Taiwania, **40** (3): 291-298, 1995

Inhibitory Effect of Sodium Floride on Activity of Acid Phosphatase in Rice Plants

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(Manuscript received 31 August 1995; accepted 7 September 1995)

ABSTRACT: Plants treated with sodium floride showed the retardation of plant growth in shoot and root systems. In vitro,sodium floride also inhibits the activity of acid phosphatase activity. It is a noncompetitive inhibitor of acid phosphatase extracted from rice seedlings. Its Km value for p-nitrophenyl phosphate (p-NPP) was 0.33 mM and Ki value for NaF is 0.23 mM. Crude extract of acid phosphatase from rice seedlings has four major groups of isozymes (group I, II, III, and VI). Group I isozymes are more tolerant to NaF, gropus III are more sensitive (group III) and the rest are intermediate.

KEY WORDS: Acid phosphatase, isozymes, NaF, inhibition, kinetics.

INTRODUCTION

Acid phosphatases (APases, EC 3.1.3.2.) involve in the metabolism of phosphate which is essential for plant growth and development (Basha, 1986). They are widely distributed in plants and present in multiple isozymes differing in their biochemical properties (Panara *et al.*, 1990). The patterns of isozymes vary with plant species, tissues, developmental stage of plants (Baker and Tadakazu, 1973; Mizuta and Suda, 1980). Changes in isozyme patterns of acid phophatase are also affected by hormones (Gabard and Jones, 1986; Hooley, 1984) and environmental stress (Gabbreilli *et al.*, 1989). Cytochemical studies showed that acid phosphatase occurs in various organelles (Hall and Sexton, 1974; Nishizawa and Mori, 1980; Schulz and Jensen, 1981; Chen *et al.*, 1990) and their appearance in plant cells are subcellular-specific (Huang and Chen, 1989; Mizuta and Suda, 1980).

Sodium floride is a well known inhibitor of acid phosphatase for both biochemical and cytochemical studies (Hall and Sexton, 1974; Mizuta and Suda, 1980). However, the inhibitory results of NaF on acid phosphatase are inconsistency in these two studies (Hall, 1978; Hasegawa *et al.*, 1975; Panara *et al.*, 1990). The ultrastructural study on the distribution of acid phosphatase in root cap of NaF-treated rice showed that NaF had partial inhibition on acid phosphatase activity in extranuclear region (Chen *et al.*, 1992).

In the present study, the kinetics of the inhibitory effect of NaF on acid phosphatase activity in rice seedlings were examined.

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MATERIALS AND METHODS

Plant materials.

Surface-sterilized rice (*Oryza sativa* Tainon No. 67) seeds were imbibed with water and germinated as previously described (Chen *et al.*, 1980). Young seedlings were transfered to hydroponic culture containing Hoagland's solution with a 14 h light and 10 h dark period at 30 ℃ day and 25 ℃ night temperature. Eight-day-old plants were transfered to fresh Hoagland solutions containing 0, 0.1, 0.5, 1.0, 2.0, 5.0, 10, 20, 50, 100 , and 200 mM NaF. Culture media were changed every day. After 7-day treatment, both control and NaF-treated plants were collected for biochemical assays and morphological comparisons. To examine the effect of osmotic shock on plant growth at different concentrations of NaF, 1, 5, 20 and 100 mM NaCl were added to Hoagland's solution.

Enzyme preparation

Plant tissues, frozen in liquid nitrogen, were first ground into small powders with commercial dry-type blender, then transfered to Polytron and homogenized with two fold (w/v) of 0.05 M Tris-HCl buffer (pH 7.5) containing 0.007% (V/V) β-mercaptoethanol. The homogenate was strained with four layers of cheese-cloth and centrifuged in a Sorval RC 5C at 8000 rpm for 10 min. The supernatant was collected. The pellet was homogenized with same extraction buffer, centrifuged at 8,000 rpm , and the supernatant was collected again. Supernatants were pooled together and used as an enzyme extract.

Enzyme assay

The assay of acid phosphatase activity was followed the method of Hooley (1984). Assay medium contained 30 μl enzyme extract, 125 μl of 0.1 N sodium acetate (pH 5.0) and 100 μl of 0.05 M p-nitrophenyl phosphate. The reaction was carried out at 37 ℃ for 10 min and terminated by adding 4 ml of 0.6 N Na_2CO_3. The absorbance at 400 nm was measured by a Hitachi 3210 spectrophotometer. Units of enzyme activity was defined as μmol of nitrophenol released per min. Protein content was determined by Bradford's method (1976).

Electrophoretic analysis of the crude enzyme was performed at pH 8.8 on 7.5 % slab polyacrylamide gels. Sample containing 50 μg protein was applied to each well and run in a Hoefer mini-gel at 150 V for 2 h. The enzyme activity of acid phosphatase was detected by incubating the gels in a solution containing 0.2 M sodium acetate (pH 5.0), 0.1% α-naphthyl phosphate, 0.1% Fast red TR salt, and 5 mM $MgCl_2$ at 30℃ for 3 h.

RESULTS

Effect of NaF on plant growth

As shown in Table 1, hydroponic culture of Hoagland's solution containing different concentrations of NaF affect the growth of rice seedlings. Rice plants grown in culture medium containing less than 10 mM of NaF do not show a significant change in their morphological characteristics. However, culture media containing NaF higher than 20 mM severely retard plant growth. The symptoms (yellowing and growth retardation of leaves) of shoot system are

more distinct than those of root system.

Table 1. Effect of NaF on shoot and root growth of rice plants

NaF (mM)	shoot length (cm)	root length (cm)	numbers of roots (per plant)
Control	12.02 ± 0.86	6.77 ± 1.99	7.20 ± 1.48
0.1	11.82 ± 0.99	4.50 ± 0.71	7.00 ± 0.67
0.5	10.57 ± 1.27	6.37 ± 1.17	6.70 ± 1.25
1	10.51 ± 1.67	5.25 ± 0.75	6.30 ± 1.06
2	9.45 ± 0.68	7.22 ± 1.38	5.40 ± 0.84
5	10.04 ± 1.15	3.48 ± 0.32	5.90 ± 0.88
10	5.82 ± 0.83	2.27 ± 0.32	4.90 ± 0.57
20	4.36 ± 0.82	3.26 ± 1.57	2.10 ± 0.88
50	4.10 ± 0.57	3.32 ± 1.04	1.50 ± 0.53
100	4.31 ± 0.69	5.21 ± 1.52	1.20 ± 0.42
200	3.44 ± 0.41	5.06 ± 1.17	1.20 ± 0.63

For tracing the osmotic effect of NaF on rice plant, a similar study on the effect of NaCl on plant growth was also studied. As shown in Tables 1 and 2, the response patterns of rice growth to NaCl treatment are quite different from that of NaF-treated plants. The effective concentration of NaCl on plant growth is around 200 mM and meanwhile, both root and shoot systems show the significant decreases of their growth in culture medium containing NaCl higher than 20 mM.

Table 2. Effect of NaCl on the growth of rice plants.

NaCl (mM)	shoot length (cm)	root length (cm)	numbers of roots (per plant)
control	12.02 ± 0.86	6.77± 1.99	7.20± 1.48
1	11.86 ± 0.53	5.84 ± 0.71	7.20 ± 0.92
5	11.69 ± 0.39	6.94± 1.17	6.50 ± 1.27
20	10.48 ± 0.62	6.23 ± 0.90	6.60 ± 0.84
200	5.07 ± 0.15	2.03 ± 0.50	2.50 ± 0.71

Inhibitory effect on acid phosphatase activity

As shown in Figure 1, rice acid phosphatase is only partially blocked by NaF and its activity still remains about 25% of its initial activity in 20 mM NaF. However, NaF concentrations at lower than 5 mM, the activity of acid phosphatase is severely inhibited by NaF on the basis of % decrease of activity per mM NaF. The NaF concentration for remained 50% enzyme activity is around 3 mM.

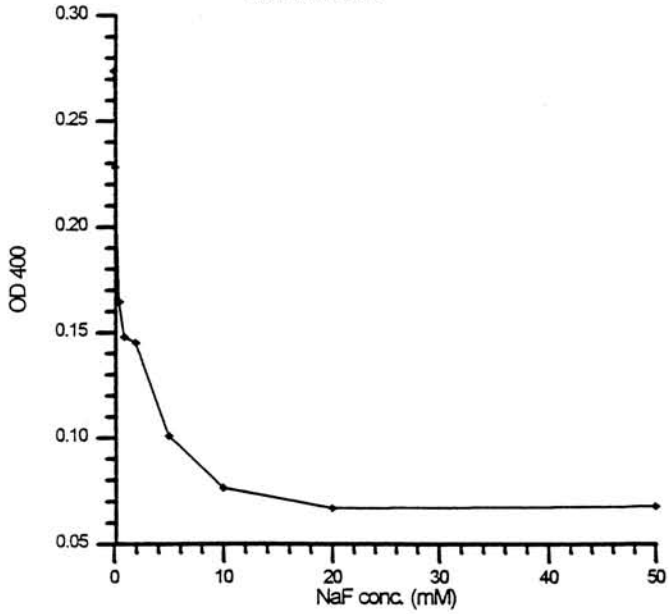

Fig. 1. Inhibitory effect of NaF on crude enzyme activity of acid phosphatase. OD $_{400}$ denotes the amount of p-NP production with maximal absorption at 400 nm..

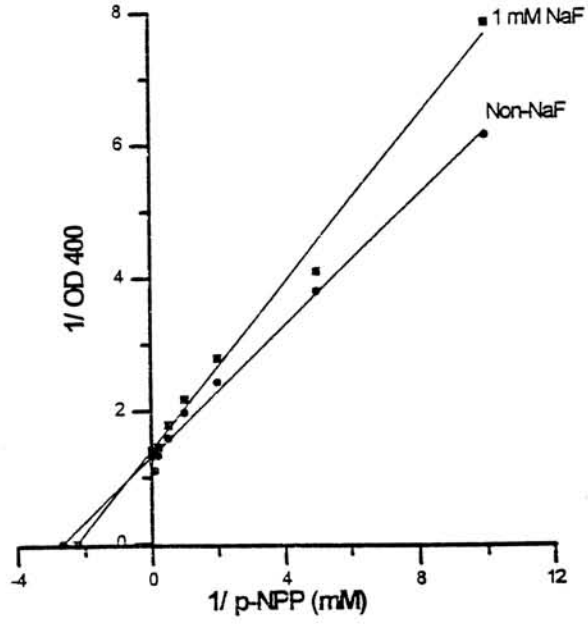

Fig. 2. Kinetic study of 1 mM NaF on crude enzyme activity of acid phosphatase. OD $_{400}$ denotes the amount of p-NP production.

The kinetic study of 1 mM NaF inhibition on acid phosphatase activity is shown in Figure 2. In Lineweaver-Burk plot, their regressional equations are $Y = 0.49x + 1.3$ and $Y' = 0.63x + 1.4$, their Vmax are 0.76 μmol/min and 0.71 μmol p-NPP/min, and their Km are 0.37 mM and 0.45 mM for control and 1 mM NaF-treated plants, respectively. Similar study on a purified isozyme conducted in our laboratory showed its Km was 0.33 mM. Its Vmax values were 0.34 μmol/min and 0.06 μmol/min for control and 1 mM NaF-treated enzyme solutions, respectively (Figure 3). Sodium floride is a non-competitive inhibition on rice isozyme and in hydrolysis of p-NPP and the Ki value for NaF is about 0.23 mM.

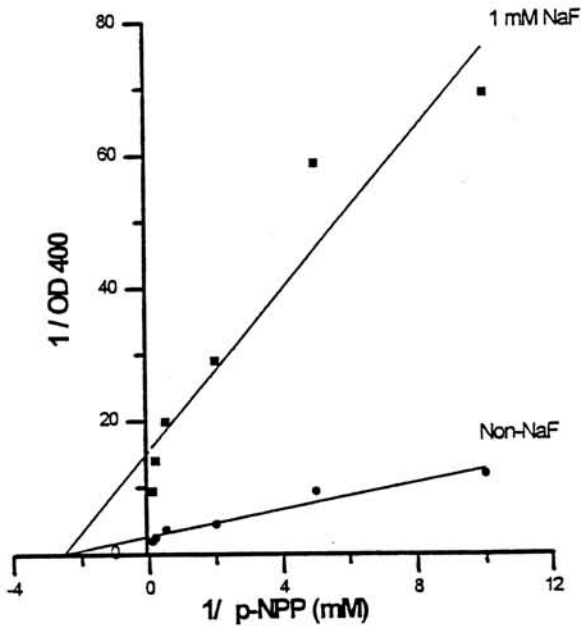

Fig. 3. Kinetic study of 1 mM NaF on purified group III isozyme activity of acid phosphatase. OD_{400} denotes the amount of p-NP production.

Effect on isozyme patterns of acid phosphatase

As shown in Figure 4, there are four groups (I, II, III and IV) of acid phosphatase in rice seedlings. The electrophoretic mobility of these four group of isozymes follows the series of IV > III > II > I. In 1 mM NaF treated enzyme extract, all four gruops of isozyme persisted regular pattern in native-PAGE zymogram. However, staining activity of group III is significantly decreased in the presence of NaF concentration at 5mM. Isozyme group II and IV are also strongly affected by NaF at 20 mM. All the above facts indicate that different isozymes of acid phosphatase have different responses to NaF treatment .

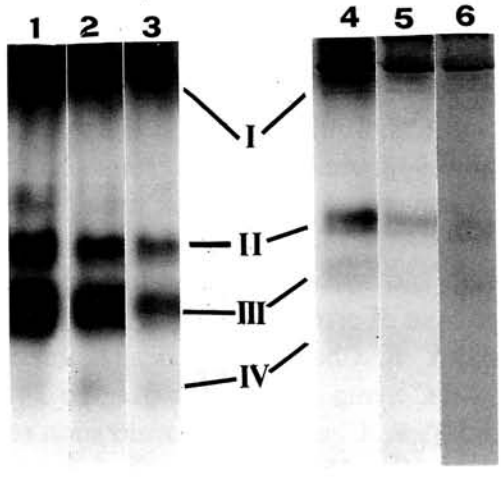

Fig. 4. Zymograms of acid phosphatase in response to NaF treatment at concentrations of 0 (lane 1), 1(lane 2), 5 (lane 3), 10 (lane 4), 20 (lane 5), and 100 mM (lane 6).

DISCUSSION

At supraoptimal concentrations of NaCl and NaF, both chemicals affect the growth of rice seedlings. However, the effective concentration of NaF (20 mM) is much lower than that of NaCl (200 mM). It indicates that salt-stress inducing osmotic shock on plant growth does not match with the retardation effect of NaF-treatment. Sodium floride inhibits the activity of acid phosphatase could provide another way for studying the relationships.

Sodium floride is a non-competitive inhibitor of acid phosphatase and its inhibition on enzyme activity is highly reversible through the kinetic study (NagDas and Bhattacharyya, 1984). However, the degree of enzyme inhibition by NaF treatment varies with sources of enzyme extraction (Hall, 1978; Moore *et al.*, 1987). Different isozymes of acid phosphatase from several plant species also show various degree of inhibition by floride (Arnold *et al.*, 1987; Chen *et al.*, 1992; Mizuta and Suda, 1980).In the present study, the kinetic study of the NaF inhibitory effect on crude enzyme extract of acid phosphatase from rice seedlings seems to show mixed-typed inhibition. However, NaF effect on activity of the purified group III isozyme do as show a non-competitive inhibition

The diversity of acid phosphatase in response to floride could provide information for using acid phosphatase as a developmental marker for plants or/and defense marker toward environmental stress (Gabard and Jones, 1986; Hooley, 1984; Gabbreielli *et al.*, 1989). In yeast-like cells of *Sporothrix schenckii*, selective inaction of extra-cytoplasmic acid phosphatase by treatment of low concentration of NaF was observed (Arnold *et al.*, 1987). It is consistent with differentially inhibitory effect of NaF on rice acid phosphatase isozymes in the present study. Different compartmentation of acid phosphatase isozyme may also play important role in their physiological meanings (Mizuta and Suda, 1980) Cytochemical localization of acid phosphatase did show extranuclear isozymes were more sensitive than intranuclear ones in response to NaF treatment (Chen *et al.*, 1992).

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氟化鈉對酸性磷酸酶活性的抑制作用

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(收稿日期：1995年8月31日；接受日期：1995年9月7日)

摘　　要

水稻幼苗經氟化鈉處理後，植株的根部及地上部生長皆受妨礙。離體下，氟化鈉也會抑制酸性磷酸酶的活性，其屬非競爭性的抑制作用。以 p-NPP 爲酵素受質時的 Km 值爲 0.33 mM，而氟化鈉的 Ki 值爲 0.23 mM。粗抽出液在電泳圖上酸性磷酸酶呈出四群同功酵素。第一群同功酵素較耐氟化鈉的作用；第三群對氟化鈉則較敏感；其餘屬中度敏感者。

關鍵詞：酸性磷酸酶，同功酵素，氟化鈉，抑制作用，動力學。

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