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TEMPORAL SHIFTS IN THE APPARENT *IN VIVO* TRANSLATIONAL EFFICIENCIES OF TOMATO LEAF PROTEINASE INHIBITORS I AND II mRNAs FOLLOWING WOUNDING[1]

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SUMMARY

The apparent *in vivo* translational efficiencies of tomato leaf proteinase Inhibitors I and II mRNAs in wounded tomato leaves increased 6- to 9-fold between the 9th and 14th hour following wounding. During this period the *in vitro* translation of mRNA for Inhibitor I and II decreased over 2-fold while the rates of Inhibitors I and II accumulation (synthesis) increased 3- to 5-fold. Some type of cellular reorganization is occurring after 9 hours that appears to be involved in regulating the *in vivo* translation efficiencies of Inhibitor I and Inhibitor II mRNA.

INTRODUCTION

Mechanical wounding in leaves of tomato plants results in the appearance in unwounded leaves of *in vitro* translatable mRNAs for two serine proteinase inhibitors, Inhibitors I and II (1), and the subsequent accumulation of the two inhibitors in the central vacuole of the cells (2). The mRNAs for Inhibitors I and II are translated *in vitro* as pre-inhibitors 2000-3000 daltons larger than the proteins found in the central vacuoles (1). We have now compared the *in vitro* translation capacities of the mRNAs for Inhibitors I and II with *in vivo* rates of inhibitor synthesis during the first 18 hours after wounding and have found that the apparent *in vivo* translational efficiency of both inhibitors increased. In this communication, we present evidence that such a change is apparently occurring abruptly between the 9th and 14th hours after wounding.

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MATERIALS AND METHODS

Cultivation of tomato plants, isolation of tomato leaf mRNA and *in vitro* translation in a rabbit reticulocyte lysate system using [^{35}S]methionine have been previously described (1). Seventeen-day-old tomato plants having but two expanding leaves were wounded by crushing the lower leaf with a hemostat and incubated under continuous light for appropriate time periods. *In vivo* synthesis of Inhibitors I and II was monitored by use of an immuno-radial diffusion assay as described previously (3,4). The tomato leaf mRNA (4 µg) was translated for 60 minutes, after which the reaction was terminated by the addition of cold 10 mM NaPhosphate pH 7.4, 0.15 mM NaCl, 1% (vol/vol) Triton-X 100, 1% (wt/vol) NaDeoxycholate and 50 µM methionine. Pre-Inhibitors I and II were specifically immunoprecipitated through the use of preformed double antibody precipitates (1,5). Analysis of pre-inhibitors was accomplished by SDS-urea slab gel electrophoresis (1,6). The gels were stained with Commassie blue to locate standards of Inhibitors I and II and then sliced into 2 mm sections. The gel slices were digested in 0.5 ml or 30% H_2O_2 at 60°C for 6 hours and [^{35}S] was counded in the presence of Scintiverse (Fisher). Characteristically this resulted in one peak of radioactivity in each gel corresponding to either pre-Inhibitor I or II (1). Total cpm of the pre-Inhibitor was calculated by integration of this peak.

RESULTS AND DISCUSSION

The rate of *in vivo* synthesis of Inhibitors I and II induced by leaf wounding is shown in Table I. Gustafson and Ryan (7) demonstrated that large quantities of Inhibitors I and II in tomato leaves accumulate in tomato leaves primarily because they are not degraded. Thus, their rates of synthesis can be calculated from the rates of *in vivo* appearance. In leaves of wounded tomato plants, as shown in Table I, the rates of both Inhibitors I and II synthesis steadily increased during the first hours after wounding. The rates

TABLE I. Rates of *in vivo* synthesis and accumulation of Inhibitors I and II in leaves of Wounded Tomato Plants[1].

Time After Wounding (hr)	Rate of Inhibitor Accumulation[2] (µg/hr/g leaf tissue)	
	Inhibitor I	Inhibitor II
0 - 4	1.6	0.9
5 - 9	3.4	2.3
10 - 14	6.2	3.4
15 - 18	5.7	3.3

[1] Young tomato plants about 5 cm high with two expanding leaves and an apical leaf were wounded on both sides of leaf by crushing with a surgical hemostat.

[2] Determined Immunologically (3,4).

TABLE II. Incorporation of [^{35}S]Methionine into Pre-Inhibitors I and II in Reticuolocyte Lysate Translation System Directed by mRNA Isolated from Wounded Tomato Leaves[1].

Time After Wounding (hr)	[^{35}S]Methionine Incorporation[2] (CPM x 10^{-3}) into	
	Inhibitor I	Inhibitor II
0	0.1	0.1
4	11.5	9.6
9	13.1	11.6
14	5.4	4.9
18	5.5	5.1

[1] For wounding protocol see Table I.

[2] See Reference (1) for mRNA isolation and translation procedure.

of both Inhibitor I and II's accumulation were essentially linear (7,8). The rates of inhibitor synthesis remained constant for at least another 30 hours.

Successful isolation and translation of tomato leaf mRNA in an *in vitro* translation system and the subsequent isolation of pre-Inhibitors I and II (1) allowed us to examine the amounts of *in vitro* translatable mRNA for each inhibitor per 4 µg of total mRNA during the early and linear phase of the *in vivo* Inhibitor I and II synthesis and accumulation. Table II shows the incorporation of [^{35}S]methionine specifically into the pre-Inhibitor proteins at the times indicated. The data in Table II indicate that during the first 4 hours of the wounding response a large quantity of translatable mRNA for the two inhibitors had already been produced. At 12 hours this quantity had increased but 14 hours after the initial wound the amount of translatable mRNA for both inhibitors had decreased by nearly 60% and then remained constant through 18 hours.

The relative efficiencies of the translatable mRNA for the two inhibitors or the ratios of the rates of *in vivo* inhibitor synthesis divided by the relative amounts of *in vitro* translatable mRNA, are shown in Table III. This table provides evidence that the translatable mRNA at 14-18 hours after wounding became 6-9 times more efficient than that present at 4 hours.

TABLE III. Apparent Efficiencies of Translatable mRNAs for Inhibitors I and II in Wounded Tomato Leaves at Various Times After Wounding.

Time After Wounding (hr)	Apparent Translational Efficiencies: Inhibitors Accumulation[1] (μg/hr/g tissue) / *In Vitro* Translation[2] (CPM x 10^{-3})	
	Inhibitor I	Inhibitor II
4	0.14	0.09
9	0.26	0.20
14	1.15	0.69
18	1.04	0.58

[1] From Table I.

[2] From Table II.

A similar change in total translatable mRNA in plant tissues has been reported recently in wheat aluerone tissue, treated with gibberillic acid, in which an initial burst of translatable mRNA was observed, followed by a decline after 12 hours (9). Herein we find that translatable mRNA for pre-proteins of Inhibitors I and II initially increased (4-9 hours) and then declined. The large (6-9) increase in apparent translational efficiency between 4-9 and 14-18 hours suggests that some sort of post-transcriptional change(s) must be occurring in the cell that leads to either a more efficient translation system or to changes in the processing, or turnover, and subsequent accumulation of the newly synthesized inhibitors. An alternative explanation to account for the high rates of synthesis of the two inhibitors at 14 and 18 hours is that total mRNA has increased 6-9 fold. Although the recoveries of poly(A)+ RNA vary somewhat among preparations, we consistantly found a decrease of total poly(A)+ RNA in leaves at 14 and 18 hours, further supporting a post-transcriptional control of inhibitor synthesis.

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