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Ribosome Inactivating Protein and Lectin from Bitter Melon (*Momordica charantia*) Seeds: Sequence Comparison with Related Proteins

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One of the ribosome inactivating proteins (RIPs), β-momorcharin, and a lectin were isolated from seeds of the bitter melon *Momordica charantia* (Family Cucurbitaceae) in accordance with published procedures. Both β-momorcharin and *M. charantia* lectin were then subjected to amino acid sequencing. β-momorcharin exhibited considerable homology in sequence to other Cucurbitaceae RIPs, and also to the A chains of abrin and ricin which are type II (double-chained) RIPs. The resemblance between α-momorcharin and other RIPs is closer than that between β-momorcharins and other RIPs. *M. charantia* lectin manifested a certain extent of sequence similarity to β-momorcharin and other RIPs, and some degree of homology in sequence to lectins from *Cucurbita maxima, Cucurbita argyrosperma, Sambucus nigra* and *Ricinus Communis.*

Key Words: **ribosome inactivating protein; lectin; *Momordica charantia* seeds; sequence.**

Ribosome inactivating protein (RIPs) are a family of proteins which have captured the attention of many researchers on account of their potentially exploitable bioactivities. Their manifold pharmacological activities comprise antiviral and most notably anti-human immunodeficiency virus activity, antitumor, immunosuppressive, and enzymatic (DNase, RNase and N-glycosidase) activities (1-3). RIPs can be coupled to monoclonal antibodies to form immunotoxins which have highly specific tumoricidal activity (4-5). Many immunotoxins based on momordin, a *Momordica charantia* RIP (7) with sequence similarity to α-momorcharin (3), were used in the study of bladder carcinoma (8), Hodgkin's disease (9), lymphoma (10) and myeloma (11). RIPs are divided into type I and type II depending on the number of subunits they have. Type I RIPs such as RIPs from family Cucurbitaceae (α-momorcharin, β-momorcharin, momordin, trichosanthin) are single-chained whereas type II RIPs such as ricin and abrin possess 2 subunits, an RIP subunit (A chain) and a lectin subunit (B chain). Type II RIPs, e.g. ricin, are in general much more toxic than type I RIPs by virtue of the ability of the carbohydrate-binding lectin chain to interact with the cell membrane, although Girbes et al. (12,13) have isolated two relatively non-toxic type II RIPs, ebulin I and nigrin b from *Sambucus ebulus.*

Ho et al. (14) have published a comparison of the amino acid sequences of α-momorcharin, trichosanthin, abrin-A chain and ricin-A chain, and observed a striking resemblance among them. β-Momorcharin is an RIP with a molecular weight, chromatographic behavior and biological activities similar to those of α-momorcharin, another RIP also from *M. charantia* seeds. Since only the first 20 N-terminal amino acids in β-momorcharin are known (7), we decided to obtain the purified RIP in greater quantities for a more complete amino acid sequencing which would facilitate a more thorough comparison with related RIPs.

In the Cucurbitaceae family, lectins have been purified from seeds (15) and roots (16,17) of *Trichosanthes kirilowii,* tubers of *T. japonica* (18,19), seeds of *T. anguina* (20,21) fruits of *Cucurbita pepo* (22), phloem of *C. argyrosperma* and *C. maxima* (23), roots of *C. ficifolia* (24), and fruit exudate of *Sechium edule* (25).

A galactose-binding lectin with insulinomimetic activity has been isolated from *M. charantia* seeds. The lectin, which is a glycoprotein, possesses a molecular weight of 12.4 kDa and is made up of three subunits. It displays potent hemagglutinating and insulin-like (antilipolytic and lipogenic) activities (25). The purpose of the present study was to compare the sequence of the lectin with those of the RIPs (α- and β-momorcharins) isolated from the same source i.e., *Momordica charantia* seeds.

MATERIALS AND METHODS

β-momorcharin and *M. charantia* lectin were isolated from *M. charantia* seeds as described by Fong et al. (3) and Ng et al. (26) respectively. Briefly, for isolation of β-momorcharin, decorticated

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TABLE 1

Comparison of the N-Terminal Portions of the Amino Acid Sequences of *Momordica charantia* Lectin (MCL) and the Ribosome Inactivating Proteins (RIPs) β-Momorcharin (βMMC), α-Momorcharin (αMMC), Trichosanthin (TCS), Abrin A Chain (abrin A), and Ricin A Chain (Ricin A)

	1 10 20
MCL:	NIQISQSNFSADTYKRFIKN
βMMC:	DVNIDLSTATAKTYTKIFED
αMMC:	DVSFRLSGADPRSYGMFIKD
TCS:	DVSFRLSGATSSSYGVFISN
Abrin A:	••KFSTEGATSQSYKQFIEA
Ricin A:	•INFTTAGATVQSYTNFIRA
	21 30 40
MCL:	LRPQLTIGAS••YGRAGIYPLK•••HQ
βMMC:	IRATLPISHKV•YD•••F•PLL••YST
αMMC:	LRNALPFREKV•YN•••I•PLL••LPS
TCS:	LRKALPNERKL•YD•••I•PLL••RSS
Abrin A:	LRERLRGGL•I•HD•••I•PVLPDPTT
Ricin A:	VRGRLTTGADVRHD•••I PVLPNRVG
	50 60
MCL:	VPIQCRRC
βMMC:	FSD•SRRIFLLALTSYAC•ETF
αMMC:	VSG•AGRYLLMHLFNYDG•KTI
TCS:	LPG•SQRYALIHLTNYAD•ETI
Abrin A:	LQE•RNRYITVELSNS•DTESI
Ricin A:	LPI•NQRFILVELSNHAE•LSV
	61 70
βMMC:	GCPFAV•N•Y
αMMC:	TVAVDVTNVY
TCS:	SVAIDVTNVY
Abrin A:	EVGIDVTNAY
Ricin A:	TLALDVTNAY

Note. Sequences of MCL and β-MMC were determined in this study. Those of αMMC, TCS, abrin A and ricin A were from reference 14. Identical residues in RIPs are underlined.

seeds were extracted with phosphate buffered saline. The extract was subjected to ion exchange chromatography on DEAE-cellulose. The unadsorbed fraction was purified by adsorption on Affi-gel Blue gel. The final chromatographic separation between α- and β-momorcharins was achieved by fast protein liquid chromatography on Mono S.

Momordica charantia lectin was isolated from the seeds by delipidation with petroleum ether (1:3, w/v), extraction with phosphate buffered saline (1:5, w/v), addition of $(NH_4)_2SO_4$ to 60% saturation to precipitate proteins and affinity chromatography on lactogel and elution with a 0-0.1M galactose gradient in phosphate buffered saline.

The purified proteins were subjected to N-terminal amino acid analysis using an HP G100A Edman degradation unit and an HP-1000 HPLC system.

RESULTS

An inspection of the amino acid sequences presented in Table 1 reveals that out of the 70 N-terminal amino acid residues examined, 33 (47%) were identical between α- and β-momorcharin, 28 (40%) were the same in β-momorcharin and trichosanthin, 16 (23%) were invariant between β-momorcharin and abrin A chain, and 18 (26%) were unaltered in β-momorcharin and ricin A chain.

α-Momorcharin demonstrated 63% (44/70), 34% (24/70) and 34% (24/70) identity to trichosanthin, abrin A chain and ricin A chain respectively. The resemblance between α-momorcharin and other ribosome inactivating proteins was thus closer than that between β-momorcharins and other ribosome inactivating proteins. α-Momorcharin resembled trichosanthin to a greater extent (63%) than the similarity between the two momorcharins (47%). Trichosanthin displayed 43% (30/70) and 44% (31/70) identity to ricin A chain and abrin A chain respectively (Table 1).

Momordica charantia lectin exhibited 26% (13/50), 28% (14/50), 26% (13/50), 24% (12/50) and 26% (13/50) identity to β-momorcharin, α-momorcharin, trichosanthin, abrin A chain and ricin A chain respectively (Table 1). When portions of the molecule demonstrating sequence similarity are compared, *Momordica charantia* lectin manifested about 25% identity to lectins from *Cucurbita maxima* and *Cucurbita argyrosperma* (all three belonging to the Cucurbitaceae family) and approximately 30% identity to lectin from elderberry (*Sambucus nigra*) bark (Family Adoxaceae) (Table 2) *Momordica charantia* lectin exhibited about 40% identity to lectin from elderberry fruits (Table 3) and about 30% identity to lectin from *Ricinus communis* (Table 4).

DISCUSSION

Two RIPs designated α- and β-momorcharins have been isolated from seeds of the bitter gourd *Momordica charantia*. They have a molecular weight of about 29,000 which is within the range of 26,000-32,000 reported for most known RIPs. The momorcharins are immunologically different from each other as revealed by immunoprecipitation and immunoelectrophoresis (27). Comparison of the first 70 N-terminal amino acid residues of α-and β-momorcharins in the present study

TABLE 2

Comparison of the Portions of the Amino Acid Sequences of *Momordica charantia* Lectin (MCL), *Cucurbita argyrosperma* Lectin (CAL), *Cucurbita maxima* Lectin (CML) and *Sambucus nigra* Bark Lectin (SNBL) which Show Similarity

MCL1:	N•IQISQSNFS••ADTY••••KRFIKN
CML43:	N•IELKKPDGSKIARQECLLGKP••QN
CAL141:	N•IELKKPNGSKIERQECLLGKP••KN
SNBL261:	NSIE••VTNFRLFELTY••••IAVLLY
MCL21:	LR•PQLTIGASYGR•AGIYPL••KHQV
SNBL282:	GCAP•VTSS•SYSNNA•IDAQIIKMPV
MCL44:	PIQCRRC
SNBL307:	FRGGEYE

Note. The number after the abbreviated name of a lectin refers to the position of the first amino acid in that row. e.g., CML43 refers to N being the 43rd amino acid in CML. The sequences of CML, CAL and SNBL were from references 46, 46 and 47 respectively. Residues identical to MCL are underlined.

further corroborates the distinctiveness of these two RIPs. The profiles of biological activities of the momorcharins are in general similar to that of trichosanthin, encompassing antifertility, abortifacient, embryotoxic, immunosuppressive, antitumor, enzymatic (DNase, RNase, N-glycosidase), protein synthesis-inhibitory and anti-human immunodeficiency virus (2,3,28-36) activities. The fact that α-and β-momorcharins and trichosanthin are type 1 RIPs with similar biological activities and that they all originate from plants of the Cucurbitaceae family offers a possible explanation of the 40% sequence identity observed among these proteins. The RIP components represented by the A chains in the type 2 RIPs abrin and ricin also manifest resemblance, albeit to a lesser extent, with β-momorcharin and α-momorcharin. Of the three type 1 RIPs examined, β-momorcharin was outstanding in that it exhibited the smallest extent of identity with other type 1 RIPs and type 2 RIPs. This finding, together with the observation of different antigenicities between α- and β-momorcharins (16), (27), infer that sequential/alternate administration of β-momorcharins and another RIP may circumvent the problem of allergenicity. The desired pharmacological efficacy will be maintained because of the overlap in biological activities. The sequence similarity between *M. charantia* lectin and the RIPs, though not striking, is interesting. RIPs and lectins exert antiproliferative activity against tumor cell lines (36,37) and display antitumor effects (2,38). Both types of proteins exhibit immunomodulatory action (31,38). RIPs as well lectins serve a function of defense against pathogen attack. Lectins may have antiviral activity just like the RIPs (2,39). Type 2 RIPs represent conglomerates of RIPs and lectins which constitute their A chain and B chain respectively. Indeed, *Sambucus nigra* fruit lectin is a lectin derived from a truncated type 2 RIP (40). The B chain of RIP and the lectin from *S. elder* rhizomes show structural homology (41). It remains to be elucidated if the structural similarity between RIPs and lectins is accountable for their aforementioned functional resemblance. On the other hand, N-glycosidase activity on ribosomal RNA is a specific property of RIPs (3,42) not shared by lectins. *M. charantia* lectin (25), like a diversity of other lectins (42,43), possesses antilipolytic and lipogenic activities owing to interaction with insulin receptors on adipocytes. The RIPs are devoid of this insulinomimetic action (44).

TABLE 3

Comparison of the Portions of Amino Acid Sequences of *Momordica charantia* Lectin (MCL) and *Sambucus nigra* Fruit Lectin (SMFL) which Show Similarity

MCL1:	NIQISQSNFSADTYKR••FI
SNFL32:	SFNLAQA•KSA•TY•R•DFL
MCL1:	KNLRPQL•TIGASYGRAGIY
SNFL32:	KNLRTIVAT•GT•YEVNGL•
MCL:	P•LKH••••QVP
SNFL:	PVLRRESEVQVK

Note. The number after the abbreviated name of a lectin refers to the position of the first amino acid in that row. The sequence of SNFL was from reference 40. Residues identical to MCL are underlined.

TABLE 4

Comparison of the Portions of Amino Acid Sequences of *Momordica charantia* Lectin (MCL) and *Ricinus communis* Lectin (RCA) which Show Similarity

MCL:	NI••QISQSNFSADTYKRF•
RCA:	•IFKQII••NTTADTVESTN
MCL:	IKNLRPQLTIGASY••••G
RCA:	I••VRSHLTT•ADVRHEIPN
MCL:	RAGIYPLKHQVPIQCRRC
RCA:	RVGL•PI••QIESHAELS

Note. The sequence of RCA is from reference 47. Residues identical to MCL are underlined.

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