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Enhancement of IgG Purification by FPLC for a Serological Study on the *Turnip mosaic virus* P1 Protein

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ABSTRACT

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The P1/HC-Pro of *Potyvirus* is the first discovered viral gene-silencing suppressor in which helper-component-proteinase (HC-Pro) function in concert with P1 to improve the suppression of post-transcriptional gene silencing (PTGS). However, the mechanism of P1/HC-Pro remains unclear. The P1 protein is the most divergent protein regarding length and the amino acid sequence in potyviruses. An effective serological tool is required to examine the function of P1. To improve the titer of the antiserum to P1 of *Turnip mosaic virus* (TuMV), we developed a procedure using fast protein liquid chromatography (FPLC) to purify immunoglobulin G (IgG). Moreover, the purified IgGs were further concentrated using appropriate centrifugal filter device to enhance the sensitivity. The results indicated that the new procedure improved the efficiency of IgG purification in a few hours. The low P1 signal was difficult to be detected with unpurified antersum to P1 in the TuMV-infected *N. benthamiana* plants, whereas the purified IgG to P1 enhanced 100-fold of the sensitivity for detection. These results imply that P1 may have a rapid turnover rate *in vivo* and the new IgG purification procedure is suitable for any serological study to enhance the detection sensitivity.

Keywords: *Turnip mosaic virus*, P1, immunoglobulin G, fast protein liquid chromatography

INTRODUCTION

The *Potyvirus* encodes a large polyprotein that is cleaved by P1, helper-component-proteinase (HC-Pro), and nuclear inclusion a (NIa) proteases [16]. The P1 protein is located at the beginning position in the viral genome and belongs to the serine-type protease, whereas HC-Pro is a second viral protein that belongs to the cysteine-type protease [5]. Therefore, P1 and HC-Pro remain fused, when the viral polyprotein is in the early stage of virus infection, but dissociate at a later stage. The P1/HC-Pro protein combination represents the fusion states of the 2 proteins, whereas P1 and HC-Pro indicate the dissociated state.

The P1/HC-Pro protein of the *Potyvirus* is the first identified viral suppressor [2]. Several studies in the past 2 decades have demonstrated the functions of P1/HC-Pro, but the suppression mechanism of P1/HC-Pro remains unclear. The P1/HC-Pro combination causes an abnormal accumulation of microRNA (miRNA) and short-interfering RNA (siRNA) in transgenic and virus-infected plants [10], affecting the RNA-induced gene-silencing complex (RISC) assembly [6, 20].

P1 is the most diverse protein regarding amino acid sequence (0% to 71.6% identity) and protein size among potyviruses [1]. The conserved motifs were identified near the C-terminal region of P1, which relates to protease catalytic activity. Certain studies have shown that the variable feature facilitates host adaptation [13, 17]. However, the real function of P1 remains unknown. Moreover, the P1 of *Tobacco vein mottling virus* (TVMV) is highly positively charged and has RNA-binding ability *in vitro* [4]. P1 of *Turnip mosaic virus* (TuMV) also has a non-specific RNA-binding property *in vitro* [15], and P1 of *Tobacco etch virus* functions as an accessory factor for viral genome amplification [18]. P1 enhances the suppression of HC-Pro in post-transcriptional gene silencing (PTGS) [7]. In addition, the separation of P1 and HC-Pro is required for viral infectivity [18]. The P1 subcellular localization of *Potato virus Y* (PVY) occurs in the cytoplasm and forms inclusion bodies [3]. This suggests that P1 and HC-Pro may have a direct or indirect interaction [10].

The P1/HC-Pro (112K protein) of *Papaya ringspot virus* (PRSV) was purified from the infected *Cucumis metuliferus* tissues that was used for producing monoclonal antibodies (MAbs) by Wang et al. (1989) [19]. Total 31 MAbs reacting with the P1/HC-Pro and HC-Pro (51K protein) were obtained [19]. However, only 2 isolated MAbs, which recognized the 112 K of the P1/HC-Pro fusion proteins, also weakly recognized the 64K of the P1 *in vitro* and *in vivo*. These results indicate that immunodetection of P1 may be difficult [19]. Certain similar studies have demonstrated the weak reactivity of P1 when detecting *Plum pox virus* (PPV) [12]. This implies that P1 has a rapid turnover rate in infected cells [12].

In this study, a rapid IgG purification procedure using FPLC was developed, and the *in vivo* detection of P1 protein can be improved by the purified and concentrated IgG. Hence, our approach provides a feasible tool to study the function of the unstable potyviral P1 protein.

MATERIALS AND METHODS

Plant materials and virus inoculation

Nicotiana benthamiana plants were grown at 25ºC in a greenhouse with 16 hr light and 8 hr dark cycles. TuMV was maintained in *N. benthamiana* plants, and the virus-infected tissues were collected at 7 days post inoculation (dpi) and ground with 10 volumes (w/v) of 10 mM inoculation buffer (200 mM NaH_2PO_4 and 200 mM Na_2HPO_4, pH 7.0). The 3-week-old seedlings of *N. benthamiana* were used for mechanical inoculation of TuMV.

Recombinant protein purification and antisera production

For the recombinant proteins expressed in *E. coli*, the *P1* reading frame of TuMV was amplified from p35S-TuMV-YC5 [11] by PCR with the primers PTu-P1-NdeI (5'-CCAACAGCAAACCAACATATGGCAGTAGTTAC-3') and MTu-P1-XhoI (5'-CACTCTCGAGTTAAAAGTGCAC AATCTTGTGACTCATTG-3'), which contains *Nde*I and

*Xho*I sites (underlined), respectively. The PCR fragments were digested with *Nde*I/*Xho*I and ligated with the same restriction enzyme-digested pET-28b (Novagen, San Diego, CA) to generate pET-TuP1.

The *E. coli* cells containing pET-TuP1 or pET-TuCP (positive control) [9] plasmids were individually cultured in 500 ml LB medium at 37°C for 3 hr and were induced with 0.5 mM isopropyl-D-1-thiogalactopyranoside (IPTG) and grown at 20°C for 6 hr until the absorbance at 600 nm reached 0.5. The *E. coli* cells were pelleted down and suspended in a denaturing buffer (8 M Urea, 100 mM NaH_2PO_4, and 100 mM Tris-HCl, pH 8.0) and disrupted with the EF-C3 Emulsiflex Homogeniser (Avestin, Canada). The lysate supernatant was loaded onto a 1 ml HisTrap FF column (GE Healthcare, Uppsala, Sweden) and mounted on an FPLC (AKTApurifier, GE Healthcare, Uppsala, Sweden). The column was washed with 5 column volumes of a denaturing buffer and eluted using the same buffer with a pH gradient of 8.0 to 4.5 for 30 min.

The recombinant P1 (his-P1) was used for antiserum production. One mg recombinant protein was mixed with Freund's adjuvant (1:1 v/v) and injected into New Zealand white rabbits once per week; the procedure was repeated 4 times [21]. For the first injection, the “complete” adjuvant was used, and the “incomplete” adjuvant was used for the following infections. The titer of the antiserum was analyzed using the indirect enzyme-linked immunosorbent assay (indirect ELISA) or western blotting.

Immunoglobulin purification by FPLC

Ten ml of antiserum were filtered through a 0.45 μm membrane filter (Millipore, Billerica, MA, USA) and loaded into a Hitrap rProtein A FF 1 ml column (GE healthcare, Uppsala, Sweden) by FPLC. After washing with a 10 ml binding buffer (20 mM sodium phosphate, pH 7.0), the pH gradient of 0.1 M citric acid was reduced from 6.0 to 3.0 for elution for 10 min. The eluted fraction was immediately neutralized with 200 μl neutralization buffer (1 M Tris-HCl, pH 9.0), dialyzed overnight at 4°C in 1x PBS buffer (137 mM NaCl, 2.7 mM KCl, 10 mM Na_2HPO_4, and 1.8 mM KH_2PO_4, pH 7.5) and concentrated through a Vivaspin 20 Centricon (GE healthcare, Uppsala, Sweden) by centrifugation at 10,000 rpm for 30 to 60 min.

Western blot

The plant extracts or recombinant proteins were denatured in a 2 volumes (w/v) of 2x protein sample buffer (2% SDS, 10% glycerol, 1% 2-mercaptoethanol, 0.005% bromophenol blue, and 50 mM Tris-HCl, pH 6.8) and boiled at 100°C for 10 min. Ten μl samples were separated by electrophoresis on a SDS-polyacrylamide (12%) gel and analyzed by western blotting using unprocessed antiserum to P1 (1:8,000) or enriched IgG to P1 (1:10,000). The goat anti-rabbit horseradish peroxidase (HRP)-conjugated IgG (GE healthcare, Uppsala, Sweden) was used (1:10,000) as secondary antibody and the subsequent chemiluminescent detection was performed with WesternBright™ ECL HRP Substrate Kit (Advansta, Menlo Park, CA, USA).

LC- MS/MS analysis for recombinant P1

The desired band of his-P1 was excised from the Coomassie blue-stained SDS-PAGE gel and in-gel digestion was performed following manufacturer’s instructions (trypsin:proteins = 1:50) (Promega, Fitchburg, Wisconsin, USA) [14]. The peptide extracts were resolved with 0.1% trifluoroacetic acid (TFA), and C_{18}ZipTip (Millipore, Billerica, MA, USA) was used for desalting and cleaning for high-performance liquid chromatography (HPLC) with tandem mass spectrometric (LC-MS/MS) analysis (Synapt G1, Waters, Milford, MA, USA) in the National Taiwan University proteome core. The acquired MS/MS spectrum was searched against the NCBI nr protein database using the MASCOT algorithm (Matrix Science, Boston, MA, USA).

RESULTS

Expression and purification of recombinant P1 protein

For the production of the antiserum against the P1 of

TuMV, the recombinant P1 (his-P1) was purified from *E. coli* carrying the pET-P1 plasmid (Fig. 1A). The pET-CP plasmid was used as a positive control for purification by FPLC to generate the recombinant CP (his-CP) of the TuMV (Fig. 1A) [(9)]. The his-P1 (42 kDa), and his-CP (34 kDa) proteins were expressed after 0.5 mM IPTG induction for 2 hr. Only the his-P1 formed inclusion bodies in *E. coli*. Therefore, a denaturing method was used for the purification of insoluble his-P1. The FPLC-eluted fractions of his-P1 and his-CP indicated that both proteins were efficiently purified using FPLC (Fig. 1B and C). The purified his-P1 protein was used to produce antiserum in rabbits.

In vitro and *in vivo* detection efficiency of antiserum to P1

The specificity of the antisera for recombinant proteins (his-P1 and his-CP) was analyzed by western blotting (Fig. 2A). All antisera specifically recognized their respective antigens, which were expressed in the induced fractions of *E. coli*. No background was noticed at the same position of the un-induced fractions (Fig. 2A, left panel). These results indicate that these antiseria have a high specificity for detecting recombinant proteins.

For detection of viral proteins in TuMV-infected *N. benthamiana* plants, the antiserum to CP specifically detected the TuMV CP *in vivo* (Fig. 2A, right panel), however, the P1 was poorly detected in the TuMV-infected tissues using antiserum to P1 in western blotting (Fig. 2A, right panel). Moreover, his-P1 was detected, suggesting that the western analysis was successful (Fig. 2A).

To ensure that the expressed protein used for antiserum production was TuMV P1, we analyzed the eluted protein from *E. coli* by LC-MS/MS for protein identification (Fig. 2B, left panel). The LC-MS/MS results showed that P1 had the highest score within the sequence of the polyprotein of TuMV (Fig. 2B, right panel). Other identified proteins with higher scores were the elongation factor complex, Ef-TuEF-Ts of *E. coli*, the leech-derived tryptase inhibitor/trypsin complex, and keratin (Fig. 2B, right panel).

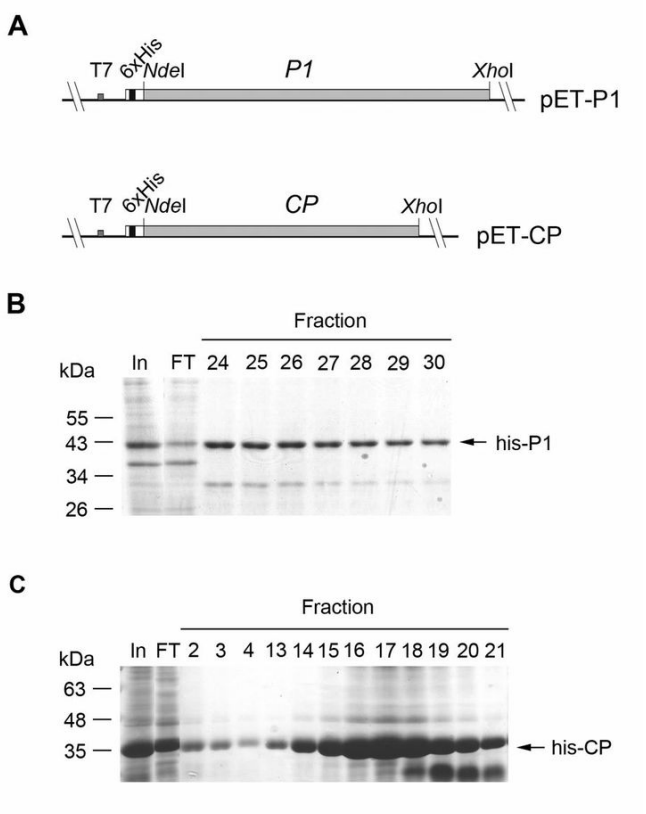

Figure 1. Schematic diagram of the construction for recombinant proteins expression and large-scale purification of recombinant proteins. (A) The schematic diagram of construction for *P1* and *CP* gene that were cloned in pET28b vector with *Nde*I and *Xho*I clone sites. The 6xhis-tag sequence was fused at N-terminal P1 (his-P1) or CP (his-CP) for Ni-NTA purification. T7 promoter was indicated on the map. FPLC-eluted fraction of recombinant P1 (his-P1) **(B)** and the CP (his-CP) **(C)** were analyzed by SDS-PAGE with Coomassie blue staining.

The homologues or proteins related to these proteins might be co-purified in FPLC (Fig. 2B, right panel). Moreover, the results showed that the optimal match was the polyprotein of TuMV with more than 50% coverage of a peptide hit (Fig. 2C). The signal of western blotting also confirmed that it was the P1 protein (Fig. 2A). These results indicate that the recombinant protein was the his-P1 that was constructed from TuMV. Overall, the present results indicate that P1 is a protein that is difficult to be detected or unstable in TuMV-infected plants. We hypothesize that the difficult

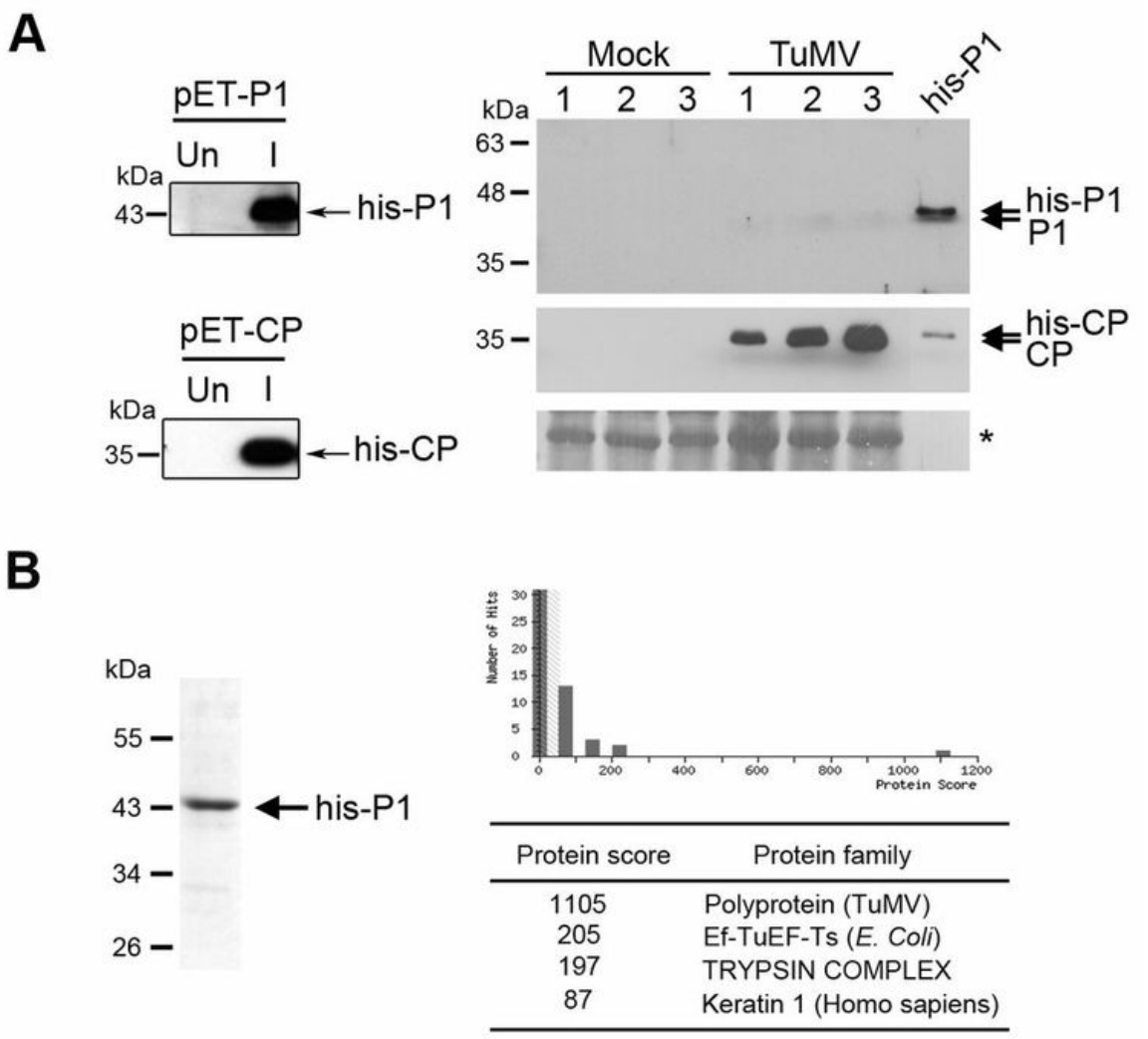

Figure 2. Difficult detectability of P1 *in vivo* and LC-MS/MS analysis for recombinant P1. (A) The detection specificity analysis for antiserum to P1 (upper panel**)** and the CP (lower panel**)** of TuMV. The antiserum was diluted 5,000-fold for recombinant protein or TuMV-infected *N. benthamiana* plant (*in vivo*) detection. For recombinant proteins detection (left panel), the inducted (I) and the non-inducted (Un) fractions of *E. coli* cultures were analyzed by western blotting. For *N. benthamiana* plant extracts (right panel), TuMV-infected plants and mock plants were repeated with 3 individual samples and analyzed by western blotting with antiserum to P1 and CP. **(B)** The eluted his-P1 was analyzed by SDS-PAGE with Coomassie blue staining. The Mascot results showed the distribution of family members scores in the top 50; the table shows 4 major hits. The protein with the highest score (1,105) was P1 of TuMV. **(C)** The matched peptides are emboldened.

detectability of P1 may be because of its unstable nature or rapid turnover rate in the host cells [12]. However, enhancing the sensitivity of the antiserum to P1 may assist in detecting the low amounts of P1 *in vivo*.

Enhancement of the titer of the antiserum to P1

We developed an IgG purification procedure using FPLC, which can rapidly purify IgG in 1 day, enhancing the titer efficiency and specificity of the antiserum to P1 for *in vivo* detection. The IgG was purified with protein A resin using FPLC, and the purified IgG was concentrated using a Centricon to enhance the titer. The process of IgG purification using FPLC requires only a few hours, and dialysis can be conducted on the same day. The heavy and light chains of IgG were enriched after purification, compared to the original antiserum (Fig. 3A). The corresponding peak and fractions were shown in Figure 3B.

To enhance the titer of the IgG to P1, the IgG must be concentrated using a centrifugal filter device (Centricon). Ten- and 20-fold concentrations of IgG to P1 were used for comparing the titer with the original IgG to P1. The results showed that the end-point detection of the 10- and 20-fold-concentrated IgG can reach 100 ng and 10 ng of his-P1, respectively, whereas the original IgG to P1 was detected 1 μg of his-P1 (Fig. 3C). The sensitivity of IgG increased after the concentration procedure.

IgG specificity assay

The specificity of IgG for his-P1 was analyzed by western blotting. The IgG to P1 recognized the antigens of the his-P1 that were expressed in the induced fractions of *E.coli* (Fig. 3C). No background was observed at the same position in the empty vector control (Fig. 3C). These results indicate that the IgG to P1 has a high specificity for detecting recombinant proteins. In addition, the sensitivity of the IgG titer increases after concentration. The data showed that 1x of IgG to P1 can only detect 1 μg of his-P1, whereas the 10x and 20x of IgGs to P1 can detect 100 ng and 10 ng, respectively. This indicates that the concentrating IgG can increase the detection sensitivity.

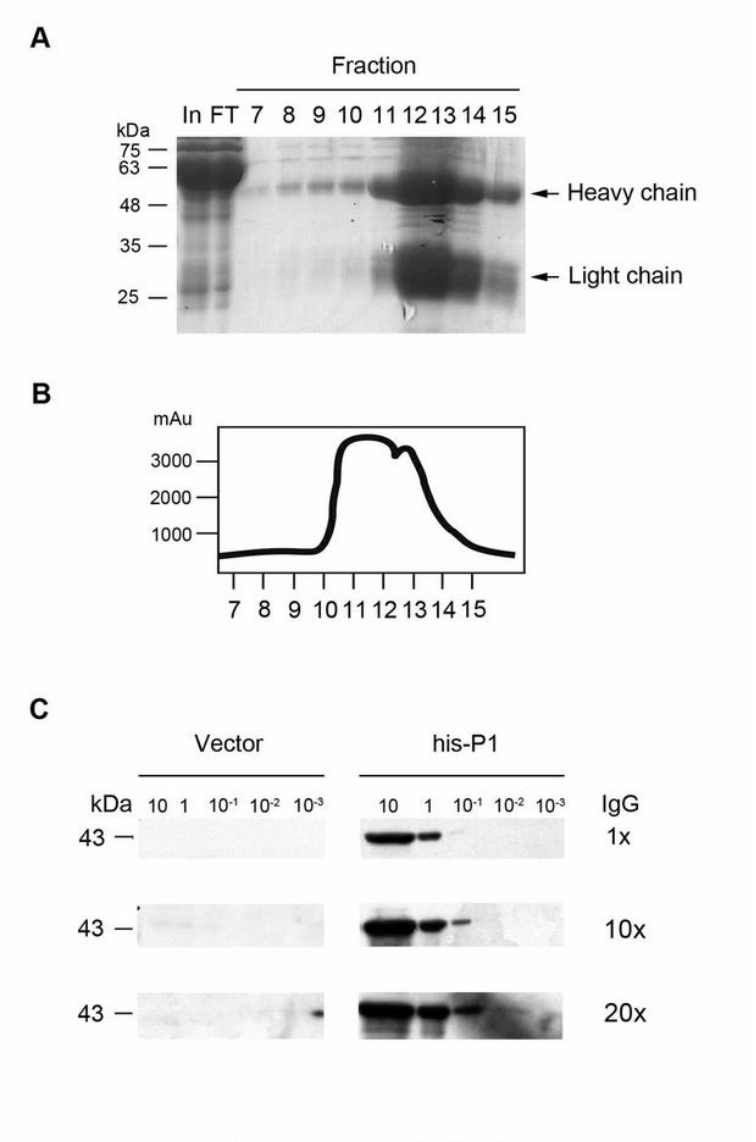

Figure 3. The efficiency of IgG purification by FPLC and titer measurement for various concentrations of purified IgG to P1. (A) The FPLC-eluted fractions for IgG purification were analyzed by SDS-PAGE with Coomassie blue staining. The heavy chain (approximately 50 kDa; upper arrowhead) and the light chain (approximately 25 kDa; lower arrowhead) of IgG are indicated. **(B)** The curve represents the UV absorbance (mAu) of IgG from FPLC. **(C)** Titer measurement for various concentrations of purified IgG to P1. The varying protein concentrations (10 μg, 1 μg, 100 ng, 10 ng and 1 ng) of his-P1 and total proteins from the pET28b vector control were analyzed by western blotting with various concentrations (1x, 10x, and 20x) of purified IgG to P1 (1:8,000).

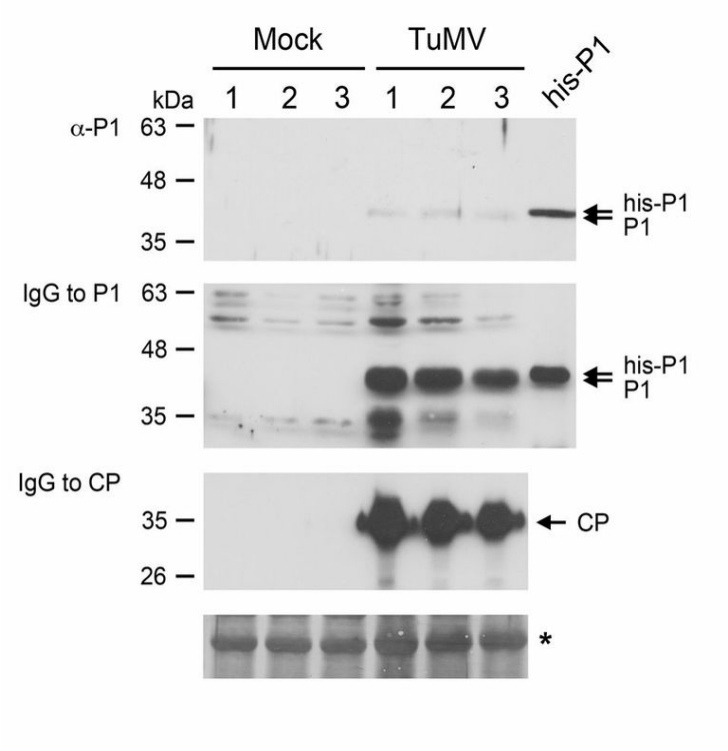

Figure 4. Evaluation of P1 delectation efficiency with antiserum or purified IgG to P1 in TuMV-infected *N. benthamiana* plants. TuMV-infected and mock-inoculated *N. benthamiana* plants were used for the evaluation. Each sample was repeated with 3 individual plants. The western blottings were hybridized with antiserum to P1 (1:8,000) (upper panel), purified IgG to P1 (1:10,000) (the second panel), and purified IgG to CP (the third panel). RUBISCO (lower panel) was used as the loading control.

IgG to P1 enhances the *in vivo* detection signal

IgG to CP specifically detected the viral proteins in TuMV-infected *N. benthamiana* plants (3 repeats) *in vivo*, which indicated that the TuMV infection was successful (Fig. 4, lower panel). However, the P1 signals elicited by the antiserum to P1 remained difficult to detect from the TuMV-infected *N. benthamiana* plants (Fig. 4, upper panel). However, the P1 signal was enhanced significantly when IgG to P1 was used for reaction (Fig. 4, mid panel). These results indicated that the purified and concentrated IgG to P1 can be used to detect the trace amounts of P1 protein *in vivo*. Moreover, these results imply that P1 may be significantly unstable with a rapid turn over rate *in vivo*.

DISCUSSION

Potyvirdae are the largest virus group with the widest host range in the world. The high variation of the P1 protein in potyviruses is an unusual characteristic. Several studies have indicated that P1 determines the host range [13, 17]. However, the importance of the potyviral P1 protein is unclear, and scientific evidence regarding the function of P1 is limited. Although the P1 proteins vary considerably in different potyviruses, these proteins share a common unknown function that is essential to HC-Pro for suppressing the miRNA pathway [10]. The transgenic *Arabidopsis* expressing *35S-P1/HC-Pro* cloned from TuMV (unpublished data) or ZYMV (27.5% similarity between 2 viruses) exhibited the serrated leaf phenotype, which is a typical miRNA pathway-suppressed phenotypes [8].

The recombinant proteins of P1 were purified from *E. coli* to produce an antiserum for the detection of P1. The denaturing method for purifying his-tagged recombinant proteins was effective for recovering the insoluble protein and increasing the protein amount for antigen injection. Moreover, the entire process can be accelerated by conducting IgG purification with the protein A column using FPLC, which has a real-time detection and does not require ammonium sulfate precipitation. The titer was enhanced to improve the detection by concentrating IgG with a Centricon. The traditional method for IgG purification requires 3 days, including overnight ammonium sulfate precipitation. Purifying IgG using FPLC has several advantages, such as real-time detection and a high yield of specific proteins. Ammonium sulfate precipitation is performed on Day 1 for traditional IgG purification. IgG purification with protein A is conducted on Day 2, and dialysis is conducted on Day 3.

Several studies have confirmed the difficulty in the detection of P1 of PRSV and PPV [12, 19]. The titer of 20x IgG to P1 was enhanced 100-fold in this study for recombinant protein detection (Fig. 3C). Moreover, the 20x IgG to P1 can detect P1 in TuMV-infected *N. benthamiana* plants, whereas the poor detection of *in vivo* P1 with original antiserum (Fig.

4). Based on the gene expression strategy of the *Potyvirus*, the amount of P1s should equal CPs. However, detecting P1 with an unpurified antiserum is difficult, whereas the CPs were easily detected with an unpurified CP antiserum. We imply that P1 might have a fast turnover rate in host cells.

In this study, we developed a procedure for IgG purification to shorten the purification time, and to enhance the P1 detection *in vivo*. The rapid turnover rate of P1 may result in the low amounts of P1, causing the detection problem. The generation of efficient IgG to P1 may assist in clarifying the role of P1 in the P1/HC-Pro-mediated suppression of the miRNA pathway.

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Author contributions

S.-S.L. designed the research. M.-T.C. and P.-C.L. performed the experiments. C.-P.L. provides important comments and suggestions. All authors discussed the results and commented on the manuscript that was written by S.-S.L.

LITERATURE CITED

1. Adams, M. J., Antoniw, J. F., and Fauquet, C. M. 2005. Molecular criteria for genus and species discrimination within the family Potyviridae. Arch. Virol. 150:459-479.
2. Anandalakshmi, R., Pruss, G. J., Ge, X., Marathe, R., Mallory, A. C., Smith, T. H., and Vance, V. B. 1998. A viral suppressor of gene silencing in plants. Proc Natl Acad Sci USA 95:13079-13084.
3. Arbatova, J., Lehto, K., Pehu, E., and Pehu, T. 1998. Localization of the P1 protein of potato Y potyvirus in association with cytoplasmic inclusion bodies and in the cytoplasm of infected cells. J. Gen. Virol. 79:2319-2323.
4. Brantley, J. D., and Hunt, A. G. 1993. The N-terminal protein of the polyprotein encoded by the potyvirus tobacco vein mottling virus is an RNA-binding protein. J. Gen. Virol. 74:1157-1162.
5. Carrington, J. C., and Dougherty, W. G. 1988. A viral cleavage site cassette: identification of amino acid sequences required for tobacco etch virus polyprotein processing. Proc. Natl. Acad. Sci. USA 85:3391-3395.
6. Chapman, E. J., Prokhnevsky, A. I., Gopinath, K., Dolja, V. V., and Carrington, J. C. 2004. Viral RNA silencing suppressors inhibit the microRNA pathway at an intermediate step. Genes Dev. 18:1179-1186.
7. Kasschau, K. D., and Carrington, J. C. 1998. A counterdefensive strategy of plant viruses: suppression of posttranscriptional gene silencing. Cell 95:461-470.
8. Kasschau, K. D., Xie, Z., Allen, E., Llave, C., Chapman, E. J., Krizan, K. A., and Carrington, J. C. 2003. P1/HC-Pro, a viral suppressor of RNA silencing, interferes with Arabidopsis development and miRNA unction. Dev. Cell 4:205-217.
9. Lin, Y. Y., Fang, M. M., Lin, P. C., Chiu, M. T., Lin, C. P., and Lin, S. S. 2012. Improving initial infectivity of the Turnip mosaic virus (TuMV) infectious clone by an mini binary vector via agro-infiltration. Botanical Studies Accepted.
10. Mallory, A. C., Ely, L., Smith, T. H., Marathe, R., Anandalakshmi, R., Fagard, M., Vaucheret, H., Pruss, G., Bowman, L., and Vance, V. B. 2001. HC-Pro suppression of transgene silencing eliminates the small RNAs but not transgene methylation or the mobile signal. Plant Cell 13:571-583.
11. Niu, Q. W., Lin, S. S., Reyes, J. L., Chen, K. C., Wu, H. W., Yeh, S. D., and Chua, N. H. 2006. Expression of artificial microRNAs in transgenic Arabidopsis thaliana confers virus resistance. Nat. Biotechnol. 24:1420-1428.
12. Riedel, D., Lesemann, D. E., and Maiss, E. 1998. Ultrastructural localization of nonstructural and coat proteins of 19 potyviruses using antisera to bacterially expressed proteins of plum pox potyvirus. Arch Virol. 143:2133-2158.
13. Salvador, B., Saénz, P., Yangüez, E., Quiot, J. B., Quiot,

L., Delgadillo, M. O., García, J. A., and Simón-Mateo, C. 2008. Host-specific effect of P1 exchange between two potyviruses. Mol. Plant Pathol. 9:147-155.

14. Shevchenko, A., Tomas, H., Havlis, J., Olsen, J. V., and Mann, M. 2006. In-gel digestion for mass spectrometric characterization of proteins and proteomes. Nat. Protoc. 1:2856-2860.
15. Soumounou, Y., and Laliberté, J. F. 1994. Nucleic acid-binding properties of the P1 protein of turnip mosaic potyvirus produced in Escherichia coli. J. Gen. Virol. 75:2567-2573.
16. Urcuqui-Inchima, S., Haenni, A. L., and Bernardi, F. 2001. Potyvirus proteins: a wealth of functions. Virus Res. 74:157-175.
17. Valli, A., López-Moya, J. J., and García, J. A. 2007. Recombination and gene duplication in the evolutionary diversification of P1 proteins in the family Potyviridae. J. Gen. Virol. 88:1016-1028.
18. Verchot, J., and Carrington, J. C. 1995. Evidence that the potyvirus P1 proteinase functions in trans as an accessory factor for genome amplification. J. Virol. 69:3668-3674.
19. Wang, G. X. 1989. Purification and characterization of the 112K gene product of Papaya ringspot virus. National Chung-Hsing University, Taichung.
20. Wu, H. W., Lin, S. S., Chen, K. C., Yeh, S. D., and Chua, N. H. 2010. Discriminating mutations of HC-Pro of zucchini yellow mosaic virus with differential effects on small RNA pathways involved in viral pathogenicity and symptom development. Mol. Plant Microbe Interact. 23:17-28.
21. Yeh, S. D., and Gonsalves, D. 1984. Evaluation of induced mutants of Papaya ringspot virus for control by cross protection. Phytopathology 74:1086-1091.

摘　要

邱敏慈[1,4,5]、林長平[4,5]、林品均[1]、林詩舜[1,2,3,6] 2013. 利用快速流速層析儀發展改良式免疫球蛋白 G 純化流程進行蕪菁嵌紋病毒 P1 蛋白血清學之研究.植病會刊 22: 21-30. ([1]台灣大學生物科技研究所及[2]基因體與系統生物學學程；[3]中央研究院農業生物技術研究中心；[4]國立台灣大學植物病理暨微生物學系; [5]共同第一作者；[6]通訊作者，電子郵件：linss01@ntu.edu.tw)

馬鈴薯 Y 群病毒 (potyvirus) 產生的 P1/HC-Pro 基因是第一個被發現的病毒抑制子，其作用主要是藉由 P1 蛋白協助 HC-Pro 針對後轉錄時期基因靜默機制進行抑制作用。然而 P1/HC-Pro 的真正功能尚未了解十分透徹。P1 蛋白在 *Potyvirus* 屬中是大小及序列差異最大的蛋白。要有效率的研究 P1 功能，必須要有好的血清學工具。本研究，我們開發了一個新的利用快速液相層析儀純化蕪菁嵌紋病毒 (*Turnip mosaic virus*, TuMV) 的 P1 免疫球蛋白 IgG 流程。此外，所純化出的免疫球蛋白 IgG 配合 Centricon 濃縮技術，增加免疫球蛋白 IgG 的濃度。我們結果顯示，新的純化方式可快速地在數小時內有效率地純化出免疫球蛋白 IgG。此外，在 TuMV 所感染的菸草中，病毒產生的 P1 蛋白難以被未經純化的 P1 抗血清所偵測；而濃縮及純化的 P1 免疫球蛋白 IgG 具高於未純化的 P1 抗血清的 100 倍靈敏度，可以順利地偵測出植物體內病毒所產生的 P1 蛋白。因此，我們推測 P1 蛋白具有快速代謝降解率，導致所感染之植物體內含量不高，難以偵測。此新的免疫球蛋白 IgG 純化流程，適合任何血清學研究，有助於提升偵測靈敏度。

關鍵詞：蕪菁嵌紋病毒、P1 蛋白、免疫球蛋白 IgG、FPLC