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BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Reciprocal Dependence between Pectinmethylesterase Gene Expression and Tobamovirus Reproduction Effectiveness in *Nicotiana benthamiana*

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The transport protein (TP) of tobacco mosaic virus (TMV) ensures intercellular transport of viral RNA through plasmodesms, apparently by interacting with the cell proteins of endoplasmic reticulum, cytoskeleton, and cell wall [1]. During the past years, several cell proteins that specifically interact with VTM TP have been identified. These are cytoskeletal tubulin, myosin, and actin [2, 3]; protein kinanses [4–6]; transcriptional coactivator KELP [7]; and cytoskeletal protein MPB2C [8]. The functional role of these proteins in the transport of viral infection remains unknown.

The analysis of cell wall (CW) proteins of TMV by the methods of ligand blotting and affine chromatography revealed several proteins that specifically bind TMV TP [9]. Subsequent analysis of the amino acid sequence showed that one of these CW-associated proteins was a 34-kDa pectinmethylesterase (PME) [9, 10]. The data on the ability of PME to specifically bind TMV TP allowed us to assume that PME is involved in viral infection development [9, 10].

The purpose of this study was to reveal the relationship between the synthesis of PME and reproductive capability of TMV. First, we developed a test system that allowed detecting the ability of TMV TP and its deletion variants to affect the enzymatic activity of PME *in vitro* (demethylation of pectin) [11]. For this purpose, we added TMV TP or its deletion variants [12] to CW preparations enriched in PME [12] and determined enzymatic activity of PME by the method of diffusion in gel, as described in [13]. Figure 1 shows that TP almost completely suppressed PME activity, compared to the control sample with bovine serum albumin (BSA). The deletion variants of TP, Nt96 (with the deletion of 96 amino acid residues at the N terminus) and Del4 (with the deletion of internal 55 amino acid residues) had no effect on enzymatic activity of CW PME. Mutant Del10 (with the deletion of 85 amino acid residues at the C terminus) slightly decreased PME activity. We assumed that the suppression of enzymatic activity of PME was due to a direct effect of TP on the active center of PME. The determination of changes in the enzymatic activity of PME upon its interaction with PME may serve as a sensitive indirect test to assess the interaction between TP and PME (in addition to the methods of ligand blotting and affine chromatography, used earlier [9]).

Then, we used the agroinoculation technique to study the effect of PME on the reproductive capability of TMV TP. For this purpose, we performed coagroinoculation of binary vectors (Fig. 2a), which express different PME forms, and the binary vector *cr*TMV:GFP, constructed on the basis of infectious cDNA copy of tobamovirus of crucifers (crTMV), which express green fluorescent protein (GFP), as a marker of reproductive capability of the virus. The results shown in Fig. 2b indicate that, unlike mature 34-kDa PME, which had no effect on the level of GFP expression in

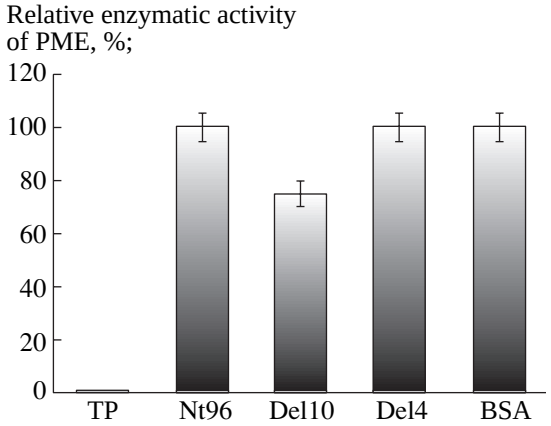

Fig. 1. The effects of TMV transport protein and its deletion fragments on the enzymatic activity of PME from tobacco cell walls.

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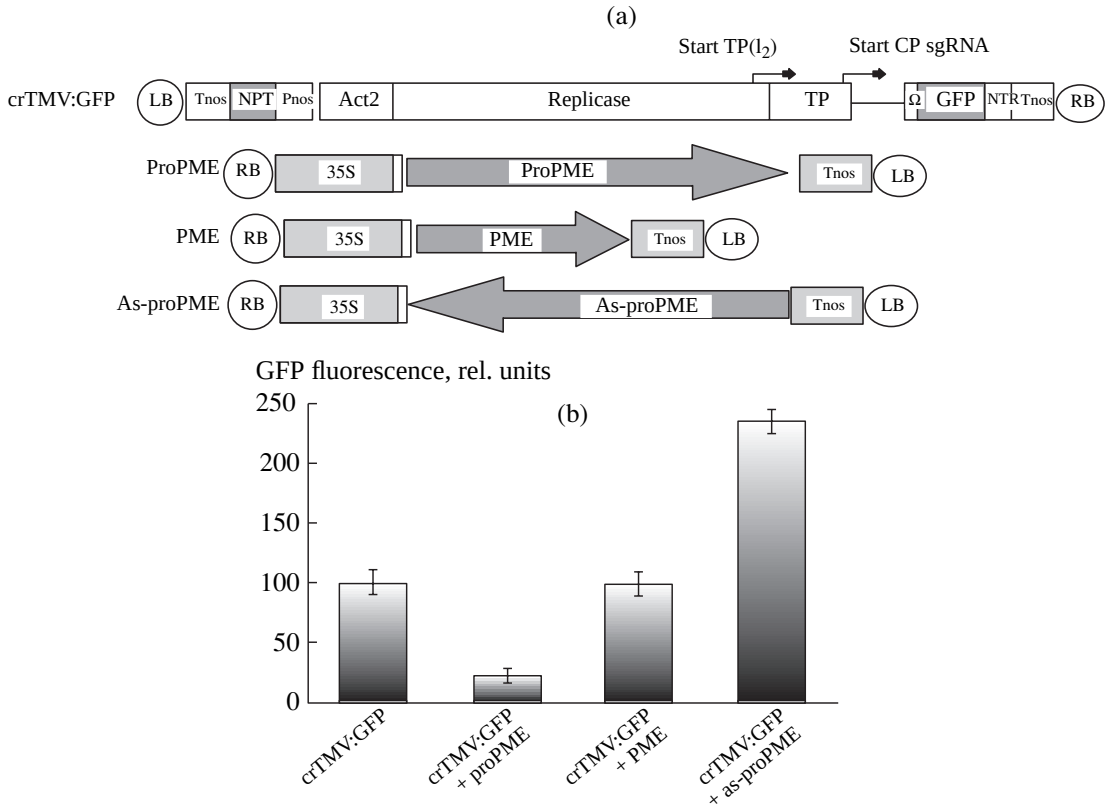

Fig. 2. The study of the effect of PME on TMV reproduction in the experiments on agroinoculation of *T. bentamiana* leaves with binary vectors. (a) Schematic structure of binary vectors. Designations: crTMV:GFP, binary vector on the basis of infectious cDNA of cruciferan tobamovirus; *LB*, the left part of the insertion site of the binary vector into the plant genome; *Tnos*, neopalinsynthase terminator; NPT, neopalinsynthase transcriptional promoter (neomycin phosphotransferase gene); *Arab.Act2*, actin transcriptional promoter 2 from *Arabidopsis*; *Replicase*, crTMV replicase gene; ω, TMV translational enhancer; *GFP*, green fluorescent protein from *Aequorea*; NTR, nontranslated region of crTMV genomic RNA; *RB*, the right part of the insertion site of the binary vector into the plant genome; proPME, binary vector controlled by the 35S-promoter of cauliflower mosaics virus (CMV) and containing the gene encoding unprocessed 70-kDa proPME; PME, binary vector containing the gene encoding mature 34-kDa PME; as-proPME, binary vector containing the gene encoding 70-kDa antisense proPME. (b) Comparative determination of GFP expression, directed by the vector crTMV:GFP, at coagroinoculation of *N. bentamiana* leaves with binary vectors expressing different forms of PME.

the leaves inoculated with the crTMV:GFP vector, the unprocessed PME form (proPME) markedly suppressed GFP production. It can be assumed that proPME, involved in cell secretion, somehow interferes and suppresses the reproduction of the virus not only in the experimental system specified but also in normalcy, i.e., the synthesis of endogenous PME has a negative effect on TMV reproduction. To test this assumption, we used suppressed of the synthesis of endogenous PME by introducing into the cell of the viral vector and the vector that produced antisense mRNA of proPME (as-proPME). It is known that the introduction of antisense mRNA into cells induces the defensive mechanism of endogenous mRNA damage and, therefore, the suppression of a specific endogenous target protein. As seen from Fig. 2b, the coexpression of the TMV virus and the vector expressing as-proPME mRNA results in a more than twofold increase in accumulation of GFP synthesized by the virus. This result is in a good agreement with our assumption of a negative effect of PME synthesis on TMV reproduction. It could be expected that any effect on the expression of PME mRNA would finally alter the enzymatic activity of PME in CWs and, as a result, would affect TMV reproductive capability. To test this assumption, in agroinoculation experiments we simultaneously measured the level of accumulation of GFP and enzymatic activity of TMV in CWs on infected leaves. These experiments demonstrated a reciprocal correlation between the level of GFP accumulation and PME activity (Fig. 3). Overproduction of proPME, induced by agroinoculation with the *pro*PME vector (Fig. 2a), not only suppressed the accumulation of GFP but also considerably increased PME enzymatic activity in CWs. Conversely, the introduction of as-proPME, resulting in the stimulation of GFP accumulation in leaves, is accompanied by the suppression of PME enzymatic activity in CWs. Agroinoculation of cells with the vector expressing mature PME does not affect the GFP accumulation and PME enzymatic activity in CWs (data not shown), which may be due to the

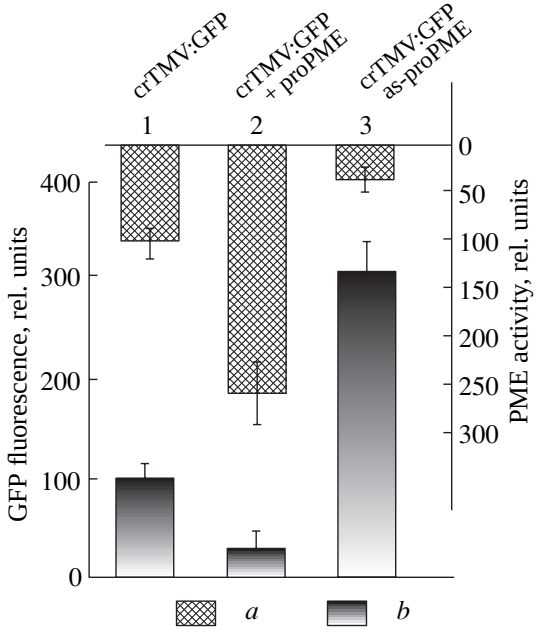

Fig. 3. Reciprocal correlation between the level of PME enzymatic activity in cell walls of *N. bentamiana* leaves *a* and the capability of the viral vector to produce GFP *b.*

absence of a signal sequence and, therefore, inability of exogenous mature PME to migrate to CWs.

Thus, the results obtained in this study are suggestive of existence of the reciprocal dependence between the level of PME enzymatic activity in CWs and TMV reproductive capability.

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