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Purification and characterisation of a hypoglycemic peptide from *Momordica Charantia* L. Var. *abbreviata* Ser.

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ABSTRACT

A water-soluble peptide MC2-1-5 from *Momordica charantia* L. Var. *Abbreviata* Ser., with hypoglycemic effect, was purified by ultrafiltration, gel filtration chromatography and reverse-phase high performance liquid chromatography (RP-HPLC). The infrared (IR) spectra showed characteristic absorption peaks and the molecular mass of MC2-1-5 was found to be 3405.5174 Da by matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF-MS). The sequence of its first 10 N-terminal amino acids was GHPYYSIKKS as determined by a protein sequencer. MC2-1-5 reduced the blood glucose level in alloxan-induced diabetic mice by 61.70% and 69.18% at 2 and 4 h, respectively, after oral administration at a dose of 2 mg/kg. The oral glucose tolerance test (OGTT) showed MC2-1-5 produced a reduction of 25.50%, 39.62% and 41.74% in blood glucose level after 1, 2 and 3 h, respectively, of oral administration compared with a diabetic control.

1. Introduction

Diabetes mellitus (DM) has been considered a major health problem in the world today. DM is a metabolic disorder of carbohydrate, fat and protein metabolism characterized by elevation of both fasting and postprandial blood glucose levels. Although different types of synthetic oral hypoglycemic agents and insulin are available for the treatment of DM, insulin cannot be taken orally and the synthetic agents in use can produce serious side effects and toxicity (Akhtar & Iqbal, 1991; Tolman & Chandramouli, 2003; Yamamoto, Nakajima, Yamazaki, & Yokoi, 2001). Hence, the demand for safer and more effective oral hypoglycemic agents is on the rise. In many parts of the world, traditional medicinal plants have been used for the treatment of diabetes and therein exists a hidden wealth of potentially useful natural products for diabetes control (Gray & Flatt, 1997). In recent years, much attention has been paid to the investigation of natural antidiabetic drugs from various medicinal plants. These include *Gongronema latifolium* (Ugochukwu & Babady, 2003), *Chamaemelum nobile* (Eddouks, Lemhadri, Zeggwagh, & Michel, 2005), *Rehmannia glutinosa* (Zhang, Zhou, Jia, Zhang, & Gu, 2004), *Gentiana olivieri* (Sezik, Aslan, Yesilada, & Ito, 2005), Du-Zhong (*Eucommia ulmoides* Oliv.) (Lee et al., 2005), *Calotropis Procera* (Roy, Sehgal, Padhy, & Kumar, 2005), *Picrorrhiza kurroa* (Joy & Kuttan, 1999) and *Murraya koenigii* (Yadav, Vats, Dhunnoo, & Grover, 2002). Despite this, few traditional anti-diabetic plants have received proper scientific screening. The World Health Organization (WHO) has recommended that this area warrants further evaluation (World Health Organization, 1980). Such an evaluation might reveal effective dietary adjuncts either for the treatment of DM or the discovery of natural products for the development of new anti-diabetic drugs.

Momordica Charantia L. Var. *abbreviata* Ser. (MCV) belongs to a short-fruited group of the Cucurbitaceae family. Derived from a wild plant, it was domesticated and improved by Jiangsu High-quality Farm Product Development Center, China and has been successively planted over large areas. Many species of the same genus in the Cucurbitaceae family, such as, *Momordica Charantia* (Ahmed, Lakhani, Gillett, John, & Raza, 2001; Akhtar, Athar, & Yaqub, 1981; Cakici et al., 1994) and *Momordica Cymbalaria* (Rao, Kesavulu, & Apparao, 2001) have been reported to have significant hypoglycemic and antidiabetic effects. Furthermore, some investigators have attempted to purify the active fractions from fruits of *M. charantia* and reported that saponins (Lotlikar & Rajarama, 1966; Matsuda et al., 1998) and peptides (Khanna, Jain, Panagariya, & Dixit, 1981; Nag, Medicherla, & Sharma, 2000; Zhang et al., 1980) from it had hypoglycemic effects. Therefore, it was speculated that there are bioactive saponins and peptides in MCV. Zhao (2005) reported crude saponins in MCV could significantly lower the blood glucose level in alloxan-induced diabetic mice. In a previous study, it was shown that semi-purified peptides MC2-1 from MCV have hypoglycemic effects in alloxan-induced diabetic mice (Yuan, Gu, Tang, & Wasswa, 2008). However, the exact peptide(s) with hypoglycemic effect in MC2-1 remains to be established. The present

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investigation was undertaken to further purify and characterize hypoglycemic peptide(s) from the semi-purified peptide fraction.

2. Materials and methods

2.1. Materials and chemicals

Fresh fruits of MCV were collected in July 2006 locally and authenticated by Jiangsu Academy of Agricultural Sciences. Glibenclamide was purchased from Tianjin Pacific Pharmaceutical Co., Ltd (Tianjin, China). Alloxan was purchased from Sigma Co. (St. Louis, MO, USA). The other chemicals and reagents used in the experiment were of food or analytical grade quality.

2.2. Preparation of the aqueous extract

Fresh MCV fruits were washed thoroughly and the seeds removed. The fruits were sliced into approximately 1.5-in. pieces. To 2 kg of the sliced fruits, 4 l of distilled water were added, blended and then stirred for 8 h at 20 °C. This process was repeated three times. The resultant slurry was centrifuged at 4000*g* for 20 min. The clear supernatant was concentrated under reduced pressure in a rotary evaporator (Rotavapor R-114, Büchi Labortechnik AG, Flawil, Switzerland) and then freeze dried. The dried extract was stored at −15 °C prior to further analyses.

2.3. Purification of the hypoglycemic peptides in the extract

MCV aqueous extracts were utilized to purify potent hypoglycemic peptide(s) and hypoglycemic effect was tested at each purification step. The supernatant of the aqueous extract was passed through a microfiltration (MF) membrane having a pore size of 0.2 μm (Tianjin Motian Membrane Engineering and Technology Co., Ltd., Tianjin, China). The filtrate was subsequently passed through an ultrafiltration (UF) membrane with 10 kDa molecular weight cut-off (MWCO) (Tianjin Motian Membrane Engineering and Technology Co., Ltd., Tianjin, China) and divided into a large molecular weight fraction (MC1) and a small molecular weight fraction (MC2). The two fractions were lyophilized and tested for hypoglycemic effect. The higher active fraction MC2 obtained after UF was fractionated by gel filtration chromatography on a column (2.6 cm × 150 cm, Shanghai Qingpu Huxi Instrument Factory, Shanghai, China) of Sephadex G-25 (Pharmacia Co., Uppsala, Sweden). The sample was dissolved in distilled water and loaded onto the column. The column was pre-equilibrated with distilled water and eluted at a flow rate of 0.6 ml/min. The absorbance at 220 nm was measured to monitor the peptide during chromatography separation. These fractions were then concentrated under reduced pressure in a rotary evaporator (Rotavapor R-114, Büchi Labortechnik AG, Flawil, Switzerland) and then lyophilized. The hypoglycemic effect of the six fractions was determined. The highest active fraction was further purified on a HPLC column (Lichrospher C_{18} column, 10 mm × 250 mm, Jiangsu Hanbon Science and Technology Co., Ltd., Huaiyin, China). Separation was carried out under linear gradient elution conditions using acetonitrile as the organic modifier and trifluoroacetic acid (TFA) as the volatile buffer. Eluent A consisted of 0.1% TFA in 5% acetonitrile (v/v) while eluent B consisted of 0.1% TFA in 80% acetonitrile (v/v). The hypoglycemic fraction was applied on the C18 column and eluted by increasing eluent B concentrations, that is, 0–20 min, 0–50%B; 20–30 min, 50–100%B and 30–40 min, 100%B. The eluent was monitored at 220 nm and fractions or individual peaks were collected for assay of hypoglycemic effect. The most active fraction was concentrated by lyophilization and then analyzed on a HPLC column (Diamonsil C_{18} column, 4.6 mm × 250 mm, Beijing Dikma Technologies Co., Beijing, China) to check its purity with a linear gradient of acetonitrile (5–80%, v/v, in 30 min) with a flow rate of 0.5 ml/min at 220 nm.

2.4. FT-IR spectra

The purified hypoglycemic peptide was incorporated into KBr (spectroscopic grade) and pressed into a 1-mm pellet. Spectra were recorded in absorbance mode from 500 to 4000 cm^{-1} on a Nicolet Nexus FT-IR spectrometer (Thermo Electron Co., Madison, WI, USA).

2.5. Molecular mass determination

The molecular mass of the purified hypoglycemic peptide was determined by a MALDI-TOF mass spectrometer (4700 Proteomics Analyzer, Applied Biosystems, Foster City, CA, USA) equipped with a nitrogen laser (355 nm). The spectrometer was operated in reflection mode with delayed extraction and the acceleration voltage in the ion source was 20 kV. The accuracy of mass determinations was within 0.02%.

MALDI–TOF MS samples were prepared as follows: a lyophilized peptide was dissolved in water to a concentration of 0.5 mg/ml. Then 5 μl of the sample solution was mixed with 5 μl matrix solution containing 10 mg α-cyano-4-hydroxycinnamic acid. Subsequently, 2 μl of the mixture was directly spotted onto a MALDI target plate. MALDI-TOF MS analysis was performed directly after spotting.

2.6. Amino acid sequence analysis

Automated Edman sequencing was performed by standard procedures using an automated protein sequencer (ABI491A, PE Co., Boston, USA) equipped with on-line HPLC to determine the N-terminal amino acid sequence of the purified hypoglycemic peptide.

2.7. Hypoglycemic effect

2.7.1. Animals

Male Kunming mice weighing 25–30 g were obtained from Shanghai SLAC Laboratory Animal Co. Ltd. (Shanghai, China). All mice were maintained in plastic cages under standard environmental conditions of temperature, relative humidity and dark/light cycle. Mice were fed on a standard chow diet and water ad libitum. The mice were used for experimentation after a 6-day acclimatization period. All the experiments were done during daytime. The guidelines for the care of animals were strictly followed throughout the study.

2.7.2. Experimental induction of diabetes in mice

After an 18 h fasting, mice were intraperitoneally injected with ice cold alloxan monohydrate (Sigma chemicals, St. Louis, MO, USA) freshly dissolved in distilled water (2%) at a dose of 200 mg/kg bw (Zheng, 1999). After one week, the fasting blood glucose (FBG) level of mice was measured and only mice with FBG level of between 13 mmol/l and 20 mmol/l were used for the experiments.

2.7.3. Estimation of blood glucose level

Blood samples were collected from the mice tail tip and blood glucose levels (BGL) were estimated using an electronic glucometer (Roche Diagnostics GmbH, Mannheim, Germany).

2.7.4. Hypoglycemic effect in alloxan-induced diabetic mice

Normal and diabetic mice were randomly divided into groups of 12 mice each. After an overnight fast of the mice, the FBG level (0 h) was measured. Then distilled water, samples tested and a reference

drug (glibenclamide) were orally administered to the different groups. BGL was determined at 0, 2 and 4 h after administration.

2.8. Effect of the hypoglycemic peptide on oral glucose tolerance test

Normal and diabetic mice were randomly divided into groups of 12 mice each. After an overnight fast of the mice, the FBG level was measured. Distilled water, the hypoglycemic peptide and glibenclamide were orally administered to different groups. One hour later, glucose (2 g/kg) was orally administered to the mice in all groups. BGL was measured just prior to glucose administration (0 h) and at 1, 2 and 3 h after glucose loading.

2.9. Statistical analysis

All data were expressed as mean ± S.D. for all experiments and a two-tailed student's *t*-test was used to calculate the significant difference between groups and among group means. A p value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. Purification of hypoglycemic peptide(s)

Alloxan-induced diabetes is a well-documented model of experimental diabetes. It induces diabetes by damaging the insulin secreting β-cells in the pancreas leading to hyperglycaemia (Chattopadhyay, Ramanathan, Das, & Bhattacharya, 1997). In a previous study (Yuan et al., 2008), alloxan-induced diabetic mice were used to screen the peptide(s) with hypoglycemic effect in an aqueous MCV extract. The results showed that semi-purified peptides MC2-1 from Sephadex G-25 at a dose of 20 mg/kg could significantly lower the BGL in diabetic mice. To obtain a single peptide, MC2-1 was pooled and subjected to further purification using RP-HPLC. Ten different fractions of MC2-1 were obtained through RP-HPLC (Fig. 1). Among them, MC2-1-1 (Peak 1 peptide) and MC2-1-5 (Peak 5 peptide) at a dose of 2 mg/kg could significantly lower the BGL in diabetic mice, while other fractions had no significant effect on BGL in diabetic mice (Table 1). MC2-1-1 produced a reduction of 43.84% and 54.57% in BGL after 2 and 4 h of oral administration, respectively. MC2-1-5 produced a reduction of 61.70% and 69.18% in BGL after 2 and 4 h of oral administration respectively. As shown in Fig. 1, MC2-1-1 was still a mixture of many compounds, while MC2-1-5 was relatively pure. Moreover, the hypoglycemic effect of MC2-1-5 was stronger than that of MC2-1-1 at the same dosage. Therefore, MC2-1-5 was selected for further elevation. MC2-1-5 was analyzed by analytical RP-HPLC to confirm its purity. MC2-1-5 showed a single peak at 220 nm (Fig. 2), an indication of its high purity.

Table 1
Effect of fractions from HPLC chromatography on the blood glucose level in alloxan-induced diabetic mice

Group	Dosage (mg/kg)	Blood glucose level (mmol/l)		
		0 h	2 h	4 h
Normal control	–	4.63 ± 0.62	4.40 ± 0.59	4.30 ± 0.65
Diabetic control	–	15.95 ± 3.01	15.83 ± 1.70	15.69 ± 2.78
MC2-1-1	2	15.85 ± 2.91	8.90 ± 0.93[a]	6.20 ± 0.87[b]
MC2-1-2	2	16.30 ± 3.07	13.01 ± 2.39	12.01 ± 2.38
MC2-1-3	2	16.23 ± 3.12	14.49 ± 2.87	13.51 ± 2.43
MC2-1-4	2	15.91 ± 3.25	13.91 ± 2.77	12.98 ± 2.73
MC2-1-5	2	16.19 ± 3.00	6.20 ± 0.71[b]	4.99 ± 0.66[b]
MC2-1-6	2	15.90 ± 2.69	14.85 ± 2.76	13.28 ± 2.60
MC2-1-7	2	15.99 ± 2.90	13.20 ± 2.12	12.30 ± 2.28
MC2-1-8	2	16.01 ± 3.09	14.20 ± 2.62	13.11 ± 2.55
MC2-1-9	2	15.99 ± 2.90	14.15 ± 2.12	12.99 ± 2.19
MC2-1-10	2	15.12 ± 2.66	13.90 ± 2.02	12.59 ± 2.22
Glibenclamide	20	16.27 ± 3.12	9.08 ± 1.92[a]	7.84 ± 2.68[a]

Values are given as mean ± S.D. for 12 mice in each group.
[a] $P < 0.01$, compared with initial value.
[b] $P < 0.001$, compared with initial value.

3.2. Effect of MC2-1-5 on glucose tolerance in diabetic mice

The results of OGTT are shown in Fig. 3. Among the three groups, the initial blood glucose levels were not significantly different. However, untreated diabetic mice had significantly higher BGL than that in the treated diabetic mice during the OGTT. Compared with diabetic control, MC2-1-5 (2 mg/kg) produced a reduction of 25.50%, 39.62% and 41.74% in BGL 1, 2 and 3 h, respectively, after oral administration. Glibenclamide (20 mg/kg) produced a reduction of 9.08%, 33.74% and 28.02% in BGL 1, 2 and 3 h, respectively, after oral administration. The results indicated that MC2-1-5 enhanced glucose utilization because it significantly decreased the BGL in glucose loaded mice. This may be due to restoration of delayed insulin response or due to inhibition of intestinal absorption of glucose (Pari & Saravanan, 2002).

3.3. Identification of MC2-1-5

MC2-1-5 was identified by IR, mass spectrometry and peptide sequencing. The infrared spectra showed MC2-1-5 to have characteristic absorption peaks of the peptide in the spectral region 500–4000 cm^{-1} (Fig. 4). The protein amide I band (polypeptide C=O stretching, Krimm & Bandekar, 1986) was observed as a strong

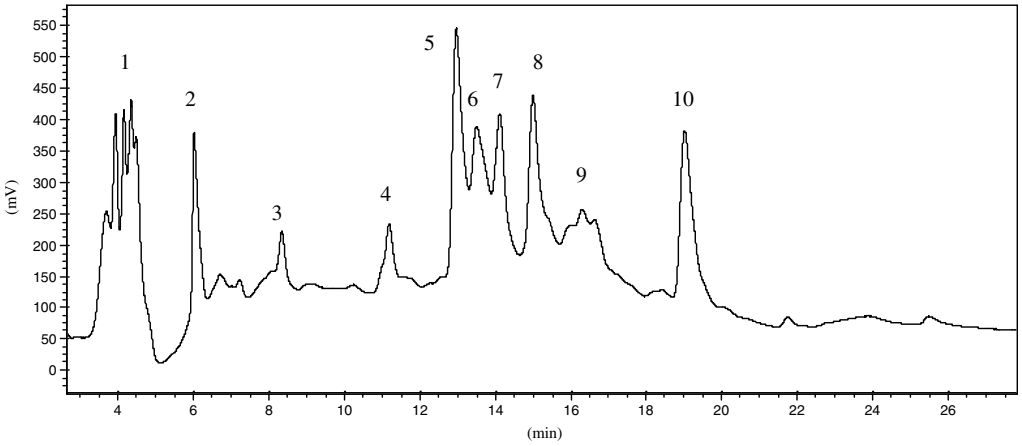

Fig. 1. HPLC profile of MC2-1 from Sephadex G-25 gel filtration chromatography.

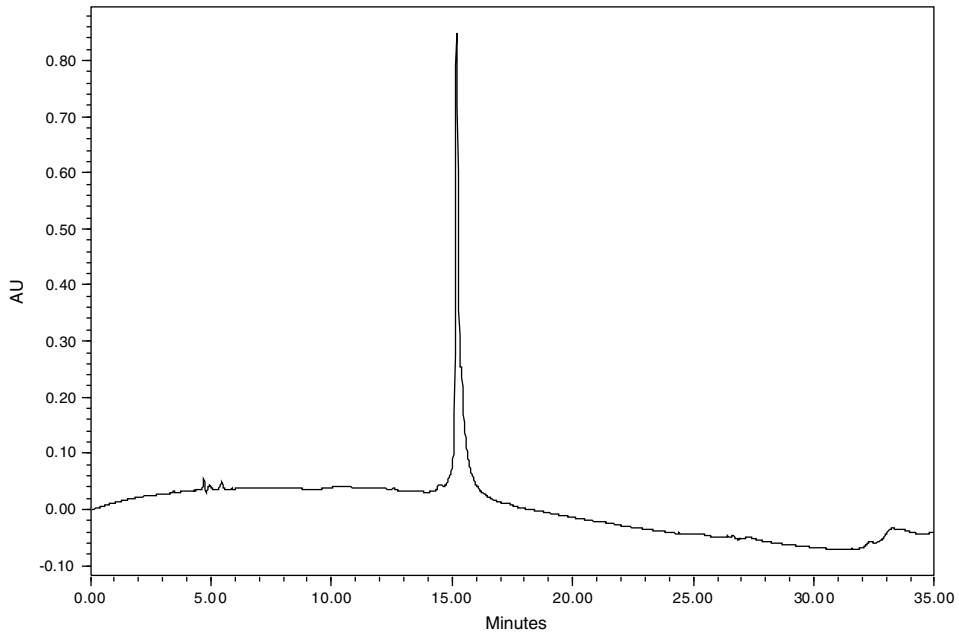

Fig. 2. HPLC profile of the purified peptide from *Momordica Charantia* L. Var. *abbreviata* Ser.

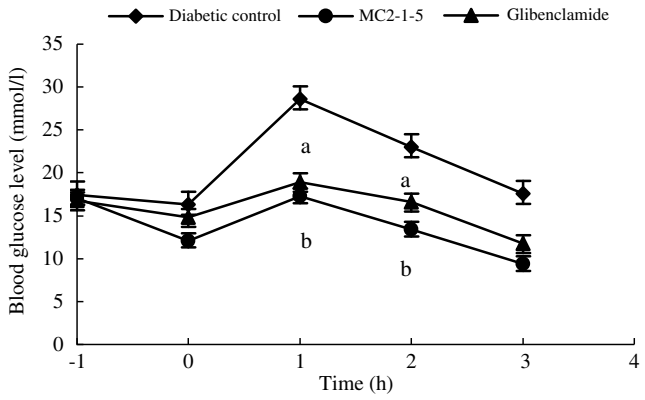

Fig. 3. Effect of the purified hypoglycemic peptide on glucose tolerance in alloxan-induced diabetic mice. Values are given as mean ± S.D. for 12 mice in each group. $^{a}P < 0.01$, $^{b}P < 0.001$, compared with diabetic control.

band at 1653 cm^{-1}, while a band of medium intensity at 1546 cm^{-1} was attributed to the amide II (C–N stretching and N–H bending modes) vibrations (Beauchemin, Harnois, Rouillon, Tajmir-Riahi, & Carpentier, 2007). The molecular mass of MC2-1-5 was determined to be 3405.5174 Da by MALDI-TOF-MS (Fig. 5). The small peaks at *m/z* 3276.4272 and 1703.2615 may be due to impurities or peptide fragments. The hypoglycemic peptide was then analyzed using a peptide sequencer and its sequence of the first 10 N-terminal amino acids was identified to be GHPYYSIKKS. Edman degradation requires peptides with high purity. However, the separation of peptides is known to be very difficult due to their complexity. In the present study, only the first 10 N-terminal amino acid residues were determined due to the insufficient purity of MC2-1-5. Two hypoglycemic peptides from *M. charantia* have been reported. Khanna et al. (1981) extracted P-polypeptide from fruit and seed of *M. Charantia* with a similar hypoglycemic effect as insulin. The polypeptide had 166 amino acid residues and its

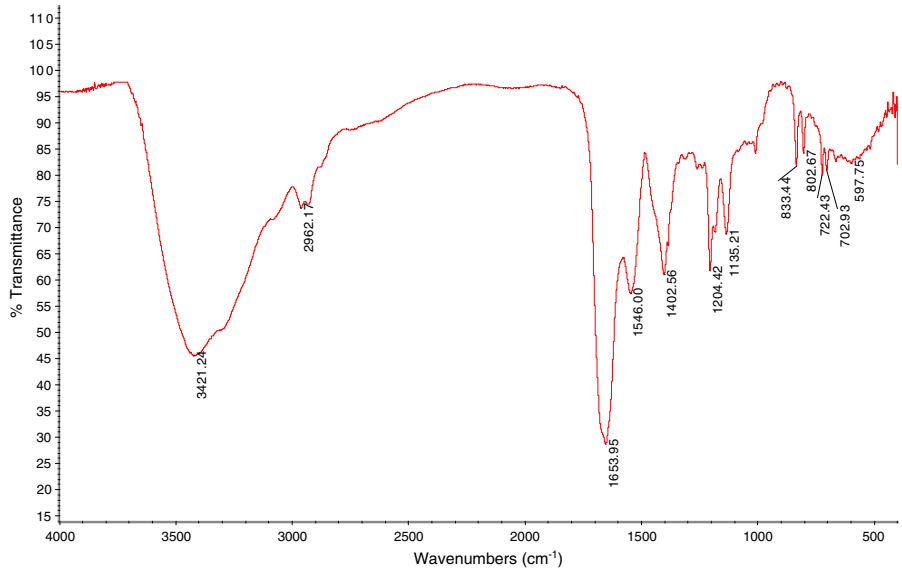

Fig. 4. Fourier transform infrared spectra of the purified hypoglycemic peptide from *Momordica Charantia* L. Var. *abbreviata* Ser.

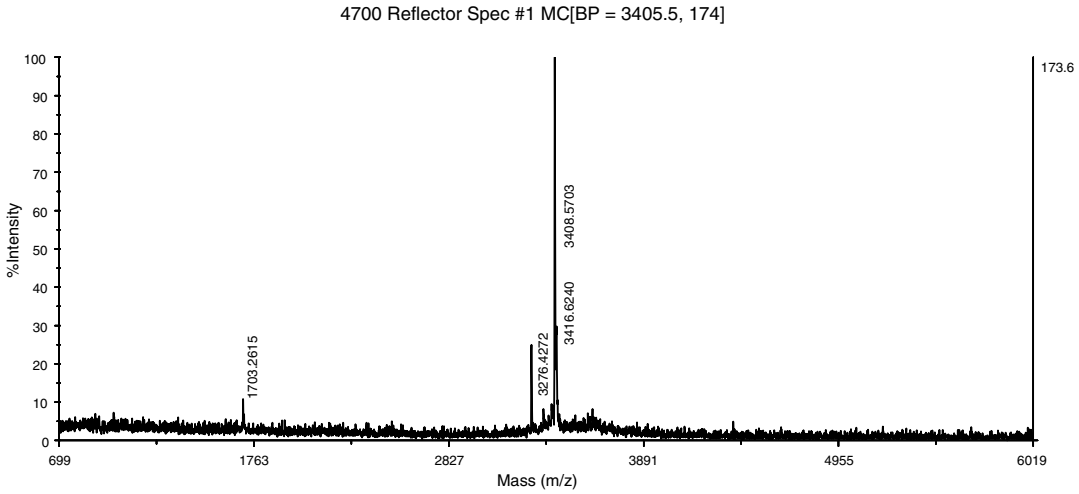

Fig. 5. MALDI-TOF mass spectra of the purified hypoglycemic peptide from *Momordica Charantia* L. Var. *abbreviata* Ser.

molecular mass was about 11 kDa. Nag et al. (2000) found a water-soluble insulin-like peptide in *M. Charantia*, which exhibited hypoglycemic effect in mice by oral administration and injection, while it had no effect on serum insulin. Its sequence was KTNMKHMAGAAAAGAVVG. The sequence of the first 10 N-terminal amino acids of MC2-1-5 was different from that of the peptides from *M. Charantia* and therefore it was considered to be a novel hypoglycemic peptide. However, the amino acid sequence of these three peptides was unique making comparisons difficult. Moreover, the hypoglycemic mechanism of these peptides may be different from each other.

4. Conclusions

In conclusion, there was a water-soluble hypoglycemic peptide in MCV aqueous extract. It could significantly lower the BGL in alloxan-induced diabetic mice. However, the complete amino acid sequence of the hypoglycemic peptide remains to be determined. Further studies will focus on determining the complete amino acid sequence of MC2-1-5 and its hypoglycemic mechanism.

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References

Ahmed, I., Lakhani, M. S., Gillett, M., John, A., & Raza, H. (2001). Hypotriglyceridemic and hypocholesterolemic effects of anti-diabetic *Momordica charantia* (karela) fruit extract in streptozotocin-induced rats. *Diabetes Research and Clinical Practice, 51*, 155–161.

Akhtar, M. S., & Iqbal, J. (1991). Evaluation of the hypoglycemic effect of *Achyranthes aspera* in normal and alloxan diabetic rabbits. *Journal of Ethnopharmacology, 31*, 49–57.

Akhtar, M. S., Athar, M. A., & Yaqub, M. (1981). Effect of *Momordica charantia* on the blood glucose level of normal and alloxan diabetic rabbits. *Planta Medica, 42*, 205–212.

Beauchemin, R., Harnois, J., Rouillon, R., Tajmir-Riahi, H. A., & Carpentier, R. (2007). Interaction of polyamines with proteins of photosystem II: Cation binding and photosynthetic oxygen evolution. *Journal of Molecular Structure, 833*, 169–174.

Cakici, I., Hurmoglu, C., Tunctan, B., Abacioglu, N., Kanzik, I., & Sener, B. (1994). Hypoglycemic effect of *Momordica charantia* extracts in normoglycemic or cyprohepta-diene induced hyperglycaemic mice. *Journal of Ethnopharmacology, 44*, 117–121.

Chattopadhyay, S., Ramanathan, M., Das, J., & Bhattacharya, S. K. (1997). Animal models in experimental diabetes mellitus. *Indian Journal of Experimental Biology, 35*, 1141–1145.

Eddouks, M., Lemhadri, A., Zeggwagh, N. A., & Michel, J. B. (2005). Potent hypoglycaemic activity of the aqueous extract of *Chamaemelum nobile* in normal and streptozotocin-induced diabetic rats. *Diabetes Research and Clinical Practice, 67*, 189–195.

Gray, A. M., & Flatt, P. R. (1997). Nature's own pharmacy: The diabetes perspective. *Proceedings of the Nutrition Society, 56*, 507–517.

Joy, K. L., & Kuttan, R. (1999). Anti-diabetic activity of *Picrorrhiza Kurroa* extract. *Journal of Ethnopharmacology, 167*, 143–148.

Khanna, P., Jain, S. C., Panagariya, A., & Dixit, V. P. (1981). Hypoglycemic activity of polypeptide-P from a plant source. *Journal of Natural Products, 44*(6), 648–655.

Krimm, S., & Bandekar, J. (1986). Vibrational spectroscopy and conformation of peptides, polypeptides, and proteins. *Advances in Protein Chemistry, 38*, 181–364.

Lee, M. K., Kim, M. J., Cho, S. Y., Park, S. A., Park, K. K., Jung, U. J., et al. (2005). Hypoglycemic effect of Du-zhong (*Eucommia ulmoides* Oliv.) leaves in streptozotocin-induced diabetic rats. *Diabetes Research and Clinical Practice, 67*, 22–28.

Lotlikar, M. M., & Rajarama, R. M. R. (1966). Pharmacology of a hypoglycemic principle: Isolated from the fruits of *Momordica charantia* Linn. *Indian Journal of Pharmacy, 28*, 129–133.

Matsuda, H., Li, Y., Murakami, T., Matsumura, N., Yamahara, J., & Yoshikawa, M. (1998). Antidiabetic principles of natural medicines III structure-related inhibitory activity and action model of oleanolic acid glycosides on hypoglycemic effect. *Chemical and Pharmaceutical Bulletin, 46*, 1399–1403.

Nag, B., Medicherla, S., & Sharma, S. D. (2000). Orally active fraction of *Momordica charantia*, active peptides thereof, and their use in the treatment of diabetes. US Patent 6127338.

Pari, L., & Saravanan, G. (2002). Antidiabetic effect of Cogent db, a herbal drug in alloxan-indued diabetes mellitus. *Comparative Biochemistry and Physiology, Part C, 131*, 19–25.

Rao, B. K., Kesavulu, M. M., & Apparao, C. (2001). Antihyperglycemic activity of *Momordica cymbalaria* in alloxan diabetic mice. *Journal of Ethnopharmacology, 78*, 67–71.

Roy, S., Sehgal, R., Padhy, B. M., & Kumar, V. L. (2005). Antioxidant and protective effect of latex of *Calotropis procera* against alloxan-induced diabetes in rats. *Journal of Ethnopharmacology, 102*, 470–473.

Sezik, E., Aslan, M., Yesilada, E., & Ito, S. (2005). Hypoglycaemic activity of *Gentiana olivieri* and isolation of the active constituent through bioassay-directed fractionation techniques. *Life Science, 76*, 1223–1238.

Tolman, K. G., & Chandramouli, J. (2003). Hepatotoxicity of the thiazolidinediones. *Clinics in Liver Disease, 7*, 369–370.

Ugochukwu, N. H., & Babady, N. E. (2003). Antihyperglycemic effect of aqueous and ethanolic extracts of *Gongronema latifolium* leaves on glucose and glycogen metabolism in livers of normal and streptozotocin-induced diabetic mice. *Life Science, 73*, 1925–1938.

World Health Organization (1980). WHO expert committee on Diabetes Mellitus: Second report. WHO Technical Report Series 646. Geneva: World Health Organization.

Yadav, S., Vats, V., Dhunnoo, Y., & Grover, J. K. (2002). Hypoglycemic and antihyperglycemic activity of *Murraya koenigii* leaves in diabetic rats. *Journal of Ethnopharmacology, 82*, 111–116.

Yamamoto, Y., Nakajima, M., Yamazaki, H., & Yokoi, T. (2001). Cytotoxicity and apoptosis produced by troglitazone in human hepatoma cells. *Life Science, 70*, 471–482.

Yuan, X. Q., Gu, X. H., Tang, J., & Wasswa, J. (2008). Hypoglycemic effect of semi-purified peptides from Momordica Charantia L. Var. abbreviata Ser. in alloxan-induced diabetic mice. *Journal of Food Biochemistry, 32*, 107–121.

Zhang, S. Y., Yao, W. Z., Xue, Q. F., Wang, G. Y., Han, J. H., Lei, Q. J., et al. (1980). Isolation, purification and characterization of peptides with hypoglycemic effect in *Momordica Charantia* L. *Acta Biochimica et Biophysica Sinica, 12*(4), 391.

Zhang, R. X., Zhou, J. H., Jia, Z. P., Zhang, Y. X., & Gu, G. M. (2004). Hypoglycemic effect of *Rehmannia glutinosa* oligosaccharide in hyperglycemic and alloxan-induced diabetic rats and its mechanism. *Journal of Ethnopharmacology, 90*, 39–43.

Zhao, H. W. (2005). Studies on extraction and hypoglycemic effect of saponins of *Momordica charantia* L. Var. *Abbreviata* Ser. Master dissertation. Wuxi: Jiangnan University.

Zheng, J. X. (1999). *Functional food*. Beijing: China Light Industry Press.