

**Two Functional Domains of A NIa Protease Gene of
Soybean Mosaic Virus, Expressed in *Escherichia coli*.**

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The polyprotein encoded by soybean mosaic potyvirus (SMV) is proteolytically processed to, at least, eight mature products by viral-encoded proteases. The nuclear inclusion protein a (NIa) protease plays an important role in the processing at least two-thirds of genome, catalyzing *cis*-cleavage between itself and nuclear inclusion protein b (NIb) and *trans*-cleavage between NIb and coat protein (CP). In addition, it has two functional domains, genome-linked protein (VPg) domain at the N-terminus and protease (NIa-pro) domain at the C-terminus. The full-length of NIa gene was synthesized by reverse transcription-polymerase chain reaction (RT-PCR) with gene-specific primers under concentration at 2.5 mM MgCl₂, annealing temperature at 55°C and 35 cycles of amplification. The gene product of interest, 1298 bp, ligated into pET *E. coli* expression vector, determined its nucleotide and deduced amino acid sequences, and characterized the biochemical properties of the purified recombinant protein. Optimal conditions for expression were at 1mM treatment of IPTG at 25°C for 5 hr. The released protein remains soluble and can be extracted from both supernatants and pellets, showing not only unprocessed 49 kDa protein, but also autocatalytically processed products to 27 kDa of NIa-pro and 22 kDa of VPg.

Keywords: Soybean mosaic virus, nuclear inclusion protein a (NIa), genome-linked protein (VPg)