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Insights into the Molecular Antiviral Mechanism of Pokeweed Protein from *Phytolacca americana*

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Abstract

Agriculture is an indispensable part of every person’s life, ensuring that nutritious and inexpensive food is readily available. As any other organisms, plants are subject to numerous parasitic infections. Biological evolution has allowed plants to produce a variety of toxic compounds to deal with their pathogens. American pokeweed plant (*Phytolacca americana*) manufactures pokeweed antiviral protein, a ribosome inactivating protein that disrupts protein synthesis and lowers infectivity of many plant and animal viruses. The intricate mechanism of PAP antiviral activity entails a delicate coordination and interplay of several factors, allowing the plant to battle its invaders. Here, we examine the molecular mechanism of this plant peptide, and describe a molecular model of pokeweed’s antiviral activity.

Keywords: Pokeweed antiviral protein; Ribosome inactivating protein; Depurination

Introduction

Agriculture continues to be confronted by epidemics, having devastating effects on economies and the plant sources essential for human and animal life. Plants are essential for human and animal life, and encompass natural and landscaped spaces, including forests, crops, nurseries and orchards. Multitudes of microbial pathogens invade and colonize plants, while metabolizing their tissues and disrupting a delicate balance of hormones and nutrients, and in some cases, suppressing gene activity [1-3]. Many plants have evolved to produce natural defense mechanisms that aid in the battle with foreign pathogenic invaders. Plant defense mechanisms include myriad physical and chemical defenses, which prevent pathogens from entering the plant cell, limit their availability, and/or restrict the nutrients necessary for the growth and replication of the pathogen [4-8].

Ribosome Inactivating Proteins (RIPs)

Ribosome inactivating proteins (RIPs) are a group of cytotoxic proteins possessing extremely specific rRNA N-glycosidase activity, and proficient in catalytically inactivating ribosomes, inducing cellular death [9]. The biological effects credited to these protein toxins go back to early times, owing to the high toxicities of the castor bean and jequirity bean [10]. Yet other plants, such as American pokeweed (*P. americana*) and common soapwort, synthesize pokeweed antiviral protein (PAP) and saporin, that impose lower toxicity on intact cells, and depurinate capped mRNA directly. Hudak et al. [51] generated and depurinates capped mRNA directly. Hudak et al. [51] generated several PAP mutations (PAPx, an active site mutant (E176V); PAP n, a N-terminal sequence; PAP c, N-terminal 25 amino acid residues) and showed showing a broad spectrum of antiviral activity [13]. This depurination activity, and inhibited transmission of tobacco mosaic virus (TMV) in plants; though, not until 1978 PAP was accepted as an inhibitor of protein synthesis [25]. While the mechanism of PAP antiviral activity is somewhat unclear, recent findings, produced by the Hudak and Tumer laboratories, show that this activity is not dependent exclusively on inactivation of ribosomes [34,35]. It has been postulated that a direct interaction of PAP with viral RNA (or DNA) is an alternative antiviral mechanism in play. The pokeweed plant produces several isozymes of PAP, all exerting potent antiviral properties [11,13,36-42]. PAP isoforms evoke depurination of genomic HIV-1 RNA [43-45], TMV RNA [46], poliovirus [47], herpes simplex virus (HSV) [48], influenza virus [49], and brome mosaic virus (BMV) [50], among many others, showing a broad spectrum of antiviral activity [13]. This depurination is concentration dependent.

PAP Inhibits Replication of Capped Viruses

Recent findings have put forward an interesting mechanism for the translation inhibition by PAP [51], where PAP specifically targets and depurinates capped mRNA directly. Hudak et al. [51] generated several PAP mutations (PAP+, an active site mutant (E176V); PAP-, a mutant with a substitution (G75D) in the N-terminal sequence; PAP-, a mutant lacking the C-terminal 25 amino acid residues) and showed

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that these PAP mutants do not depurinate rabbit or tobacco ribosomes, yet inhibit the *in vitro* translation of potato virus X and BMV with no notable depurination of ribosomes. These studies showed that PAP is proficient in differentiating capped from uncapped mRNAs, since wild type (WT) PAP, and several of its mutants, prompted inhibition of only capped (but not uncapped) luciferase transcripts. The presence of cap analog decreased the ability of PAP and PAP mutants to inhibit translation of viral RNA, meaning that these toxic proteins are able to identify the cap structure at the 5′ end of the mRNA. Examination of the PAP-treated luciferase transcripts revealed that only the capped RNAs were subject to cleavage by acidic aniline, and hence were depurinated *in vitro*. Based upon these important findings, it was concluded that PAP binding to the cap structure, with subsequent RNA depurination, was the main mechanism for the translation inhibition; depurination of capped viral RNA perhaps, is the primary mechanism for the antiviral activity of PAP [52]. Furthermore, Friedland team have examined interactions between PAP and m’GTP cap analog employing direct fluorescence titrations [53]; this led to quantitative characterization of these interactions.

**PAP Inhibits Replication of Uncapped Viruses**

Although only less than twenty percent of plant positive strand RNA viruses are similarly structured to host mRNA (with a 5′-m’G cap and poly(A) tail) [54], the majority lack one or both of these moieties. Interestingly, PAP also exerts its inhibitory effects on the replication uncapped viruses such as influenza and poliovirus [47,49]. Vivanco et al. [55] have inspected PAP activity against a set of capped and uncapped viral RNAs, demonstrating that PAP does not depurinate every capped RNA, while inhibiting translation of uncapped viral RNAs in *in vitro* without causing measurable depurination at multiple sites. PAP did not depurinate uncapped luciferase mRNA, while depurinating TMV and BMV transcripts, demonstrating that PAP is able to discriminate between capped and uncapped RNAs. No evident depurination of capped alfalfa mosaic virus (AMV) RNA was recorded either. This indicates that the recognition of the cap feature alone is not enough to cause multiple site depurination of RNAs [55]. Furthermore, the team did not record any evident depurination of uncapped satellite panicum mosaic virus (SPMV) RNA, tomato bushy stunt virus (TBSV) RNA, nor uncapped RNA encompassing a poliovirus internal ribosome entry site (IRES); yet, *in vitro* translation experiments illustrated PAP inhibiting translation of the above viral RNAs [55].

**Interactions with Translation Initiation Factors (eIFs) and Effects of Structured RNA**

Work published by Wang and Hudak [56] presents confirmation that PAP is able to bind eukaryotic translation initiation factor eIF4G and its isoform eIF4O4G. Studies show that PAP binds specifically to each form, and biochemical and genetic analyses present confirmation that the region of the protein between amino acids 511 and 624 is needed for PAP binding activity [56]. PAP not only binds to m’GTP-Sepharose; this binding does not reduce the binding of PAP to purified eIF4O4G, indicating that PAP simultaneously forms a complex with eIF4O4G and the cap moiety. In wheat germ lysate translational system, PAP depurinated uncapped transcripts containing a functional WT 3′ translational enhancer element (3′ TE), but did not depurinate messages containing a non-functional mutant 3′ TE [56]. These findings supports a previously postulated hypothesis that binding of PAP to eIF4G (or eIF4O4G) may offer an alternative mechanism for PAP to access both uncapped and capped viral RNA for depurination. Baldwin et al. [53] have demonstrated that PAP not only binds to the initiation factor eIF4O4G, but that binding of the cap analog to PAP is amplified by this macromolecular interactions, supporting previous findings. This suggests a novel mechanism: PAP interacts with eIF4O4G/eIF4G (as part of eIF4F/eIF4F) and interacts with the
cap moiety of mRNA. Moreover, addition of eIF4E/eIF4F (as part of eIF4E/eIF4F) competitively diminishes binding affinity of PAP for the cap, since both proteins are cap-binding [53]. These PAP-eIF interactions possibly unfold the active site of PAP, allowing PAP to recognize target adenine residues for depurination (Figure 1A) [53].

Recent work performed in our laboratory showed that PAP binds to and depurinates the 5'-leader sequence from tobacco etch virus (TEV) RNA [57]. The TEV 5'-leader is sufficient to confer cap-independent translation, even in the absence of the 5'-terminal VPg (a viral genome-linked protein) [58,59]. The TEV 5'-leader contains an IRES element [58-60] that is notable for its small size, and represents one of the most compact viral elements capable of promoting cap-independent translation yet identified. A 5'-proximal, 45-nt RNA pseudoknot-encapsiding domain (PK1) within the TEV 5'-leader is essential to promote cap-independent translation [61]. Mutations disrupting the PK1 reduce cap-independent translation, including mutations to loop 3 that exhibit complementarity to a conserved region in eukaryotic 18S rRNA [61]. Furthermore, PAP bind to both full length TEV leader sequence and the PK1, yet depurinates only the full-length leader element, indicating that PAP’s binding affinity is separate from its depurinating activity [62]. Employing a pull-down assay, we showed that PAP binds to eIF4eG and eIF4E simultaneously, and this was confirmed by fluorescence resonance energy transfer (FRET) [63]. These findings support a model, previously proposed by Wang and Hudak [56]: the recognition and depurination of uncapped mRNA by PAP is promoted by the translation initiation factors, allowing PAP to gain access to uncapped, non-polyadenylated RNAs containing either 5'- (and/or 3'-) TEs, associating with eIF4F/eIF4F4 protein complexes (Figure 1B).

Conclusion

The mechanism of PAP antiviral activity, specifically PAP recognition of its substrate viral RNAs, is complex and entails the interplay of a set of different factors. The initial recognition may occur through PAP binding to the cap structure found at the 5'-end of the capped viral genomes, thereby enabling PAP to access its substrates for depurination (Kd for PAP-m7GTP 43.3 ± 0.1 nM at 25°C) [53]. In the instances where the 5'-cap is absent, PAP directly binds to either the 5'- or 3'-UTRs (untranslated regions), containing either translational enhancer sequences or an internal ribosome entry site (Kd for PAP-uncapped full length TEV 5'-leader RNA is 28.5 ± 3.7 nM; Kd for PAP-mGpppG-capped full length TEV 5'-leader RNA is 87.5 ± 4.8 nM) [62]; binding of the eIFs increases PAP-RNA affinity, promoting depurination of RNA (presence of eIF4eG increases 2.4-fold PAP-cap interactions) [53]. Additionally, PAP isoforms selectivity for different ribosomes and RNAs varies (e.g., PAP-L found in spring leaves of the pokeweed plant, exhibits Kd for 1.5 nM towards rat liver ribosomes and 4.7 nM towards E. coli ribosomes [38]; PAP-S1, an isoform found in seeds of the plant, exhibits IC50 of 3.2 nM towards rat liver ribosomes and 280 nM towards E. coli ribosomes [41,42]; whereas a-PAP, expressed in all organs of the plant, exhibits IC50 of 1.3 nM towards rat liver ribosomes and 25 nM towards E. coli ribosomes [13,42]. In recent years, a viral protein (VPg), linked to genome of turnip mosaic virus (TuMV) was shown to inhibit PAP activity in vitro [13,57]. This viral peptide serves as an analog of the 5’-G cap of viral RNA, and has been shown to play an important role in mRNA translation since it interacts with the cap-binding proteins (e.g., eIF4E, eIF4G, eIF4F, eIF4F4) [64,65]. Our laboratory has shown that PAP interacts with VPg (Kd is 29.5 ± 1.8 nM) and inhibits PAP’s activity in a dose-dependent manner [57], perhaps providing an evolutionary advantage for the virus to overcome this plant defense mechanism. A valid question to ask would be: If PAP depurinates both ribosomal and viral RNA, how does the pokeweed plant prevent its own death? Recent study shows that PAP is able to form a homodimeric complex in the cytosol of pokeweed plant, while its monomeric form is predominantly found outside the cell, the apoplast [66]. The PAP homodimer was shown to be much less active on RNA in comparison to the monomeric PAP. Hudak et al. have shown that PAP dimerization involves an active site Tyr123; mutations of this aromatic residue prevents dimerization of PAP in vivo, supporting the biological role of homodimerization as a mechanism to limit toxicity to cells synthesizing PAP [66].

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References

1. Thatcher LF, Anderson JP, Singh KB (2005) Plant defence responses: what have we learnt from Arabidopsis? Funct Plant Biol 32: 1-19.
2. Jones JD, Dangl JL (2006) The plant immune system. Nature 444: 323-329.
3. Anderson JP, Gleason CA, Foley RC, Thrall PH, Burdon JB, et al. (2010) Plants versus pathogens: an evolutionary arms race. Funct Plant Biol 37: 499-512.
4. Hardham AR, Jones DA, Takemoto D (2007) Cytoskeleton and cell wall function in penetration resistance. Curr Opin Plant Biol 10: 342-348.
5. Thordal-Christensen H (2003) Fresh insights into processes of nonhost resistance. Curr Opin Plant Biol 6: 351-357.
6. Morrissey JP, Osbourn AE (1999) Fungal resistance to plant antibiotics as a mechanism of pathogenesis. Microbiol Mol Biol Rev 63: 708-724.
7. Bednarek P, Osbourn A (2009) Plant-microbe interactions: chemical diversity in plant defense. Science 324: 746-748.
8. Dang L, Van Damme DJ (2015) Toxic proteins in plants. Phytochemistry 117: 51-64.
9. Peumans WJ, Hao G, Van Damme DJ (2001) Ribosome-inactivating proteins from plants: more than RNA N-glycosidases? FASEB J 15: 1493-1506.
10. Ottesen S (2004) The history of ricin, abrin and related toxins. Toxicon 44: 361-370.
11. Irvin JD (1975) Purification and partial characterization of the antiviral protein from Phytolacca americana which inhibits eukaryotic protein synthesis. Arch Biochem Biophys 169: 522-529.
12. Ferreras JM, Barbieri L, Giribés T, Battelli MG, Rojo MA, et al. (1993) Distribution and properties of major ribosome-inactivating proteins (28 S rRNA N-glycosidases) of the plant Saponaria officinalis L. (Caryophyllaceae). Biochim Biophys Acta 1216: 31-42.
13. Domashevskiy AV, Goss DJ (2015) Pokeweed antiviral protein, a ribosome inactivating protein: activity, inhibition and prospects. Toxins (Basel) 7: 274-298.
14. Bonness MS, Ready MP, Irvin JD, Mabry TJ Jr (1994) Pokeweed antiviral protein inactivates pokeweed ribosomes; implications for the antiviral mechanism. Plant J 5: 173-183.
15. Barbieri L, Battelli MG, Stárpe F (1993) Ribosome-inactivating proteins from plants. Biochim Biophys Acta 1154: 237-282.
16. Sperò S, Montanaro L, Mattioli A, Testoni G (1975) Relationship between elongation factor-1- and elongation factor-2-dependent guanosine triphosphatase activities of ribosomes. Inhibition of both activities by ricin. Biochem J 148: 447-451.
17. Tumer NE, Parikh BA, Li P, Dinman JD (1998) The pokeweed antiviral protein specifically inhibits Ty1-directed +1 ribosomal frameshifting and retrotransposition in Saccharomyces cerevisiae. J Virol 72: 1036-1042.
18. Hudak KA, Hammell AB, Yasenchak J, Tumer NE, Dinman JD (2001) A C-terminal deletion mutant of pokeweed antiviral protein inhibits programmed
39. Tumer NE, Hwang DJ, Bonness M (1997) C-terminal deletion mutant of pokeweed antiviral protein inhibits viral infection but does not depurinate host ribosomes and DNA in comparison with other isoforms. J Biochem 131: 225-231.

40. Rajamohan F, Engstrom CR, Denton TJ, Engen LA, Kourinov I, et al. (1999) High-level expression and purification of biologically active recombinant pokeweed antiviral protein. Protein Expr Purif 16: 359-368.

41. Barbieri L, Aron GM, Irvin JD, Stiffe F (1982) Purification and partial characterization of another form of the antiviral protein from the seeds of Phytolacca americana L. (pokeweed). Biochem J 203: 55-59.

42. Honjo E, Dong D, Motohashi H, Watanabe K (2002) Genomic clones encoding two isoforms of pokeweed antiviral protein in seeds (PAP-S1 and S2) and the N-glycosidase activities of their recombinant proteins on ribosomes and DNA in comparison with other isoforms. J Biochem 131: 225-231.

43. Rajamohan F, Venkatatalcham TK, Irvin JD, Uckun FM (1999) Pokeweed antiviral protein isoforms PAP-I, PAP-II, and PAP-III depurinate RNA of human immunodeficiency virus (HIV)-1. Biochem Biophys Res Commun 260: 453-458.

44. Rajamohan F, Kurinov IV, Venkatatalcham TK, Uckun FM (1999) Deguanylation of human immunodeficiency virus (HIV-1) RNA by recombinant pokeweed antiviral protein. Biochem Biophys Res Commun 263: 419-424.

45. Uckun FM, Rajamohan F, Pendergrass S, Ozer Z, Waurzyckian B, et al. (2003) Structure-based design and engineering of a nontoxic recombinant pokeweed antiviral protein with potent anti-human immunodeficiency virus activity. Antimicrob Agents Chemother 47: 1092-1061.

46. Chen Z, Antoniv JF, White RF (1993) A possible mechanism for the antiviral activity of pokeweed antiviral protein. Physiol Mol Plant Path 42: 249-258.

47. Usery MA, Irvin JD, Hardes B (1977) Inhibition of poliovirus replication by a plant antiviral peptide. Ann N Y Acad Sci 284: 431-440.

48. Aron GM, Irvin JD (1980) Inhibition of herpes simplex virus multiplication by the pokeweed antiviral protein. Antimicrob Agents Chemother 17: 1032-1033.

49. Tomlinson JA, Walker VM, Flewett TH, Barclay GR (1974) The inhibition of infection by cucumber mosaic virus and influenza virus by extracts from Phytolacca americana. J Gen Virol 22: 225-232.

50. Picard D, Kao CC, Hudak KA (2005) Pokeweed antiviral protein inhibits brome mosaic virus replication in plant cells. J Biol Chem 280: 20669-20675.

51. Hudak KA, Wang P, Turner NE (2000) A novel mechanism for inhibition of translation by pokeweed antiviral protein: depurination of the capped RNA template. RNA 6: 369-380.

52. Hudak KA, Bauman JD, Turner NE (2002) Pokeweed antiviral protein binds to the cap structure of eukaryotic mRNA and deurinates the mRNS downstream of the cap. RNA 8: 1148-1159.

53. Baldwin AE, Khan MA, Turner NE, Goss DJ, Friedland DE (2009) Characterization of pokeweed antiviral protein binding to mRNA cap analogs: competition with nucleotides and enhancement by translation initiation factor iso4G. Biochim Biophys Acta 1789: 109-116.

54. van Regenmortel MH, Fauquet CM, Bishop DH, Carstens EB, Esters MK, et al. (2000) Virus Taxonomy: Seventh report of the International Committee on Taxonomy of Viruses. Academic Press, San Diego, USA.

55. Vivanco JM, Turner NE (2003) Translation Inhibition of Capped and Uncapped Viral RNAs Mediated by Ribosome-Inactivating Proteins. Phytopathology 93: 588-595.

56. Wang M, Hudak KA (2006) A novel interaction of pokeweed antiviral protein with translation initiation factors 4G and iso4G: a potential indirect mechanism to access viral RNAs. Nucleic Acids Res 34: 1174-1181.

57. Domashevsksy AV, Miyoshi H, Goss DJ (2012) Inhibition of pokeweed antiviral protein (PAP) by tRNAs in plants virus genome-linked protein (VPg). J Biol Chem 287: 29729-29738.

58. Carrington JC, Freed DD (1990) Cap-independent enhancement of translation by a plant potyvirus 5’-untranslated region. J Virol 64: 1590-1597.

59. Gallie DR, Tanguay RL, Leathers V (1995) The tobacco etch viral 5’ leader and poly(A) tail are functionally synergistic regulators of translation. Gene 165: 233-238.

60. Niepel M, Gallie DR (1999) Identification and characterization of the functional elements within the tobacco etch virus 5’ leader required for cap-independent translation. J Virol 73: 9080-9088.

61. Zeenko V, Gallie DR (2005) Cap-independent translation of tobacco etch virus is conferred by a pseudoknot in the 5’-leader. J Biol Chem 280: 26813-26824.
62. Domashevskiy AV, Cheng SY (2015) Thermodynamic Analysis of Binding and Enzymatic Properties of Pokeweed Antiviral Protein (PAP) toward Tobacco Etch Virus (TEV) RNA. J Nat Sci 1: e62.

63. Cheng S, Domashevskiy A, Kobilinsky L (2014) The Effect of Eukaryotic Initiation Factors on the Activity of Pokeweed Antiviral Protein. Society of Toxicology (SOT), The Toxicologist: Supplement to Toxicological Sciences, Phoenix, AZ.

64. Wittmann S, Chatel H, Fortin MG, Laliberté JF (1997) Interaction of the viral protein genome linked of turnip mosaic poiyvirus with the translational eukaryotic initiation factor (iso)-4E of Arabidopsis thaliana using the yeast two-hybrid system. Virology 234: 84-92.

65. Khan MA, Miyoshi H, Ray S, Natsuaki T, Suehiro N, et al. (2006) Interaction of genome-linked protein (VPg) of turnip mosaic virus with wheat germ translation initiation factors eIFiso4E and eIFiso4F. J Biol Chem 281: 28002-28010.

66. Tourlakis ME, Karran RA, Desouza L, Siu KW, Hudak KA (2010) Homodimerization of pokeweed antiviral protein as a mechanism to limit depurination of pokeweed ribosomes. Mol Plant Pathol 11: 757-767.