EFFECTS OF NaF ON AMYLASE IN MUNG BEAN SEEDLINGS

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SUMMARY

Extracts obtained from mung bean (Vigna radiata) seedlings treated with NaF at 0, 0.1, 1.0, and 5.0 mM for 48 and 72 hours have been studied for amylase activity. NaF at concentrations as low as 1.0 mM was found to inhibit the enzyme. The inhibitory effect of fluoride was markedly diminished in the presence of added $CaCl_2$. These data suggest that the F^--induced inhibition of amylase in mung bean seedlings may be due to the interaction of F^- with Ca^{2+}, which is required for enzyme activity.

INTRODUCTION

It is widely known that during germination stored energy sources such as fats and starch are broken down to provide energy as well as basic components for anabolic purposes. The growth and development of radicles and primary leaves, for example, depend on the breakdown of these energy-releasing compounds. While a large volume of literature exists dealing with starch degradation during germination, reports on the effects of F^- in this process are limited.

We reported previously that NaF inhibited fat metabolism in mung bean (Vigna radiata) seedlings [1]. Tissues treated with NaF showed qualitative and quantitative changes in fatty acids. In addition, lipase activity in cotyledon extracts from these tissues was found to be significantly inhibited. Subsequently, we observed that F^--treated seedlings contained

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higher amounts of sugars than control tissues treated with water [2]. It is not known whether the observed sugar accumulation in the F^--treated tissue is due to increase in starch degradation or decrease in glucose utilization. This work was initiated in an attempt to answer this question.

MATERIALS AND METHODS

Mung bean seeds were obtained locally. Germination of seeds and F^- treatment of the seedlings were the same as described previously [1]. Seedlings were harvested at the end of 48 hours or 72 hours after F^- treatment was started. The cotyledons were separated and used for the preparation of crude enzyme extract. Tissues were homogenized in 50 mM Tris buffer, pH 7.0, and the slurry was strained through four layers of cheesecloth, and the filtrate was centrifuged at 8,000 x g for 10 minutes at 4° C. The supernatant was recentrifuged at 22,000 x g for 30 minutes and the resulting supernatant was used as crude enzyme extract.

Enzyme assay was carried out in a mixture consisting of 3.0 ml of 0.1 M Tris buffer, pH 7.0, 3.0 ml of 0.2% starch solution, and 1.0 ml of the enzyme extract diluted 1:1 with 0.05 M of the buffer. A reaction mixture containing all components except the substrate was used as a blank. The assay mixture was incubated in a water-bath maintained at 30° C for 30 minutes, unless otherwise stated. At the end of the incubation period, a 1.0 ml aliquot was pipetted into a test tube containing 1 ml of the Nelson-Somogyi blue solution [3-5]. The mixture was heated in boiling water for 10 minutes. Upon cooling, 1 ml of arsenomolybdate [3,4] was added, followed by the addition of 12 ml water. The mixture was centrifuged, and the absorbance of the supernatant was read in a spectrophotometer at 520 nm [5]. The amount of sugar produced during the incubation period was determined by use of a calibration curve. Protein content of the enzyme extract was determined by the method of Lowry *et al*. [6]. Specific enzyme activity was defined as µg glucose produced per mg protein per 30 minutes.

Experimental data were analyzed by Student t-test for the differences between two independent means. Data were considered significant at the 95% level of confidence.

RESULTS

The amylase activity in mung bean seedlings were markedly affected by F treatment, and the effect appeared to be concentration-dependent. The activity in tissues exposed to 0.1 mM NaF for 48 hours showed an 11% increase over the control, whereas decreases of 13% and 37% were observed in seedlings treated with 1.0 mM and 5.0 mM F, respectively (Table I). At 72 hours, inhibition was shown even in tissues exposed to 0.1 mM NaF.

TABLE I

Inhibition of amylase in mung bean cotyledons treated with varying concentrations of NaF for 48 and 72 hours

NaF (mM)	Specific activity (μg glucose/mg protein/30 minutes)			
	48 hours	Percent of control	72 hours	Percent of control
0	71 ± 16.3[a]	100	150 ± 22.4	100
0.1	79 ± 10.1	111	132 ± 18.8	88
1.0	61 ± 14.3	87	131 ± 9.9*	87
5.0	45 ± 12.3*	63	120 ± 6.4*	80

[a]Values are means ± SD.

*$p < 0.05$.

It was speculated that the observed decrease in amylase activity in F-treated seedlings might be due to interaction of fluoride with calcium. The effect of added $CaCl_2$ on enzyme activity in extracts from tissues treated with NaF was thus studied. For this purpose, seedlings treated with 5.0 mM F^- for 24 hours were used for enzyme preparation. The assay mixture contained the same components as described previously except for the addition of 5 mM or 10 mM $CaCl_2$ in those where the effect of Ca^{2+} was to be tested. To maintain the volume of the assay mixture the same as previously, starch solution used as substrate was reduced accordingly. In addition, the reaction was allowed to run for 90 minutes, and at an interval of 30 minutes, a 1.0 ml aliquot was removed from the reaction mixture for sugar determination as described previously.

As shown in Fig. 1, the presence of added $CaCl_2$ markedly alleviated the inhibitory effect caused by NaF. Addition of 5.0 mM $CaCl_2$ enhanced enzyme activity by 73, 30, and 146% for an incubation period of 30, 60, and 90 minutes, respectively. Interestingly, addition of 10 mM $CaCl_2$ resulted in much smaller increases.

DISCUSSION

Several enzyme are involved in the breakdown of starch during germination. These include α-amylase, β-amylase, α-glucosidase (maltase), debranching enzymes, limit dextrinase, and starch phosphorylase. While the end products of starch breakdown by phosphorylase are glucose-1-phosphate and limit dextrin, the final product from reactions catalyzed by other enzymes is glucose. As mentioned previously, in the germinating seed, the sugar thus formed is used as a primary source of energy and as a starting material for synthesis of cellular components required for growth and development of the seedlings. Inhibition of amylase by F^- observed in this study suggests an important influence that this micromineral element has on energy metabolism in germinating seeds. The markedly impaired germination observed in seedlings exposed to even low

concentrations of NaF [2] may be partly attributed to F^- inhibition of amylase.

The observation that addition of $CaCl_2$ greatly alleviated the F^--induced inhibition of amylase (Fig. 1) implies that Ca^{2+} ions may be required for its activity. This supports earlier reports that α-amylase is Ca-dependent [7]. Recently, Mitsui et al. [8] reported the possible role of calcium in the biosynthesis and secretion of α-amylase in rice. It is possible that, once absorbed into the tissue, F^- may react with Ca^{2+} ions, causing enzyme inhibition. As shown in Fig. 1, the presence of 5 mM $CaCl_2$ resulted in much higher enzyme activity than 10 mM $CaCl_2$. The reason for this is not known. It is possible that high Cl^- ion concentrations may be inhibitory. Work is in progress to test this possibility.

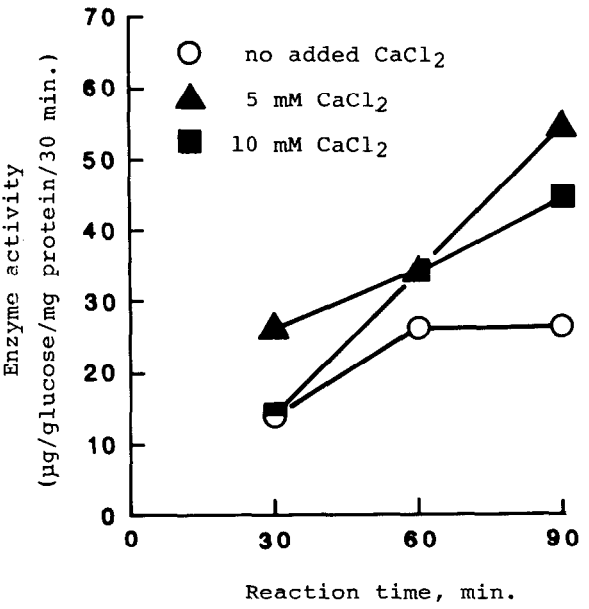

Fig. 1. Effect of $CaCl_2$ on amylase activity in mung bean seedlings exposed to NaF. Enzyme extracts were prepared from seedlings exposed to 5.0 mM NaF for 24 hours. Assay mixture contained 3 ml of Tris-buffer (pH 7.0), 2.3 ml of 0.2% starch solution, 1 ml of the enzyme extract, and 0.7 ml of water or $CaCl_2$. The reaction mixture was incubated at 30° C for 30, 60, or 90 minutes.

As mentioned previously, seedlings exposed to F^- contained more sugars than control tissues. Because amylase was inhibited by F^-, it may be concluded that the observed sugar accumulation is not due to enhanced starch breakdown. Rather, it may be due to impaired utilization of glucose produced during germination.

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