CLONING AND BACTERIAL EXPRESSION OF A GENE ENCODING RIBOSOME-INACTIVATING PROTEINS, KARASURIN-A AND KARASURIN-C, FROM *TRICHOSANTHES KIRILOWII* VAR. *JAPONICA*

Hajime MIZUKAMI,* Katsuhisa IIDA, Toshiya KONDO, and Yukio OGIHARA

*Faculty of Pharmaceutical Sciences, Nagoya City University, Tanabe-dori 3-1, Mizuho-ku, Nagoya, 467, Japan*

A genomic DNA clone of karasurin was isolated using the polymerase chain reaction from *Trichosanthes kirilowii* var. *japonica* (Cucurbitaceae). The amino acid sequence deduced from the nucleotide sequence was consistent with previously reported sequences of karasurin-A and karasurin-C except for a putative signal peptide and extra amino acids at the C-terminus, neither of which is present in the natural protein. Recombinant karasurin was synthesized in *Escherichia coli*, in which the cloned karasurin gene was expressed under the control of the *trc* promoter.

**KEY WORDS** cloning; expression; karasurin; ribosome-inactivating protein; *Trichosanthes kirilowii* var. *japonica*

Karasurins are very basic proteins accumulated in root tubers of *Trichosanthes kirilowii* var. *japonica* (Cucurbitaceae). The amino acid sequences of karasurins-A, -B and -C have been determined and shown to be ribosome-inactivating proteins and to exhibit cytotoxic, abortifacient, and immunosuppressive activities. In the present communication we describe the cloning and sequencing of a gene encoding karasurin-A and karasurin-C. We also describe the bacterial expression of the cloned gene, which will lead to the generation of muteins for investigation of the structure-activity relationship of karasurins.

Total DNA was prepared from fresh root tubers of *Trichosanthes kirilowii* var. *japonica* using the method described by Rogers and Bendich and used for subsequent PCR as a template. Based on the DNA sequence of α-trichosanthin, which is a ribosome-inactivating protein accumulated in *T. kirilowii* of which the amino acid sequence is known to be highly homologous to those of karasurins, a forward primer (KN1F=5'–CATGTGAGAAATGATGAG-3') and a reverse primer (KN1R=5'–ACGTAGCTGTGAATTTACACT-3') were designed to cover both the whole coding region and the 5' flanking region. The PCR-amplified fragment (gKRN) was ligated to pGEM-T (Promega) to form a plasmid pKRN, and the complete nucleotide sequence of gKRN which contains the sequence coding for karasurin and its flanking sequence were determined (Fig. 1).

The longest open reading frame in the gKRN sequence is capable of encoding a polypeptide of 289 amino acids. The deduced amino acid sequence is consistent with that of natural karasurin-A and -C except that gKRN codes for a putative N-terminal signal peptide and 19 extra amino acids at C-terminus. The DNA sequence shows that karasurin-A and karasurin-C are the products of the same sequence.
Fig. 1  Nucleotide Sequence of the Genomic DNA Encoding Karasurin-A and Karasurin-C with Deduced Amino Acid Sequence. A TATA-box-like motif and a putative transcription initiation site (pyApypy) are underlined and double-underlined, respectively. An asterisk indicates a stop codon. Aspartic acid (D), shown in bold, is replaced with asparagine (N) in karasurin-B. The nucleotide sequence will appear in the DDBJ, EMBL, and GenBank nucleotide sequence databases under accession number AB000666.

gene, but are proteolytically cleaved at different site of the precursor protein.

The open reading frame of gKRN was amplified by PCR with specific primers designed to incorporate flanking 5' Bsp HI and 3' Hin dIII restriction endonuclease sites. After digestion of PCR products with Bsp HI and Hin dIII, the DNA fragment was subcloned into the Nco I and Hin dIII sites of an expression cassette vector pTrc99A (Pharmacia) to yield the plasmid pTKN1. *Escherichia coli* JM105 cells harboring pTKN1 were grown at 37°C in M9ZB medium5 to A600=0.5 and
expression of the protein was induced by the addition of IPTG to a final concentration of 1 mM. The cells were collected 3 hr after the addition of IPTG, suspended in 50 mM Tris-HCl buffer (pH 7.5) containing 1 mM EDTA, and lysed by boiling in SDS-loading buffer. The lysate was analyzed by SDS-PAGE and Western blotting using a rabbit anti-karasurin antiserum \(^2\text{a}\) (Fig. 2).

A distinct band immunoreactive to the anti-karasurin antiserum was detected in the extract. The molecular weight of this protein was estimated to be about 32 kDa, which matches the size of the recombinant karasurin as deduced from the DNA sequence. Because of the presence of an N-terminal signal peptide (21 amino acids) and C-terminal peptide (19 amino acids), the recombinant protein is about 4.5 kDa larger than the natural proteins. A faint band corresponding to the recombinant protein was also detected by staining of the SDS-PAGE gel with Coomassie brilliant blue, but the amount of protein in \(E. \text{coli}\) was estimated to be less than 1% of total protein (data not presented). Overexpression of the karasurin gene using other expression systems is now under investigation, which will enable us to obtain a large amount of various mutated proteins for investigation of the pharmacological activities of karasurins.

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**REFERENCES**


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