 ml min^{-1}. The eluted fractions that showed *N*-glycosidase activity were pooled, concentrated and stored at −20°C.

Protein concentrations of crude extract and fractionated sample at each step were calculated using the Bradford method (Bradford 1976).

SDS-PAGE

The molecular weight of balsamin was determined by SDS-PAGE performed according to the procedure of Laemmli (Laemmli 1970), using a 12% resolving gel. After electrophoresis, the gel was stained with Coomassie brilliant blue R-250 (CBB). The molecular mass of the balsamin was determined by comparison with the protein markers; myosin (250 kDa), phosphorylase b (148 kDa), bovine serum albumin (66 kDa), glutamate dehydrogenase

(64), alcohol dehydrogenase (50), carbonic anhydrase (36), myoglobin red (22), lysozyme (16) and aprotinin (6).

rRNA *N*-Glycosidase activity assay

The assay was carried out as previously described (Bagga et al. 2003; May et al. 1989). Rabbit reticulocyte lysate was taken as a source of ribosome. Rabbit reticulocyte lysate (50 µl) was treated with different concentration of balsamin (10, 20 and 50 µg ml^{-1}) and incubated at 30°C for 30 min. The reaction was terminated by the addition of 10% SDS (w/v). Total rRNA was extracted with trizol reagent (Biorad). The RNA pellet was dissolved in 20 µl of water and divided into two parts. One part was treated with aniline acetate, pH 4.5, whereas the other part was left untreated. The aniline-treated and untreated samples were electrophoresed on 2% agarose gel. The gel was stained with ethidium bromide and the RNA was visualized on a UV-transilluminator.

Cell-free protein synthesis inhibition assay

The assay was performed according to the procedure described (Bagga et al. 2003; Sambrook et al. 1989). In the cell-free translation assay, 6 µl of the protein solution was incubated with rabbit reticulocyte lysate at 30°C for 60 min. The reaction was stopped by NaOH (250 µl, 1 N) containing H_2O_2 (0.2%). After further incubation at 37°C for 10 min, the proteins were precipitated with trichloroacetic acid (15%) on ice for 30 min and harvesting was done on 26 mm glass fiber filters (Whatman). The dried filters were counted using a liquid scintillation counter. Saporin (a type I RIP) from *Saponaria officinalis* was used as a positive control (Bagga et al. 2003).

N-terminal sequencing

The sequence of first 20 amino acids from N-terminal end was determined by Edman degradation method using an automated protein sequencer. Protein was subjected to SDS-PAGE using 12% separating gel. Blotting was performed at 350–400 mA for 35 min using polyvinyl fluoride membrane. After the transfer the membrane was stained and de-stained and then washed extensively with Milli-Q water. Protein blots were loaded on Blott™ cartridge (reaction chamber) of Applied Biosystems PROCISE 491 cLC Protein Sequencer and amino acid sequencing was conducted with protein sequencer.

Mass spectrometric analysis and protein identification

In gel trypsin digestion: The protein spots from coomassie stained gel were cut into 1 mm^3 pieces and transferred into a sterile micro centrifuge tube. In gel trypsin digestion was done as per the described protocol (Promega). The digested and extracted peptides were spotted onto MALDI sample plate and mixed with equal volume of α-cyano-4-hydroxyl-cinnamic acid matrix solution (10 mg/mL) in 0.1% TFA and 50% ACN. Peptide mass spectra were obtained using a MALDI-TOF/TOF 5800 mass spectrometer (ABSciex, CA) operating in reflectron mode over a window of *m/z* 700 to *m/z* 4,000. A combined MS peptide fingerprint and MS/MS peptide sequencing search was performed against the SwissProt 51.6 databases using the ProteinPilot software v4.0 (Applied Biosystems) via MASCOT search engine v2.2. Tryptic digestion with a maximum of one missed cleavage was considered. The search parameters allowed oxidation of methionine and carboxyamidomethylation of cysteine. The monoisotopic precursor ion tolerance was set to 100 ppm and the MS/MS ion tolerance to 0.4 Da. Protein identifications were accepted with a statistically significant probability based Mowse score ($p \leq 0.01$).

Sequence alignment

For multiple sequence alignment of balsamin RIP amino acid sequences, a search for sequence similarities was performed with BLAST program available on-line (http://www.ncbi.nlm.nih.gov/BLAST). Sequence submitted was then aligned using Clustal W software in the default set up and alignment was analyzed.

CD spectroscopy

CD experiments were performed on Jasco J-815 spectropolarimeter. CD spectrum of the purified balsamin (1 mg ml^{-1}) prepared in a sodium phosphate buffer (10 mM, pH 6.5) was measured in the far-UV range (200–240 nm) in 1 mm path length cuvettes. Analysis of the protein CD spectrum was performed using Spectra manager™ software (Jasco).

Results

Purification of balsamin

The proteins were precipitated by 60% ammonium sulfate saturation from *M. balsamina* seed extract. The protein precipitates were dialyzed against the buffer A and subjected to CM-Sepharose fast-flow column which was previously equilibrated with 10 mM phosphate buffer, pH 6.5 (Buffer A). Most of the protein was retained on the CM-Sepharose column; this indicates that proteins collected are mainly basic in nature. One major peak (BI) and

two minor peaks (BII and BIII) were obtained using NaCl linear gradient (0.1 and 0.2 M) to elute bound proteins. The elution at 0.1 M NaCl resulted in one protein peak, peak BI, whereas 0.2 M NaCl gave rise to two peaks (BII and BIII) (Fig. 1). Negligible amount of protein was eluted at higher salt concentrations. On SDS-PAGE peak BI contained balsamin (data not shown). The fractions from peak BI were pooled and after being desalted, further purification of those fractions was achieved using superdex 75 gel-filtration column. Four peaks (PI–PIV) were obtained (Fig. 2). Table 1 summarizes the results of purification of balsamin indicating that 1 mg balsamin was purified. The purified fraction PII was subsequently found to possess rRNA *N*-glycosidase and cell-free protein synthesis inhibition activity. The purified balsamin from *M. balsamina* showed single band with a molecular weight corresponding to ~28 kDa by 12% SDS-PAGE under denaturing conditions (Fig. 3).

N-terminal sequencing

The first 12 amino acid residues from the N-terminal sequence of balsamin are DVSFTLSGADPS which showed homology with those of other type I RIPs in the Cucurbitaceae family recorded in GenBank (Table 2). The sequence is similar to that of α-MMC (from *Momordica charantia*; 83% amino acid identity) (Fong et al. 1996), somewhat similar with TCS, TAP29 and trichokirin (from *Trichosanthes kirilowii*; 66 and 58% amino acid identity, respectively) (Shaw et al. 1991; Lee-Huang et al. 1991; Casellas et al. 1988). Balsamin has 50% similarity to several type-I RIPs purified from the Cucurbitaceae family such as *β*-MMC (from *Momordica charantia*) (Fong et al. 1996); bryodin I (from *Bryonia dioica*) (Gawlak et al. 1997) and luffin a (from *Luffa cylindrica*) (Islam et al. 1990).

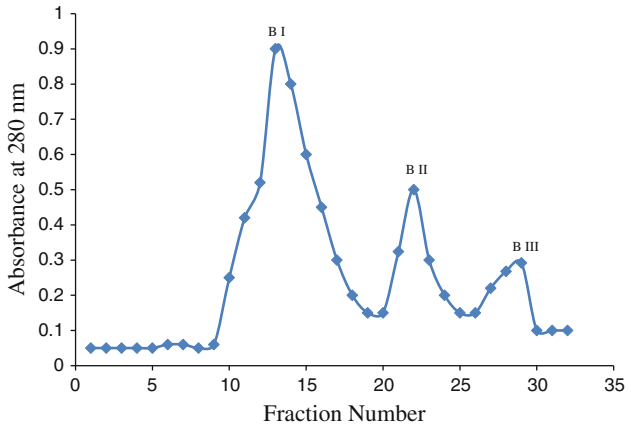

Fig. 1 Ion-exchange chromatography of extract. Profile of CM Sepharose Ion exchange chromatography. Fractions corresponding the peaks were pooled separately and designated as BI, BII and BIII

Protein identification

Balsamin, isolated from *M. balsamina* seed extract was further characterized by determining its primary structure. The protein was digested with trypsin and resulting peptides analyzed by mass spectrometry. Five peptides were identified out of which two peptides, REKVY NIPLLLPSVSGAGRY and RKITLPYSGNYERL, were unique to RIP from the seeds of Balsam apple (*Momordica balsamina*) thereby confirming the identification of the protein. The sequences of the peptide fragments were obtained with 99% confidence. The calculated molecular mass obtained from SDS-PAGE was 28 kDa. The theoretical molecular mass obtained by mass spectrometry protein identification methods was 29.1 kDa. However, both methods identified the protein to be ribosomal-inactivating protein (Table 3). The confidence/accuracy levels of peptide identification and sequences identified and the molecular masses of the fragments are listed (see supplementary information Table S1). Figure 4a shows the alignment of balsamin amino acid sequences with other type I RIPs. The amino acid sequences of balsamin were used to find homology with other type I RIPs deposited in GenBank. A BLAST search was performed to trace the possible similarity between the amino acid sequences of balsamin and other RIP sequences. Further analysis showed that balsamin has the highest similarity with α-MMC from *M. charantia* than to any other type I RIPs. Sequence from mass spectrometric analysis has 78% sequence identity with α-MMC, whereas, it has less identity with b-luffin, bryodin I, trichomislin, trichobakin and trichosanthin. From multiple alignment (as shown in Fig. 4a), a phylogenetic analysis of balsamin was carried out by comparing its amino acid sequence with those of known RIPs from other plants. Balsamin and α-MMC are located on the same branch and other RIPs originated from this branch. The phylogenetic tree shows the close relationship of balsamin with α-MMC and its distances from other RIPs. Trichomislin from *T. kirilowii* is more closely related to bryodin from *Bryonia dioica*, trichosanthin and trichobakin from *T. kirilowii* than balsamin (Fig. 4b).

RNA *N*-glycosidase activity

It has been demonstrated that RIPs are RNA *N*-glycosidases and they catalytically cleave the *N*-glycosidic bond specific to adenine residue at position 4324 in rat 28S rRNA. After further treatment with acidic aniline, the phosphodiester bond splits at the depurination site, and a specific RNA

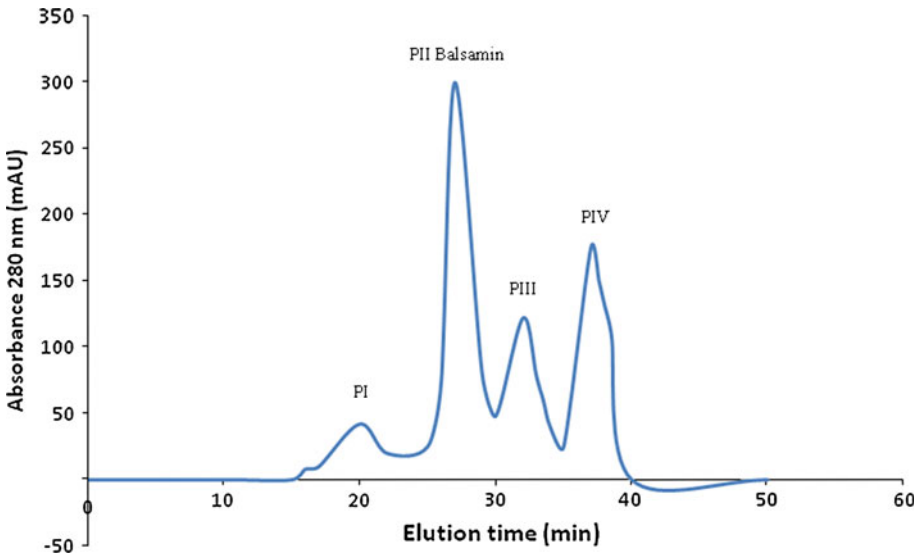

Fig. 2 Gel Filtration chromatography. Superdex 75 column profile of BI from ion exchange chromatography

Table 1 Purification summary for balsamin

Purification step	Total protein (mg)	Protein yield (%)
Extract	6.6	100
Ammonium sulfate	6.0	90
CM-Sepharose	4.0	60
Superdex 75	1.0	15

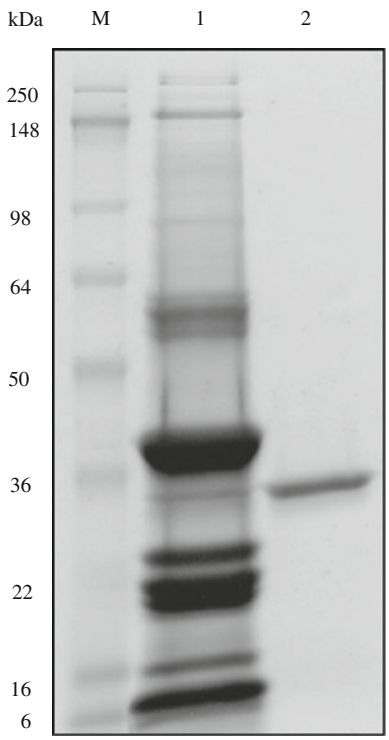

Fig. 3 SDS-PAGE of the purified balsamin fractions. Lane M contains molecular weight standards (molecular weight in kDa given on the *left*). *Lane 1*: crude extract; *Lane 2*: Superdex-75 purified fraction

fragment of about 400 nucleotides, named Endo-fragment, is released (Karumi et al. 2003). The *N*-glycosidase activity of balsamin from *M. balsamina* was examined by incubating ribosomes with different concentration of the protein. As shown in Fig. 5, reticulocyte lysate treated with 10 ng balsamin and depurinate 28S rRNA released a characteristic RNA fragment, the Endo fragment. Saporin-6 from *Saponaria officinalis* was taken as positive control and balsamin released Endo fragment same as saporin-6 (data not shown). The release of Endo fragment suggested that balsamin possesses rRNA *N*-glycosidase activity.

Cell free protein synthesis

Balsamin was tested in a rabbit reticulocyte lysate translation system to investigate whether it inhibits protein synthesis in a cell-free environment. The lysate was treated with different concentrations of sap-6 and balsamin. Balsamin from *M. balsamina* and saporin-6 (sap-6, a positive control) from *Saponaria officinalis* both inhibited protein synthesis in the rabbit reticulocyte lysate system. The lysate was treated with different concentrations of sap-6 and balsamin. Balsamin effectively inhibited protein synthesis with an IC_{50} 90 ng ml^{-1} (Fig. 6). In this experiment balsamin appeared to be less toxic than the saporin-6 which has an IC_{50} 3 ng ml^{-1}.

Secondary structure determination of balsamin

The far-UV CD spectrum suggests the presence of both α-helical and β-sheet conformations. The CD spectrum used to calculate secondary structures of balsamin by using spectra manager software (Figure 7). Balsamin contains 23.5% helix, 24.6% β-strand, 20% turn and 31.9% random coil (data not shown).

Table 2 Comparison of N-terminal amino acid sequence of balsamin with those of other type 1 RIPs

Plant	RIP	Sequence	Homology with balsamin (%)	Reference
Momordica balsamina	Balsamin	DVSFTLSGADPS	100	This paper
Momordica charantia	α-MMC	DVSFRLSGADPRSYGMFI	83	21
M. charantia	β-MMC	DVNFDLSTATAKTYTKFI	50	21
M. charantia	MAP30	DVNFDLSTATAKTTTKFIFD	50	29
Trichosanthes kirilowii	TCS	DVSFRLSGATSSSYGVFI	66	22
T. kirilowii	TAP29	DVSFRLSGATSKKKVYFISNL	66	23
T. kirilowii	Trichokirin	DVSFSLSGGGTASYEK	58	24
Bryonia dioica	Bryodin I	DVSFRLSGATTTSYGVFI	66	25
Luffa cylindrica	Luffin a	DVRFSLSGSSSTSYSKFIGDL	50	26

Residues identical to corresponding residues with balsmin are underlined. Sequence comparison of first 20 amino acids from the amino terminal of balsamin showing sequence identity with other type 1RIPs; while only 50–65% sequence identity with the RIPs from other plant families (luffin, Trichokirin and TCS)

Table 3 Identification profile of the purified protein

Protein name	Species	Protein accession number	pI/mass (kDa)		Sequence coverage (%)	No. of peptides detected	(Position) peptide sequence (peak mass)	Threshold confidence for accuracy (%)
			Observed	Theoretical				
Ribosome-inactivating protein momordin	*Momordica charantia*	RIP1_MOMCH	–/28.6	8.67/29.1	17.0	5	46–53: RNALPFREKV (974.5054) 52–69: REKVYNIPLLLPSVSGAGRY (1913.1525) 125–136: RKITLPYSGNYERL (1440.7858) 54–69: KVYNIPLLLPSVSGAGRY (1656.0001) 221–233: KQIQLAQGNNGIFRT (1458.7930)	99

Discussion

A novel type I RIP named as balsamin has been isolated and characterized from the seeds of Balsam apple (*Momordica balsamina*), a member of the Cucurbitaceae family. Balsamin has a molecular mass of 28 kDa, as determined by SDS-PAGE and gel filtration chromatography on Superdex 75. Multiple forms of RIP from *M. charantia,* namely α-momorcharin, β-momorcharin (Fong et al. 1996), γ-momorcharin (Pu et al. 1996), δ-momorcharin and ε-momorcharin (Tse et al. 1999) and MAP30 (Lee-Huang et al. 1990) have been reported. Most of the RIPs possess molecular masses in the range of 24–30 kDa and exhibit similarity in amino acid sequences (Puri et al. 2009). RIPs from Cucurbitaceae family have a notable heritage in traditional Chinese medicine. Proteins from *T. kirilowii* have been reported for centuries in abortion and in therapy for certain types of carcinoma (Casellas et al. 1988).

Balsamin cleaves a single *N*-glycosidic bond between base and ribose at position A4324 in 28S rRNA, similar to the other type-I RIPs. Balsamin displayed *N*-glycosidase activity by producing the Endo fragment and also cell-free protein synthesis inhibitory activity which are similar to other RIPs (Bagga et al. 2003). The action of balsamin on rRNA in the presence and absence of aniline is similar to that of other RIPs (Barbieri et al. 1993). Balsamin possesses a potent cell-free translation inhibitory activity ($IC_{50} = 90$ ng ml^{-1}), which is a property for type I RIPs. It adsorbed to CM-Sepharose column, similar to the chromatographic behavior of other RIPs (like α-MMC, β-MMC and MAP30) from *M. charantia* (Lifson et al. 1989; Lee-Huang et al. 1990). N-terminal sequence of balsamin shares 83% similarity with α-MMC from *Momordica* sp. Homology comparison of N-terminal amino acid sequence suggested a high similarity of balsamin to already known RIPs.

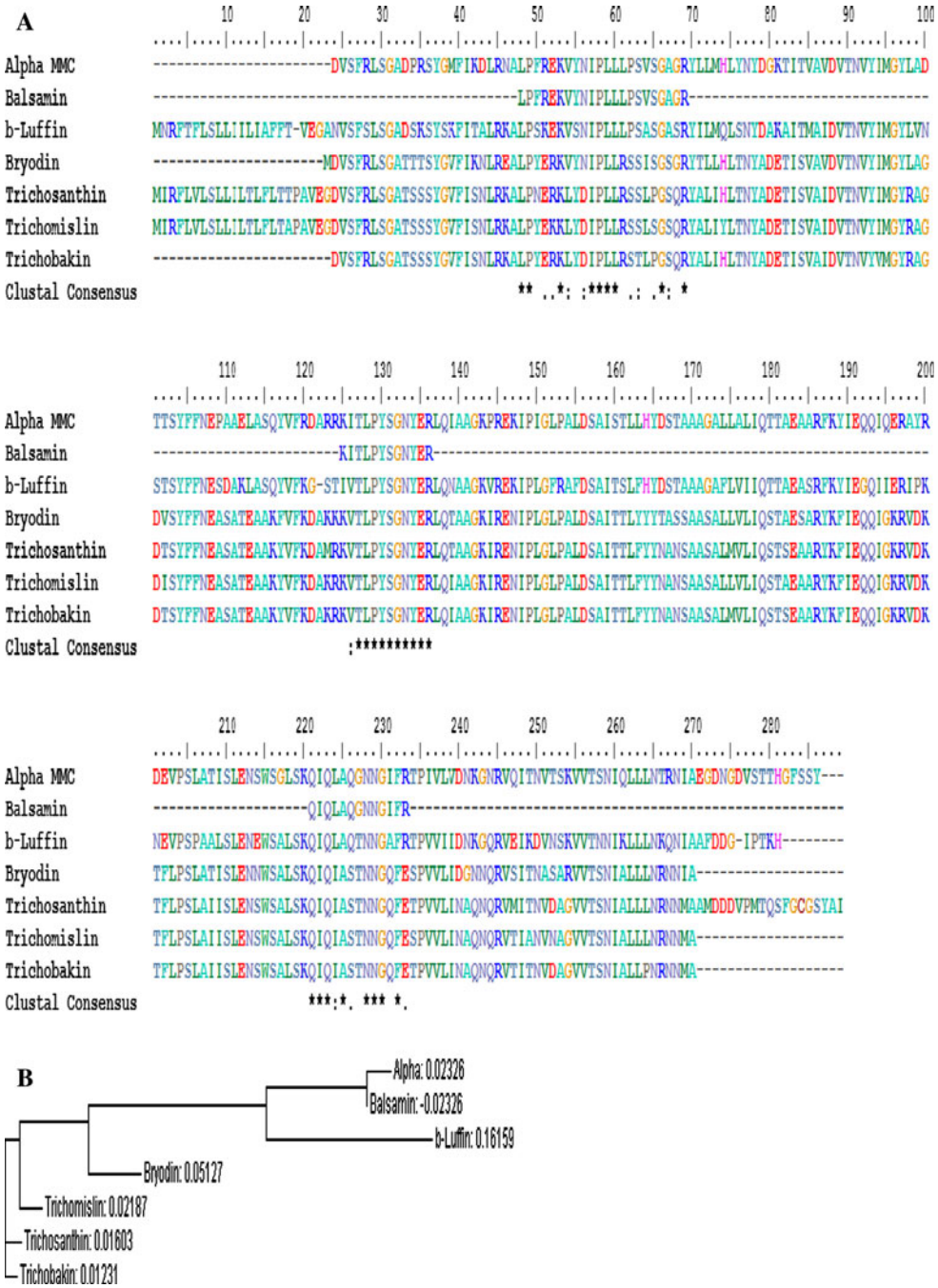

Fig. 4 **a** Alignment of amino acid sequences of balsamin from *M. balsamina* with other type I RIPs; alpha momorcharin from *M. charantia* (gi: 60459323), b-luffin from *Luffa aegyptica* (gi: 19150), bryodin from *Bryonia dioica* (gi: 2981957), trichosanthin from *T. kirilowii* (gi: 547149), trichomisilin from *T. kirilowii* (gi: 46403107), trichobakin from *Trichosanthes* sp. Bac kan 8-98 (gi: 7242890). (*star*) conserved, (*dot*) conserved substitutions, (*colon*) semi-conserved residues. **b** Phylogenetic tree is built from the sequences of *Momordica balsamina* (balsamin), *Momordica charantia* (α-MMC), *Luffa aegyptica* (b-luffin), *Bryonia dioica* (bryodin), *Trichosanthes kirilowii* (trichosanthin), *T. kirilowii* (trichomislin), *Trichosanthes* sp. Bac Kan 8-98 (trichobakin)

Balsamin matched with α-MMC according to mass spectrometry results. Alignment of the balsamin sequence shows that some residues are conserved between other RIPs like luffin b, bryodin, trichosanthin, trichomislin and trichobakin. Balsamin secondary structure contains helix (23.5%), *β*-strand (24.6%), turn (20%) and random coil (31.9%). Phylogenetic relationship showed that balsamin and alpha MMC can be grouped together and other RIPs originated from this branch. The tree showed close relationship of balsamin with α-MMC than other RIPs. Alpha MMC has abortifacient (Law et al. 1984), anti-tumor, immune response suppressor (Leung et al. 1987) and anti-HIV-1 (Lifson et al. 1989), deoxyribonuclease (Go et al. 1992) and ribonuclease (Mock et al. 1996) activities. Thus, balsamin also appears to be active as alpha MMC; it remains to be elucidated whether the balsamin is equipotent in other biological activities. These results may provide significant insights into the relationship between the various biological and enzymatic activities observed.

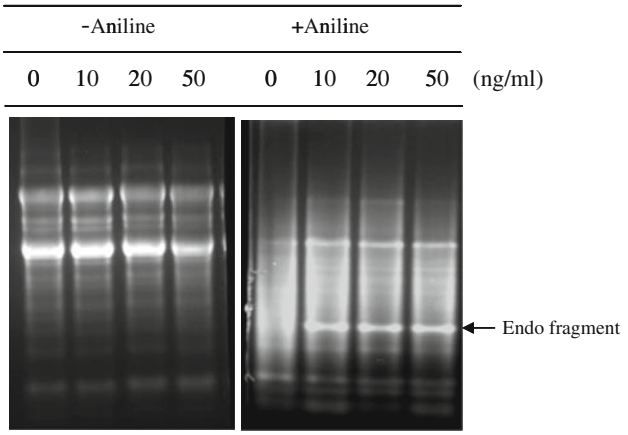

Fig. 5 RNA *N*-glycosidase activity of balsamin when incubated with rabbit reticulocyte lysate. The rRNA was extracted and treated (+) or not treated (−) with aniline-analyzed hydrolysis and analyzed by 2% agarose gel electrophoresis. The arrow denotes the position of rRNA fragments (Endo's fragment) resulting from aniline-catalyzed hydrolysis

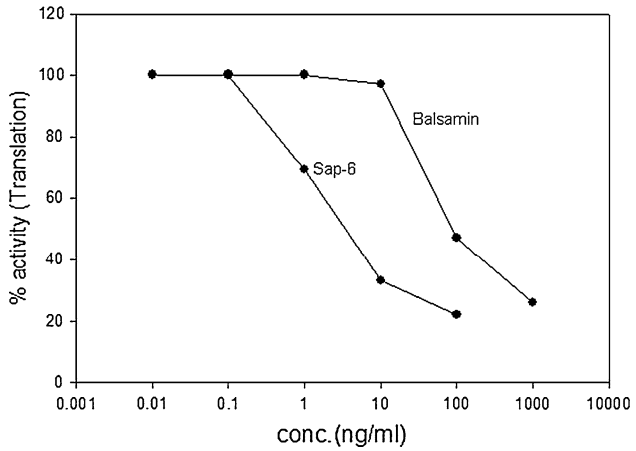

Fig. 6 Effect of balsamin on the protein synthesis of rabbit reticulocyte lysate on a cell-free system and Sap-6 as a positive control [–●– conc (ng/ml) vs. % activity]

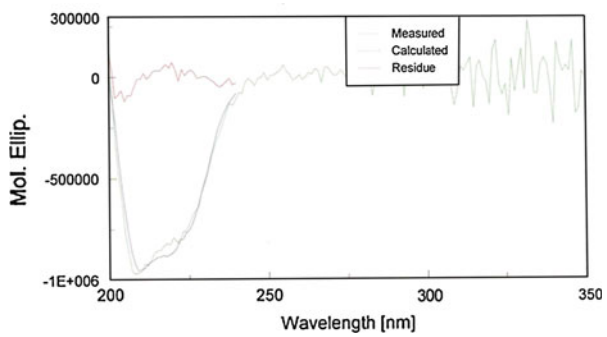

Fig. 7 CD spectra of balsamin measured in protein solution of 1 mg/mL (10 mM sodium phosphate buffer, pH 6.5). CD spectra measured in far-UV range in 1-mm path length quartz cuvettes

It is noteworthy that some of the plants containing RIPs are eaten raw by humans (e.g., spinach, pumpkin) which mean that materials containing those levels of RIPs are not harmful. Pharmaceutical-grade trichosanthin has been used for many years in China as an abortifacient and as an anticancer agent for choriocarcinoma (Lau et al. 1980). Phase I trial has been done with subcutaneous application of aviscumin in patients with progressive malignant tumors. Aviscumin induced the secretion of IL-1β and IFN-γ in plasma and stimulation of T-cells that mediated an in vivo anti-tumor T cell response (Bergman et al. 2008). Several health organizations around the world have recommended an increase in the intake of plant-derived food in order to improve human health status (Espin et al. 2007). Particularly the proteins from bitter melon are suitable for specific products (where the native form is needed) since they can resist higher temperature during processing. All the essential amino acids of bitter melon proteins with the exception of Threonine met the minimum requirements for preschool children by FAO/WHO/UNU (Horax et al. 2010). Thus, RIPs expressed in vegetables like *Momordica* sp. may be promoted as "functional ingredient" in a food system since it does not possess any toxic effect for humans and are useful as they possess anti-viral activity. Thus efforts are being made in many laboratories to purify RIPs from plants and check its activity against HIV and various tumors, which may allow their usage as potential nutraceutical.

In conclusion, balsamin, the basic protein isolated from *Momordica balsamina* has the properties of type I RIP as it (i) is a single-chain protein with a molecular mass of approximately 28 kDa; (ii) possesses enzymatic *N*-glycosidase activity on rabbit reticulocyte rRNA and (iii) releases Endo fragment after aniline treatment (Fig. 2). Furthermore, balsamin inhibits protein synthesis in cell-free system with IC_{50} value of 90 ng ml^{-1}. Based on its above-mentioned activities, balsamin may be promoted as a nutraceutical.

Acknowledgments The authors (MP, JKB and AS) acknowledge collaborative research supported by participating Institutions from Australia and India. They greatly acknowledge the help of Trayambak Basak with Mass Spec (MS) and the permission for the use of MS from Dr. Shantanu Sengupta of Institute of Genomic and Integrative Biology (IGIB), Delhi.

Conflict of interest The authors declare no conflict of interest.

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