

Function and Oligomerization Study of the Leucine Zipper-like Domain in P13 from *Leucania separata* Multiple Nuclear Polyhedrosis Virus

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The *p13* gene is uniquely present in Group II nucleopolyhedroviruses (NPVs) and some granuloviruses, but not in Group I NPVs. *p13* gene was first described by our laboratory in *Leucania separata* multiple nuclear polyhedrosis virus (Ls-*p13*) in 1995. However, the functions of Ls-P13 and of its homologues are unknown. When Ls-*p13* was inserted into *Autographa californica* nucleopolyhedrovirus, a Group I NPV, polyhedra yield was inhibited. However, this inhibition was prevented when the leucine zipper-like domain of Ls-*p13* was mutated. To determine the cause of this marked difference between Ls-P13 and leucine zipper mutated Ls-P13 (Ls-P13mL), oligomerization and secondary structure analyses were performed. High performance liquid chromatography and yeast two-hybrid assays indicated that neither Ls-P13 nor Ls-P13mL could form oligomers. Informatics and circular dichroism spectropolarimetry results further indicated marked secondary structural differences between Ls-P13 and Ls-P13mL. The LZLD of Ls-P13 has two extended heptad repeat units which form a hydrophobic surface, but it is short of a third hydrophobic heptad repeat unit for oligomerization. However, the mutated LZLD of Ls-P13mL lacks the above hydrophobic surface, and its secondary structure is markedly different. This difference in its secondary structure may explain why Ls-P13mL is unable to inhibit polyhedra yield.

Keywords: Function, Leucine zipper-like domain, *Leucania separata* multiple nuclear polyhedrosis virus, Oligomerization, P13

Introduction

The *Baculoviridae* are a family of large, enveloped double-stranded DNA (80-180 kbp) viruses that mostly infect insects (Adams and McClintock, 1991). The family has been taxonomically subdivided into two genera: *Nucleopolyhedrovirus* (NPV) and *Granulovirus* (GV) (Volkman *et al.*, 1995) based on occlusion body morphology (Winstanley *et al.*, 1993 Rohrmann, 1999). The NPVs have been further subdivided into two distinct groups based on molecular phylogenies (Herniou *et al.*, 2003). For example, *Autographa californica* multiple nucleocapsid nucleopolyhedrovirus (AcMNPV) (Ayres *et al.*, 1994), *Bombyx mori* nucleopolyhedrovirus (BmNPV) (Gomi *et al.*, 1999) and *Orgyia pseudotsugata* multiple nucleocapsid nucleopolyhedrovirus (OpMNPV) (Ahrens *et al.*, 1997) are members of group I NPVs, whereas *Spodoptera exigua* multiple nucleocapsid nucleopolyhedrovirus (SeMNPV) (Ijkel *et al.*, 1999), *Lymantria dispar* multiple nucleocapsid nucleopolyhedrovirus (LdMNPV) (Kuzio *et al.*, 1999) and *Heliothis armigera* single nucleocapsid nucleopolyhedrovirus (HaSNPV) (Chen *et al.*, 2001) are members of group II NPVs.

p13 gene was firstly discovered in *Leucania separate* multinuclear polyhedrosisvirus (LsMNPV), a member of group II NPVs, in our laboratory in 1995 (Wang *et al.*, 1995). Sequencing of baculovirus genomes has shown that about nineteen baculoviruses contain *p13* homologues (Ijkel *et al.*, 1999; Taha *et al.*, 2000; Luque *et al.*, 2001; Pang *et al.*, 2001; Li *et al.*, 2002; Lange *et al.*, 2003; Sally *et al.*, 2003; Van Oers *et al.*, 2005; Zhang *et al.*, 2005 Jakubowska *et al.*, 2006). Interestingly, all of the reported *p13* genes have been found in Group II NPVs and in some GVs, but not in Group I NPVs (Jarvis *et al.*, 1996). P13 encodes a protein of ca. 31 kDa, which is predicted to be a member of glycosyltransferase family 8 (Lange *et al.*, 2003). However, the function of P13 has not been experimentally investigated.

Although *p13* genes show sequence divergence, a conserved leucine zipper-like domain is present in most P13 proteins

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Table 1. Secondary structure prediction of LZLD^a

LZLD ^a	AA ^b						PROF(sec) ^c																																	
	(152) 1	(158) 7	(165) 14	(172) 21	(179) 28	(187) 36	(152) 1	(158) 7	(165) 14	(172) 21	(179) 28	(187) 36																												
Ls-P13	L	A	R	N	K	V	L	G	K	T	G	T	M	L	K	P	S	P	A	L	Y	S	T	V	R	K	L	N	A	N	N	D	C	L	EEE...	EEEE...	HHHHHHHHHHHHHHH...	
Ls-P13mL ^d	L	A	R	N	K	V	L	G	K	T	G	T	M	L	K	P	S	P	A	L	Y	S	T	V	R	K	L	N	A	N	N	D	C	L	E....	EE.....	HHHHHHHHHHHHH...	
Ha-P13	F	K	Y	N	K	I	L	G	K	T	G	T	V	L	N	P	D	K	D	L	S	T	I	Q	L	N	K	N	K	C	L	EEE...	EEEE...	HHHHHHHHHHHHHHH...				
Ah-P13	F	K	Y	N	K	I	L	G	K	T	G	T	V	L	N	P	D	K	D	L	S	T	I	Q	L	N	K	N	K	C	L	EEE...	EEEE...	HHHHHHHHHHHHHHH...				
Ao-P13	D	N	S	N	V	L	G	F	S	G	T	L	V	F	E	P	N	L	K	M	A	K	T	I	E	S	L	S	P	D	N	D	K	L	EEEE...	EEEE...	HHHHHHHHHHH.....	
Ce-P13	Y	K	H	N	K	I	L	C	K	A	G	T	V	L	F	E	P	S	L	K	L	N	T	I	K	S	L	V	P	T	N	R	F	L	EE...	EEEE...	HHHHHHHHHHHHH.....	
C1-P13	M	E	N	Y	N	L	G	F	T	G	T	F	V	F	I	P	S	L	K	L	S	T	I	T	S	L	T	P	T	N	K	L	I	EEEE...	EEEE...	HHHHHHHHH.....		
Cp-P13	D	E	N	H	N	L	G	F	T	G	T	F	Y	T	P	S	L	Q	M	S	T	I	L	S	L	T	P	T	N	I	L	L	EEEE...	EEEE...	HHHHHHHHHHH.....			
Mc-P13	M	R	Y	N	K	I	L	C	K	G	T	V	L	F	E	P	S	L	Q	Y	T	I	L	E	L	R	P	T	N	N	C	L	EE...	EEEE...	HHHHHHHHHHH.....			
Se-P13	M	R	Y	N	K	I	L	C	K	G	T	V	L	F	Q	P	D	M	K	Y	H	T	I	L	N	F	L	N	P	H	N	K	Y	L	EE...	EEEE...	HHHHHHHHHHHHH...	
Po-P13	L	Q	S	N	V	L	G	F	T	G	T	L	V	F	T	P	N	S	R	V	S	N	C	I	K	Y	L	S	E	E	K	S	E	L	EEEEEE...	EEEE...	HHHHHHHHHHH.....	
S1-P13	M	K	Y	N	R	V	L	C	K	T	G	T	V	L	T	P	S	K	I	L	F	E	A	I	Q	N	A	I	K	T	S	K	C	F	S	EEE...	EEEE...	HHHHHHHHHHHHH...
Gp41 ^e	L	K	Y	W	N	L	L	Q	Y	W	S	Q	E	L	K	X	S	A	V	S	L															.	HHHHHHHHHHHHHHHHHHH..			

^aLeucine zipper like domain (LZLD). ^bAmino Acid of LZLD. ^cPROF predicted secondary structure: H=helix, E=extended (sheet), dot=other (loop), PROF: Profile network prediction. ^dLZLD mutant. ^eLZLD of HIV-1 Gp41 as positive control of LZLD for oligomerization. “□” represents a mutation site. “■” represents leucine residues spaced 6-8 amino acids apart in LZLDs of reported P13s and HIV-1 Gp41. “□” represents the positions of leucines replaced by other residues. “□” represents conserved proline in LZLD of all selected P13s. Numbers in “()” show amino acids positions of Ls-P13 LZLD.

(Table 1). Moreover, leucine zipper and leucine zipper-like domains in eukaryotic proteins have been demonstrated to play essential roles in inter- and intramolecular interactions. The leucine zipper motif was initially defined as a sequence of four or five leucine residues spaced 7 amino acids (aa) apart, and its secondary structure exhibits a coiled-coil conformation (O’Shea *et al.*, 1991). In the case of DNA binding proteins, these motifs are responsible for protein dimerization and DNA binding activity (Ransone *et al.*, 1989 Hu *et al.*, 1990). The leucine zipper motif has also been identified in many viral proteins, such as, in human immunodeficiency virus type 1 (HIV-1) envelope glycoprotein gp41, Bluetongue virus (BTV) VP4 protein, baculoviruses PE38 and Gp64 proteins (Delwart *et al.*, 1990; Monsma *et al.*, 1995; Bernstein *et al.*, 1995; Ramsdale *et al.*, 1996). In addition, to protein dimerization, leucine zipper-like domains (LZLD) may also play important roles in the virus life cycle, e.g., in virus encapsidation, virus-induced fusion, and virus production.

In this article, we used the Ls-P13 that we described in 1995 to determine the importance of the putative LZLD in terms of its structural and biological activities. First, we examined what would happen if *Ls-p13* gene was inserted into AeMNPV, a group I NPV without *p13* gene in its genome. Second, we explored the function of Ls-P13 putative LZLD after the above recombinant baculovirus infection in Sf9 cells by using site-specific mutagenesis. Third, we examined whether Ls-P13 can form oligomerization oligomers *in vitro* and in yeast. In addition, we analyzed the secondary structures

of LZLD and mutated LZLD and of Ls-P13 and Ls-P13mL proteins, to determine why Ls-P13 and Ls-P13mL proteins have such different biological activities.

Materials and Methods

Cells, insects, and viruses. *Spodoptera frugiperda* pupal ovarian cell line (Sf9) cells were cultured at 27°C in Grace’s medium (Gibco/BRL), supplemented with 10% fetal bovine serum (Gibco/BRL). Cultures of larvae of *S. exigua* were maintained as previously described (Liu *et al.*, 2001). Baculovirus LsMNPV was kindly donated by Dr. Shengliang Chen (Wuhan Institute of Virology, Chinese Academic of Sciences).

Plasmids. Plasmid pMal-p2 was obtained from New England Biolabs, United Kingdom. Plasmids pGBKT7, pGADT7 were obtained from Clontech. Plasmid pFastBac1 was obtained from Invitrogen. Plasmid pIEHR3 containing hr5 enhancer and IE1 promoter fusion sequence was kindly donated by Dr. D. L. Jarvis (Jarvis *et al.*, 1996). Plasmid pBlueScriptII-KS-ph including polyhedron promoter and polyhedron expressing cassette was preserved by our laboratory.

Splice Overlap extension PCR (SOE PCR). Leucine zipper-like domains (LZLDs) are widely present in the nineteen P13 homologues reported to date. To determine the importance of the central leucine residues in putative LZLD, two substitution mutations were created in Ls-P13. Accordingly, the second (L165)

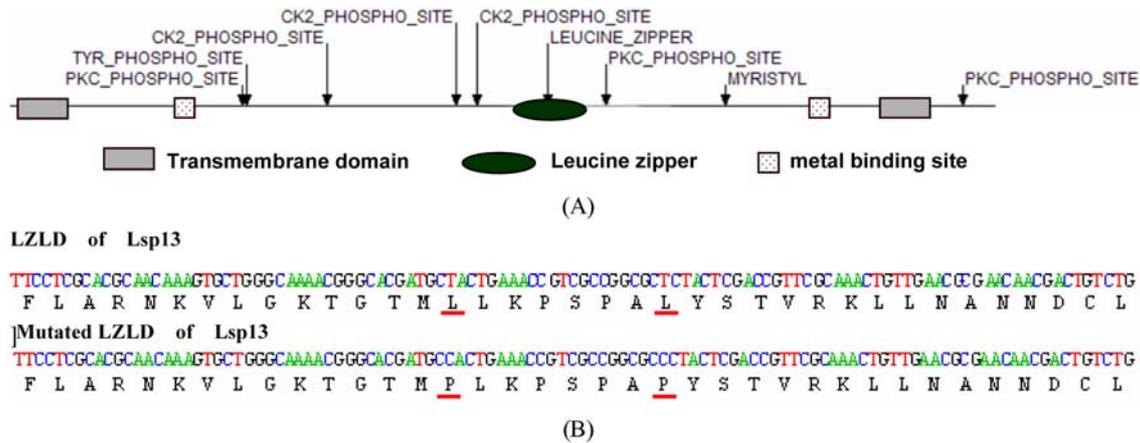


Fig. 1. The putative structure of Ls-P13 and its LZLD mutation by SOE PCR. A: Ls-P13 structures were predicted using PredictProtein Server software. B: Ls-*p13mL* sequencing result (a partial sequence containing the site mutation is shown).

and third (L172) leucines of LZLD were simultaneously mutated into prolines by SOE PCR.

Protein expression, purification and antibody production. Ls-*p13* or LZLD mutated Ls-*p13* (Ls-*p13mL*) were inserted into the bacterial expression plasmid pMal-p2. The foreign genes were fused with the MalE (maltose binding protein [MBP]) gene of *E. coli* at the N-terminus, and the expressed fusion proteins MBP/Ls-P13 and MBP/Ls-P13mL were purified by affinity chromatography by taking advantage of the affinity of MBP for maltose. Finally, the purified fusion proteins were used to raise polyclonal antibodies according to classic procedures.

Recombinant baculoviruses construction. Ac-Bac-to-Bac (Invitrogen) or Ac-Bac-to-Bac-eGFP (Liu, X *et al.*, 2005) expression systems were used to construct recombinant AcMNPVs (rAcMNPVs) to study Ls-P13 function in Sf9 cells (Fig. 2A). Briefly, the foreign genes were inserted into pFastBac1 donor plasmids and transformed into *E. coli* DH10Bac containing either Ac-Bacmid or Ac-Bacmid-eGFP using helper plasmid. After white/blue selection on kanamycin/gentamicin/tetracycline plates and PCR screening, the resulting Bacmids were transfected into Sf9 cells to yield the rAcMNPVs.

Flow Cytometry (FCM). Recombinant baculoviruses rAc-ph/G (eGFP was fused with polyhedron gene) were constructed to quantitatively identify P13 effects on polyhedra yield in Sf9 cells at different combinations these were Bacmids of rAc-hr5/IE1-Lsp13-ph + rAc-ph/G, rAc-hr5/IE1-Lsp13mL-ph + rAc-ph/G and rAc-ph + rAc-ph/G (“+” represents two baculoviruses Bacmids were used as one in combination), respectively. All Bacmids of rAcMNPVs were transfected at equivalent amounts in triplicate experiments. After 96 h cells were collected and washed three times with PBS (pH 7.4). EGFP intensities were determined by FCM (Beckman Coulter).

High Performance Liquid Chromatography (HPLC). MBP/Ls-P13 and MBP/Ls-P13mL fusion proteins were purified from bacteria and Sf9 cells by Amylose Affinity Chromatography. Eluted

proteins were dialyzed against column buffer (20 mM Tris-HCl, 200 mM NaCl, pH 7.4) and finally purified on a GF-250 HPLC column, using column buffer as eluent (Zhao *et al.*, 2003).

Yeast two-hybrid. To provide evidence of P13 self-interaction by in yeast two-hybrid, unique Ha-*p13* were fused in frame with the Gal4 DNA-binding domain (BD) or the Gal4 activating domain (AD) in pGBKT7 or pGADT7 vectors (Clontech), respectively. Yeast two-hybrid assays were carried out according to the manufacturer’s protocol (MATCHMAKER GAL4 two-hybrid system3 Clontech). Briefly, the pGBKT7 and pGADT7 constructs were co-transformed into yeast Y187 and then transferred onto -Trp/-Leu plates supplemented with X- α -Gal (20 μ g/ml) for the selection of diploids containing both plasmids. After transformation, protein-protein interactions were identified by using blue/white colony color assays.

Circular dichroism spectropolarimeter (CD). The secondary structures of Ls-P13 and Ls-*p13mL* were determined using a Jasco (Easton) J-810 spectropolarimeter at 200 nm to 250 nm and 25°C. The purified fusions MBP-Ls-P13 and MBP-Ls-P13mL were dissolved in column buffer (20 mM Tris-HCl, 200 mM NaCl, pH 7.4) at a final concentration of 200 μ g/ml and results were analyzed using the K2D program to evaluate the contents of α -helix, β -sheet and β -turn contents in of the above two fusion proteins.

Software analysis. Computer-assisted secondary structure analysis was performed using the PredictProtein Server (Rost *et al.*, 2003). LZLD of HIV-1 GP41 was used as minimal positive model for the analysis of the LZLD of Ls-P13 and the mutated LZLD of Ls-P13mL.

Results

LZLD is essential for the Ls-P13 induced suppression on of polyhedra yield. In our previous study, we confirmed Ls-P13 accelerates insect-killing rates when *S. exigua* were injected with recombinant AcMNPV expressing Ls-P13 (data not presented). To further study whether killing activity survived

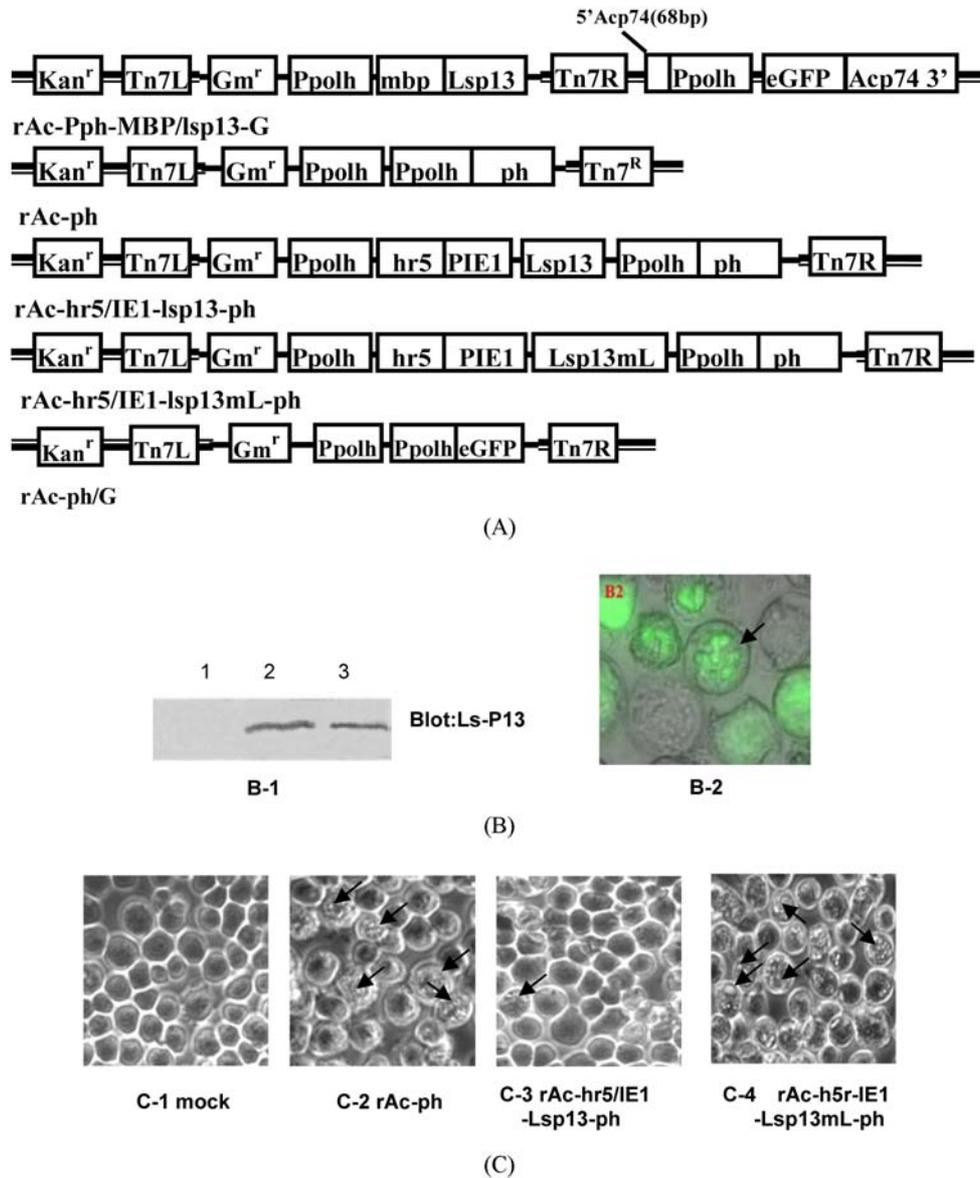


Fig. 2. Ls-P13 effect on polyhedra yield. (A) Construction the recombinant baculoviruses (rAcMNPVs) for the Ls-P13 functional study. (B) Identification of the constructed rAcMNPVs. B-1: Western blot of Ls-P13 or Ls-P13mL expressions under hr5 + IE1 promoter four days post-transfection using Ls-P13 antiserum. Lane 1, rAc-ph transfection (control). Lane 2, rAc-hr5/IE1-Lsp13-ph transfection. Lane 3, rAc-hr5/IE1-Lsp13mL-ph transfection. B-2: rAc-ph/G transfection. (C) Sf9 cells infected with different rAcMNPVs under phase-contrast and fluorescence microscopes. Black arrows indicate polyhedra.

oral ingestion, recombinant baculoviruses rAc-hr5/IE1-Lsp13-ph, rAc-hr5/IE1-Lsp13mL-ph (Fig. 2A), were constructed to express Ls-P13 or Ls-P13mL under hr5/IE1 promoter. After transfecting Sf9 cells with equivalent amounts of the above two rAcMNPVs Bacmids, Ls-P13 and Ls-P13mL expressions were examined by Western blotting (Fig. 2B-1), and the effect of Ls-P13 on rAcMNPVs was observed under a phase-contrast microscope. The results obtained indicated that Ls-P13 inhibits polyhedra yield (Fig. 2C-3), and that this inhibitory activity while the efficacy was lost when the LZLD of Ls-P13 was mutated (Fig. 2C-4).

Moreover, to measure the effects of Ls-P13 on polyhedra yield quantitatively, rAc-ph/G was constructed to express polyhedron/eGFP fusion protein (Fig. 2B-2). Because polyhedron and eGFP were fused, the fluorescence intensity of eGFP indirectly represented polyhedra yield after rAc-ph/G Bacmid transfection.

As shown in Fig. 3, most polyhedra were inhibited when Ls-P13 was uniquely droved by hr5/IE1 early promoter. However, this inhibitory efficacy was almost lost when the LZLD of Ls-P13 was mutated. Moreover, these data were consistent with our phase-contrast microscopic results.

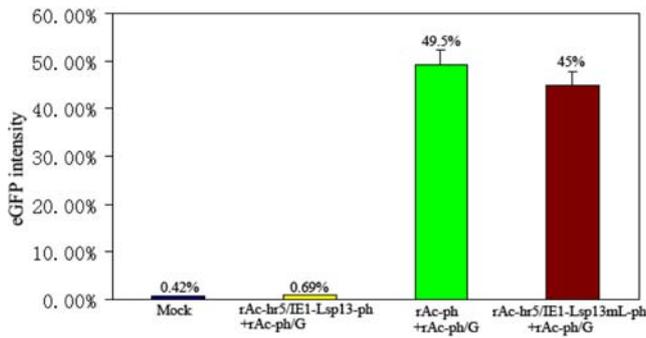


Fig. 3. Effects of Ls-P13 or Ls-P13mL on polyhedra yields as quantified by FCM 96h post-transfection. Blue: Sf9 cells control (Mock). Yellow: rAc-hr5/IE1-Lsp13-ph and rAc-ph/G co-transfection. Green: rAc-ph and rAc-ph/G co-transfection. Purple: rAc-hr5/IE1-Lsp13mL -ph and rAc-ph/G co-transfection.

From the above results we conclude that Ls-P13 inhibits polyhedra yield, and that LZLD is required for this inhibitory effect.

Ls-P13 cannot form oligomers. To verify whether Ls-P13 regulates polyhedra yield via LZLD oligomerization, the fusion proteins MBP/Ls-P13 and MBP/Ls-P13mL purified from Sf9 cells or bacteria were analyzed by gel filtration followed SDS-PAGE, using a GF-250 HPLC column, followed by SDS-PAGE. Elution was performed using 50 mM Tris-200 mM NaCl at a flow rate of 0.5 ml/min, and absorption was measured at 214 nm. Figure 4A shows that purified MBP/Ls-P13 (panels 1 and 3) and MBP/Ls-P13mL proteins (panel 2) occupied in the same horizontal line, corresponding to a molecular weight of *ca.* 72 kDa, i.e., equivalent to Ls-P13 (31 kDa) plus an MBP tag (41 kDa). Figure 4B shows that all three purified proteins eluted as a

single sharp peak at an elution time of 11.539 min, representing a single monomer of 72 kDa and indicating that no oligomers were present. Yeast two-hybrid experiments further confirmed that Ls-P13 does not interact with itself even when not fused with other proteins (Fig. 4C).

Ls-P13 secondary structure was greatly changed by LZLD mutation. The LZLD of Ls-P13 encompasses 36 residues from aa 152 to 187 (LARNKVLGKTGTMLLKPSPALYSTVRKLLNANNDCL) in the middle center of the molecule, among which 22 residues from aa 158 to 179 (refer to the underlined sequence above) contain four leucine residues spaced 7 amino acids (aa) apart, which possibly exhibit a hydrophobic 3 heptad repeat coiled-coil conformation. The above LZLD of Ls-P13 is also present in the LZLD of some P13 homologues LN₅(LN₆)₃LN₇, N represents random amino acid). A hydrophobic 3-4 heptad repeat of LZLD is required for the oligomerization of leucine zipper-like proteins. Thus, Ls-P13 and some of its homologues containing LZLD have the potential to form oligomers.

However, PredictProtein Server results showed that the LZLD of Ls-P13 only has 2 heptad repeat units available to form an extended amphipathic α -helix terminated by interval loops and β -sheets in the heptad repeat unit from 165aa to 172aa. This phenomenon was also found to be present in the LZLDs of other reported P13 proteins (Table 1). Interestingly, all the extended α -helices of P13 LZLD were terminated after one or two amino acids of the conserved prolines (open rectangle in Table 1). We presumed these conserved prolines represent an important site in terms of abrogating P13 oligomerization. Thus, as long as the central two leucines of Ls-P13 LZLD were mutated into prolines, one of the only two heptad repeat units of Ls-P13 LZLD was lost, and the hydrophobic interface formed by two heptad repeat units was also lost.

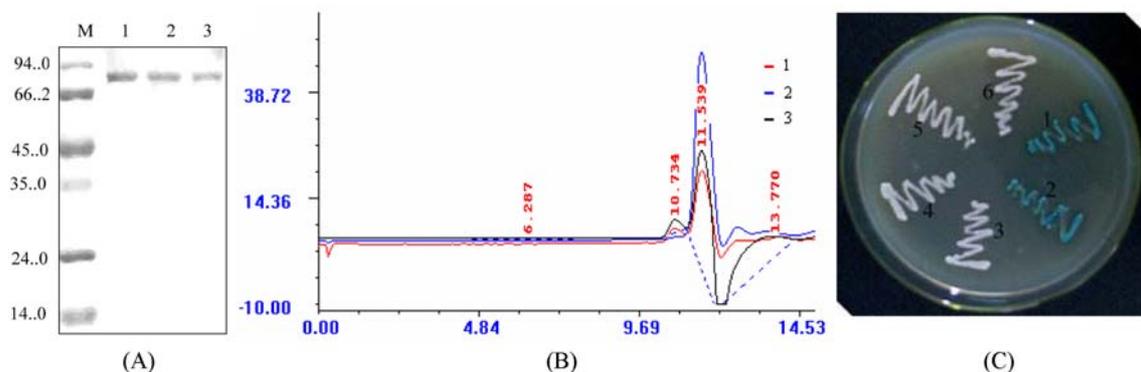


Fig. 4. Oligomerization analysis of Ls-P13. A: Ls-P13 and Ls-P13mL protein purification. Lanes 1, 2: MBP/Ls-P13 and MBP/Ls-P13mL fusion proteins purified from *E. coli* TB1. Lane 3, MBP/Ls-P13 fusion protein purification from Sf9 cells. M: represents the protein marker. B: HPLC analysis of MBP fusion proteins. Peak 1, MBP/Ls-P13 eluted from Sf9 cells. Peak 2, MBP/Ls-P13 eluted from *E. coli* TB1. Peak 3, MBP/Ls-P13mL eluted from *E. coli* TB1. C: Yeast two-hybrid assay, which was used to determine whether Ls-P13 interacts with itself. Four clones were cultured on SD/-Leu/-Trp/- plates supplemented with X-a-Gal. 1, 2: pGADT7-T and pGBKT7-53 were co-transformed into Y187 yeasts as positive controls. 3, 4, 5, 6: Four clones of pGADT7-Hap13 and pGBKT7-Hap13 were co-transformed into Y187 yeast after PCR identification.

Table 2. Secondary structure analysis by CD

	Secondary structure			
	Helix	Beta	Turn	Random
Lsp13	40.10%	0.00%	20.10%	39.80%
Lsp13mL	33.50%	27.30%	0.00%	39.20%

In addition, the secondary structure of Ls-P13 also has greatly influenced by LZLD mutation (Table 2). For example, when the central two leucines of Ls-P13 LZLD are mutated (L-P), the α -helix Ratio of the Ls-P13 secondary structure is reduced from 40.1% to 33.5%, and β -sheet and β -turn are nearly completely exchanged.

Discussion

The *p13* gene was first reported by our laboratory in LsMNPV back in 1995 (Wang *et al.*, 1995), and nearly twenty *p13* genes have been identified during recent years. Interestingly, all reported *p13* genes have been found in Group II NPVs and in some GVs, but not in Group I NPVs (Jarvis *et al.*, 1996). On the other hand, as these genes they share no homology with any other family gene and encode a putative glycosyltransferase of family 8, which has been reported in mammals, yeasts, and bacteria but not in viruses (Campbell *et al.*, 1997), we propose that *p13* genes should be viewed a novel family gene and as novel marker genes which differentiate Group II NPVs from Group I NPVs. As to the P13 function of P12 as glycosyltransferase family 8 member, we will be the subject of describe in another article.

The above begs the questions; Why is the *p13* gene not present in group I NPVs?, and, What would happen if *p13* was inserted into group I NPVs?

When the Ls-*p13* gene was inserted into AcMNPV, A Group I NPV without *p13* gene in its genome, the yield of AcMNPV polyhedra was inhibited. However, this inhibitory efficacy was lost when its leucine zipper like domain (LZLD) was mutated. We therefore concluded that LZLD is an important domain for P13 protein activity. From the molecular phylogeny viewpoint, the reason that *p13* genes are absent in Group I NPVs may be that it interferes with polyhedra yield.

Most of the P13s reported to date possess a central LZLD in the middle motif. LZLDs in eukaryotic proteins have been demonstrated to play essential roles in inter- and intra-molecular interactions, such as, during protein oligomerization and DNA binding (Monsma *et al.*, 1995). Moreover, it is known that generally oligomerization is not affected in by leucine zipper proteins when they are fused with other monomeric proteins, such as MBP or protein A (Shugars *et al.*, 1996; Ramadevi *et al.*, 1998; Prikhod'ko *et al.*, 1999) In the present study, we found using gel filtration that Ls-P13 fused with MBP (when expressed in bacteria or insect cells) does not form oligomers. Moreover, yeast two-hybrid assays

confirmed that Ls-P13 cannot interact with itself even when it is expressed alone.

As for the LZLD of Ls-P13 protein, the structure of four leucine residues spaced 7 amino acids apart may form a hydrophobic interface of three heptad repeat units, much like the leucine zipper like protein HIV-1 Gp41. However, PredictProtein Server analysis showed that the hydrophobic interface is only formed by two heptad repeat units. Another putative heptad repeat unit (165 aa to 172 aa) was abrogated by interval loops and β -sheets. Moreover, when the two central leucines were mutated to proline, one of these two heptad repeat units was abrogated disrupted and the hydrophobic interface was broken. In addition, the ability of Ls-P13 to inhibit polyhedra yield was also lost when these mutations were introduced. CD results further indicated that Ls-P13 secondary structure was greatly changed after the leucine zipper like domain of Ls-P13 was mutated, especially in terms of the β -sheet and β -turn. These differences in secondary structure may explain why Ls-P13mL lacks the ability to inhibit polyhedra yield.

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