

REGULATION OF PHOSPHOLIPASE ACTIVITY IN POTATO LEAVES BY PROTEIN
PHOSPHORYLATION-DEPHOSPHORYLATION AND PROTEOLYTIC ACTIVATION

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INTRODUCTION

We have recently reported that calcium and calmodulin stimulate the rate of autolysis of phospholipids in potato leaf homogenates (1). Further studies (2) revealed that a soluble phospholipase activity in potato leaves could be stimulated to the same degree (30-50%) by either calmodulin or protein kinase (+ATP). Two other plant enzymes, quinate:NAD⁺ oxidoreductase (3) and isofloridoside-phosphate synthase (4), have also been shown to be similarly stimulated by both calmodulin and protein phosphorylation. However, the latter enzyme was also shown to be stimulated to an even greater degree by proteolysis with trypsin or chymotrypsin (4). This study was undertaken to investigate whether the phospholipase activity in potato leaves may also respond to proteolytic activation.

MATERIALS AND METHODS

Potato (*Solanum tuberosum* c.v. Kennebec) plants were grown as previously described (1,2). Young (1-2 cm) leaves were ground with a mortar and pestle in chilled buffer containing 0.3 M sucrose, 0.1 M HEPES pH 7.5, and 5 mM each of DTT and β -mercaptoethanol. The homogenate was centrifuged at 100,000 g for 50 minutes and the supernatant fraction was used as a source of enzyme. Phospholipase activity was assayed by a new technique (5) which utilizes the fluorescent phospholipid analogue, C₆-NBD-Phosphatylcholine. The reaction mixture contained 5 μ M C₆-NBD-PC, 50 mM HEPES pH 7.0, and 10-30 μ l enzyme. The other conditions of the assay were as previously described (2).

RESULTS

Time-course studies were conducted in order to study the effects of various treatments on the levels of phospholipase activity in 100,000 g supernatant fractions from potato leaves (Fig. 1). The phospholipase activity in the 100,000 g supernatant remained constant for 60 minutes at 25° C (data not shown). In other control experiments the addition of 3 mM MgCl₂ and 0.3 mM ATP to the 100,000 g supernatant fraction had no

immediate or delayed effect on phospholipase activity (Fig. 1). When NaF, a phosphatase inhibitor, was added to the mixture of 100,000 g supernatant, $MgCl_2$, and ATP, phospholipase activity increased gradually during the 60 minute study as previously reported (2). The subsequent addition of either protein kinase or calmodulin caused a rapid increase (about 50% in phospholipase activity which peaked at 10 and 20 minutes, respectively, as previously described (2). In order to investigate the possible proteolytic activation of phospholipase activity, 25 μ grams of trypsin was added to either the 100,000 g supernatant or the 100,000 g supernatant plus $MgCl_2$ and ATP. With both treatments the addition of trypsin caused a rapid increase in phospholipase activity (about 3-fold increase in the first 20 minutes) (Fig. 1). Similar results were obtained with chymotrypsin, but the degree of stimulation was less (about 50%) than that obtained with comparable quantities of trypsin.

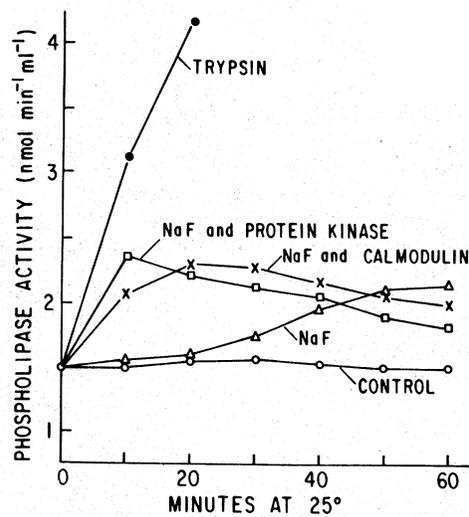


Fig. 1. Time-course study of the stimulation of phospholipase activity in the 100,000 g supernatant fraction from potato leaves. Control (○) = 1 ml 100,000 g supernatant fraction containing 3 mM Mg^{2+} and 0.3 mM ATP, control plus 50 mM NaF (△), control plus 50 mM NaF and the catalytic subunit of cyclic AMP-dependent protein kinase (500 u) (□), control plus 50 mM NaF and calmodulin (10,000 u) (×), control plus 25 μ grams trypsin (●). All reported concentrations are those which occur in the final volume of 1.1 ml.

DISCUSSION

This study served to verify our previous report (2) that phospholipase activity in potato leaves is stimulated by protein phosphorylation and by calmodulin. More importantly, it demonstrated that the degree of stimulation of the same enzyme activity by proteolytic activation was even greater than the degree of stimulation with protein kinase or calmodulin. Further work is required in order to elucidate the mechanisms of these three types of stimulation. We previously suggested that the calmodulin and protein kinase stimulations could be explained by phosphorylation of a phospholipase enzyme by an endogenous calmodulin-stimulated protein kinase (2). However, in light of the current evidence of proteolytic activation, a more complex mechanism of activation is necessary to best explain the three types of activation. The only other plant enzyme which has been reported to be stimulated by the same three treatments is isofloridoside-phosphate synthase (4) as previously described. Those authors presented evidence for the presence of an endogenous acid protease which could stimulate the enzyme. They also proposed (4) that calmodulin and protein kinase somehow stimulated this protease which in turn proteolytically-activated the isofloridoside-phosphate synthase. Such a mechanism is also conceivable for the potato leaf phospholipase. The only other plant enzyme which has been reported to be stimulated by proteolytic activation is a glucan synthase in soybean cells (6). It is also interesting to note that an animal phospholipase, pancreatic phospholipase A_2 , is activated by the proteolytic removal of a heptapeptide from the amino terminus (7).

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