GROWTH-DEPENDENT ACCUMULATION AND UTILIZATION OF PROTEINASE INHIBITOR I IN TOBACCO CALLUS TISSUES*

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Received December 26,1974

Summary: Extraordinarily large quantities of an inhibitor of serine proteinases called Inhibitor I, accumulate in growing tobacco callus tissues. As the callus ages and the growth rate declines, Inhibitor I accumulation continues until it represents over 10% of the soluble proteins of the callus tissue. When growing callus tissue is transferred to fresh medium, Inhibitor I content decreases rapidly as the callus adapts to the new conditions. Thus Inhibitor I selectively accumulates in growing and senescing callus tissues and disappears during the adaptation of the tissues to new culture conditions preceeding new growth.

In tomato and potato leaves an inhibitor of chymotrypsin and trypsin, called Inhibitor I (1), accumulates in large quantitites (up to 1-2% of the soluble proteins) during specific periods of plant development and in response to severe injury (2,3). The inhibitor usually disappears slowly over the several days following accumulation. Inhibitor also accumulates in potato tubers but rapidly disappears after the tubers sprout and during subsequent growth of the new plants (4).

In searching for a model system to study the regulation of accumulation and utilization of Inhibitor I in plant tissues our attention was drawn to the possibility of employing callus tissue cultures. We herein report that tobacco callus tissues accumulate remarkably large quantities of proteinase Inhibitor I and subsequently utilize it as function of tissue growth.

MATERIAL AND METHODS

Tobacco callus tissue was obtained from sections of a young stem that

*Supported in part by U.S.P.H.S. Grant 2K3 GM 17059 and U.S.D.A., C.S.R.S. Grant 316-15-30. Scientific Paper No.4357, Project 1791, College of Agriculture, Washington State University, Pullman, Washington 99163.

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had been surface sterilized with 95% ethanol for 2 min and 1% sodium hypochlorite for 12 min. The callus was maintained in modified Murashige-Skoog medium (5,6) containing 1 mg/l gibberellin, 3 mg/l indole-3-acetic acid, 0.3 mg/l of kinetin, and 10 g/l Bacto agar (Difco). The callus was transferred to fresh medium every 30 days.

For the study of the correlation between callus growth and Inhibitor I accumulation, 6 pieces of callus (fresh weight of 90 mg per piece) were excised from a 26-day-old mother callus and placed in a 125 ml erlenmeyer flask containing 50 ml of Murashige-Skoog medium. At various intervals two flasks (12 pieces) of callus were harvested and lyophilized. The lyophilized callus samples were weighed, ground to a powder with a mortar and pestle, and extracted with 0.1 M ammonium bicarbonate solution (60 mg dry callus/ml of NH_4HCO_3 solution) After centrifugation at 12,000 xg for 10 min, extracts were assayed for Inhibitor I and for protein. The concentration of Inhibitor I was determined by the immuno-radial diffusion technique as described by Ryan (7) using electrophoretically pure tobacco Inhibitor I as the standard. The protein concentration was determined by the method of Lowry (8).

RESULTS AND DISCUSSION

Figure 1 shows the growth of tobacco callus as measured by the increase in total dry weight of 12 transferred pieces of tissue. After a 4-day lag in the growth of the callus, the weight increased rapidly until about the 15th day after transfer, then leveled off. The callus grew very slowly after about the 20th day.

The 26-day old callus tissue, which possessed a significant quantity of Inhibitor I (130 µg/60 mg dry callus), was transferred to fresh medium. After the transfer, its Inhibitor I concentration decreased steadily for about 10 days as the callus began to grow.

Three phases of Inhibitor I utilization and accumulation could be recognized during callus growth. The first phase was the initial decrease noted above for 10 days during which the callus established a linear growth

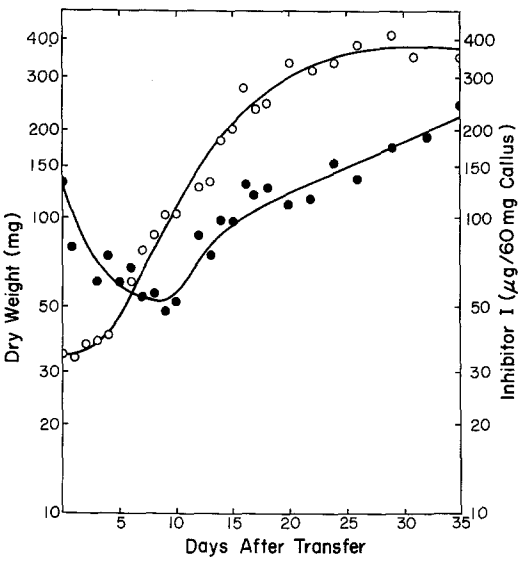

Figure 1. Time course of callus growth and Inhibitor I accumulation. Twelve pieces of callus tissue were harvested and lyophilized at various intervals as indicated. The lyophilized callus tissues were weighed and extracted with a 0.1 M ammonium bicarbonate solution. Inhibitor I concentration in extracts were quantitatively determined immunologically (7). (-o-o-) Total dry weight; (-●-●-) Inhibitor I concentration.

TABLE 1: Variation of Soluble Protein and Inhibitor I in Tobacco Callus Tissue During Growth.

Days After Transfer	Soluble Protein (mg/60 mg Callus)[a]	Inhibitor I (mg/60 mg Callus)[b]	Inhibitor I (%)
0	4.8	0.13	2.7
5	4.9	0.07	1.4
10	4.4	0.05	1.1
15	4.8	0.10	2.1
20	4.0	0.11	2.8
26	3.1	0.14	4.5
35	2.2	0.24	10.9

[a]Lowry assay (8).

[b]Immuno-radial diffusion (7).

rate. A second phase, from day 10 to about day 17, was characterized by the accumulation of Inhibitor I as the linear growth rate of the callus continued. In the final phase, from day 17 to day 35, the callus growth rate subsided while the accumulation of Inhibitor I continued at a reduced, but steady, rate.

The soluble protein concentration in the callus remained constant until about 20 days after the transfer (Table I). As the callus growth rate decreased thereafter (cf. Figure 1), soluble protein concentration also decreased. By day 35 the soluble proteins had diminished to 46% of those of the tissue at the time of transfer. Inhibitor I in transferred tissue represented 2.7% of the soluble proteins, and decreased to 1.1% ten days later, but then began to accumulate, increasing to 10.9% of the soluble proteins at 35 days, when the experiment was terminated.

The results shown in Figure 1 and Table 1 indicate that Inhibitor I increased most strikingly during the latter stage of senescence when the total soluble protein pool was decreasing (Table 1). This indicated that the inhibitor protein was preferentially accumulating while other proteins were disappearing. After the transfer of 26-day old callus tissue to fresh medium, during the period of establishing growth, Inhibitor I concentration decreased rapidly while the total soluble proteins remained constant indicating that the transfer to fresh medium signaled the cells to preferentially degrade the inhibitor or in some other manner utilize it for establishing new callus growth.

This growth-dependent regulation of the accumulation and utilization of such large quantities of a single protein species among the soluble proteins of tobacco callus is unusual for a vegetative plant tissue. The tobacco callus thus should provide an excellent model system for the study of the regulation of the turnover of proteinase inhibitors in plant tissues.

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