



Senescence of rice leaves

XXVIII. Effects of molybdate and vanadate on acid phosphatase and leaf senescence

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Abstract. Molybdate and vanadate strongly inhibited Apase activity in soluble supernatant fraction from rice leaves. However, azide had no effect on Apase activity. In the absence of molybdate and vanadate, the increase of Apase activity in detached rice leaves occurred under both light and dark conditions. Molybdate inhibited and vanadate promoted the rise of Apase activity in detached rice leaves under both light and dark conditions. However, both molybdate and vanadate promoted senescence of detached rice leaves in the light or darkness. Results suggest that Apase is unlikely to be a marker enzyme for rice leaf senescence.

Key words: Acid phosphatase; Leaf senescence; Molybdate; *Oryza sativa*; Vanadate.

Introduction

Apase (EC 3.1.3.2) is known to be widely distributed in plants and to occur in a variety of tissues. De Leo and Sacher (1970) proposed that Apase might be involved in the onset and development of senescence. Molybdate and vanadate (sodium salt) have been shown to inhibit Apase activity in plant tissues (Gallagher and Leonard, 1982; Spencer, 1954). However, azide had no effect on Apase activity (Gallagher and Leonard, 1982).

Experiments described in this paper were designed to examine the effects of molybdate and vanadate on Apase activity and senescence of rice leaves, and to test if Apase can serve as a marker enzyme for rice leaf senescence.

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Abbreviations: Apase, acid phosphatase; Pase, phosphatase; pNPP, *p*-nitrophenyl phosphate.

Materials and Methods

Rice (*Oryza sativa* cv. Taichung Native 1) seedlings were grown as previously described (Kao, 1980). The apical 3-cm segments excised from the third leaves of 10-day-old seedlings were used throughout this investigation. A group of 10 segments were floated on a Petri dish containing 10 ml of distilled water or test solutions (molybdate, vanadate and azide, sodium salt). Distilled water and test solutions were adjusted to pH 5.5. Incubation was carried out in darkness or the light provided with fluorescent lamps (16.7 Wm⁻²).

Apase was extracted and assayed following the method of Pan (1987) with some modifications. Leaf segments, weighing about 60 mg, were homogenized in prechilled mortar and pestle with 8 ml cold 50 mM of Tris-maleate buffer (pH 7.0) and 13 mM mercaptoethanol at 4°C. The resulting clear soluble supernatant fraction was used directly for enzyme assay. The reaction mixture contained 0.6 ml 100 mM of acetate buffer (pH 5.0) and 13 mM mercaptoethanol, 0.1 ml 200 mM of

pNPP and 0.3 ml of enzyme extract. Reactions were carried out at 37°C for 10 min, and terminated by adding 4.0 ml 0.3 M of sodium carbonate. The amount of *p*-nitrophenol liberated in the assay medium was determined spectrophotometrically at 400 nm. One unit of Apase activity is defined as the amount of enzyme that liberated 1 μ mol *p*-nitrophenol per min.

Chlorophyll was extracted and determined as described before (Kao, 1980). Chlorophyll was expressed as A_{665} per 10 segments after extraction in 10 ml of 80% (v/v) ethanol.

Results

In vitro Effects of Molybdate and Vanadate on Apase Activity

Apase activity in the soluble supernatant fraction from rice leaves, when assayed at pH 5.0, was inhibited by vanadate and molybdate, but was not affected by azide (Fig. 1). The concentrations of molybdate and vanadate which gave 50% inhibition (I_{50}) were about 10 and 85 μ M, respectively. Both vanadate and molybdate were effective inhibitors of phosphatase activity between pH 4.0 and 8.0 (Fig. 2). At low pH, these inhibitors were less effective.

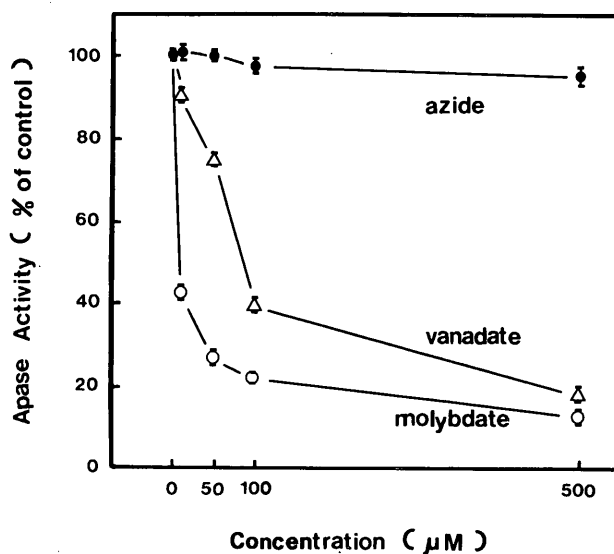


Fig. 1. Effects of molybdate, vanadate and azide on Apase activity in soluble supernatant fraction from rice leaves. Values are averages with standard errors ($n=4$).

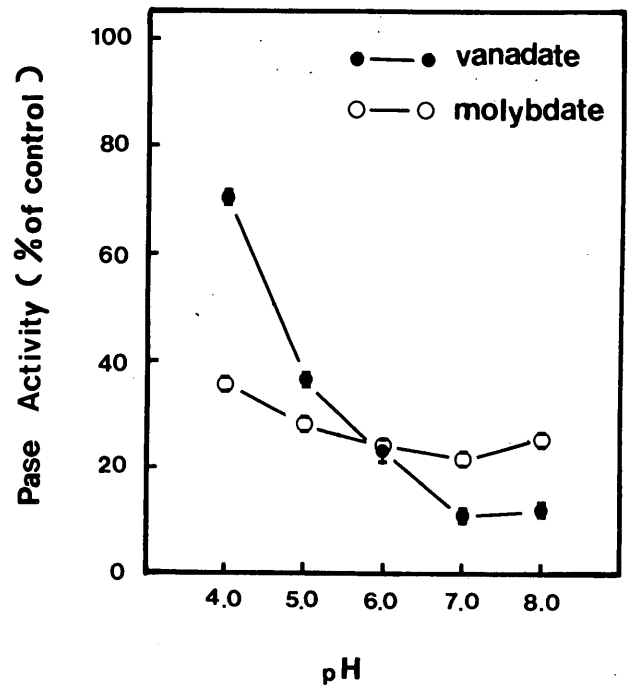


Fig. 2. Effects of molybdate and vanadate on phosphatase (Pase) activity in soluble supernatant fraction from rice leaves at various pH. Values are averages with standard errors ($n=4$).

In vivo Effects of Molybdate and Vanadate on Apase and Senescence

Since molybdate and vanadate were found to be potent inhibitors of *in vitro* Apase, these inhibitors were then used to test if they affected the senescence of and the Apase activity in detached rice leaves. Detached rice leaves were incubated in molybdate and vanadate, respectively, under light or dark condition. Prior to chlorophyll determination and enzyme extraction at various times, detached rice leaves were thoroughly washed with distilled water to remove contaminated vanadate or molybdate. In the absence of vanadate or molybdate, the increase of Apase activity in detached rice leaves occurred under both light and dark conditions (Figs. 3 and 4). Senescence of detached rice leaves, as measured by the decrease of chlorophyll, was promoted by molybdate in the light or darkness (Fig. 3). Similar to *in vitro* experiments, molybdate strongly inhibited the rise of Apase activity in detached rice leaves (Fig. 3). Vanadate was also found to promote senescence of detached rice leaves incubated in the light or darkness (Fig. 4). However, vanadate, un-

like molybdate, promoted the increase of Apase activity in detached rice leaves (Fig. 4). It should be noted that no toxicity (rolling or loss of turgor) was visually evident at the test concentrations of molybdate and vanadate.

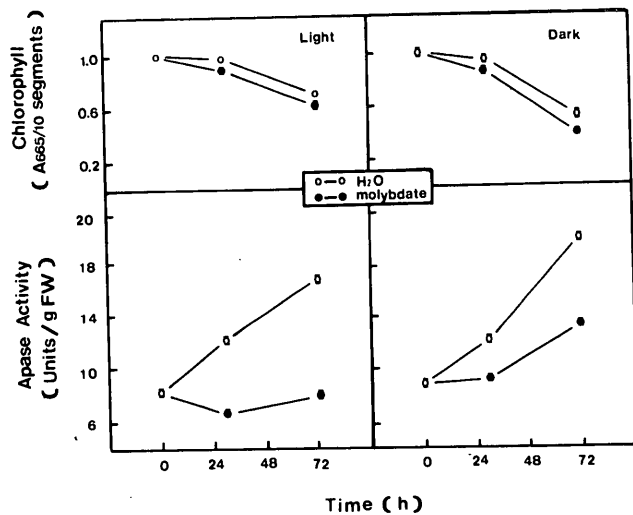


Fig. 3. Effects of molybdate on Apase activity and chlorophyll level in detached rice leaves. Detached rice leaves were incubated in 2 mM molybdate under light or dark condition. Values are averages with standard errors ($n=4$).

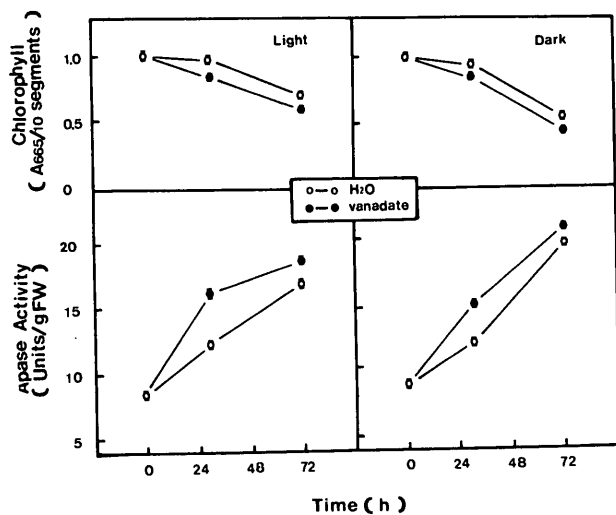


Fig. 4. Effects of vanadate on Apase activity and chlorophyll level in detached rice leaves. Detached rice leaves were incubated in 4 mM vanadate under light or dark condition. Values are averages with standard errors ($n=4$).

Discussion

In agreement with other reports (Gallagher and Leonard, 1982; Spencer, 1954), Apase preparations from rice leaves were significantly inhibited by molybdate or vanadate. Vanadate has been shown to inhibit the plasma membrane ATPase and Apase activities (Gallagher and Leonard, 1982). In a recent report, we have shown that Mg^{2+} -dependent alkaline inorganic pyrophosphatase was also inhibited by vanadate (Lin and Kao, 1990). The present work further supports the suggestion that vanadate is not a specific inhibitor of the plasma membrane ATPase.

The idea is prevalent that decompartmentation of hydrolytic enzymes in plant cells is involved in senescence. There is some evidence, at the ultrastructural level, that Apase is confined to vacuoles in healthy root cap cells of *Zea mays* and *Lepidium sativum*, but is released into the cytoplasm under conditions of senescence (Berjak, 1972; Berjak and Villers, 1972). In certain plant tissues, such as senescing *Rhoeo discolor* leaf sections and ripening banana, Apase activity increases markedly (De Leo and Sacher 1970, a,b). De Leo and Sacher (1970, a,b) proposed that an increase in Apase activity was a change common to senescing fruit and leaf tissue. If this is also the case in our rice leaf system, then vanadate and molybdate, potent inhibitors of *in vitro* Apase, would be expected to retard senescence. Contrary to our expectation, both vanadate and molybdate promoted senescence of detached rice leaves.

Similar to *in vitro* experiments, molybdate strongly suppressed the rise of Apase in detached rice leaves. However, vanadate was found to promote the rise of Apase in detached rice leaves. The contradictory *in vitro* and *in vivo* effects of vanadate on Apase activity indicate that the observed increase in enzyme activity during the 72 h of vanadate treatment is an indirect effect. In a recent study, we demonstrated that proton secretion activity of detached rice leaves, which originated from ATP-driven proton pump located in the plasma membrane, played an important role in regulating senescence (Chen *et al.*, 1990). Vanadate, which was found to inhibit proton secretion activity, promoted senescence of detached rice leaf system (Chen *et al.*, 1990). At the present, we offer no explanation on the promotive effect of molybdate on senescence of detached rice leaves. Whether molybdate

exerts its promotive effect on senescence via inhibition of proton secretion activity remains to be seen. Thus, Apase is unlikely to be involved in the promotion of senescence of rice leaves by vanadate and molybdate.

Senescence of detached rice leaves was demonstrated to be retarded by light (Figs. 3 and 4). However, the rise of Apase activity in detached rice leaves in the absence of inhibitors occurred under both light and dark conditions. Thus, the rise of Apase activity in detached rice leaves may be in response to a changing metabolic requirement after leaf detachment, rather than a senescent change *per se*. Conventionally, the biochemical parameters used to indicate senescence of leaves are the decreases in the amount of chlorophyll and protein in the leaves (Thimann, 1980). However, efforts have been made to seek a marker enzyme for leaf senescence (Lauriere, 1983). The present investigation provided support to our recent findings (Huang and Kao, 1991) that Apase does not seem to be a marker enzyme for rice leaf senescence.

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水稻葉片老化之研究 (28) 鉬酸鹽與釩酸鹽對酸性磷酸鹽水解 酵素與葉片老化之影響

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鉬酸鹽與釩酸鹽可顯著的抑制由台中在來一號水稻葉片分離出酸性磷酸鹽水解酵素活性。然而，azide 對分離之酸性磷酸鹽水解酵素活性則無抑制作用。光線與黑暗下，水稻切離葉片之酸性磷酸鹽水解酵素活性，隨着處理時間之增長而增加。鉬酸鹽抑制光線或黑暗處理之水稻切離葉片的酸性磷酸鹽水解酵素活性的增加，但釩酸鹽則促進酸性磷酸鹽水解酵素活性的增加。不論在光線或黑暗下，鉬酸鹽與釩酸鹽處理均加速葉片老化。本研究之結果顯示，酸性磷酸鹽水解酵素，不能做為水稻切離葉片老化之標示酵素。