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Enzymatic property analyses of hexokinase MeHXK3 from cassava

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Abstract: Gene expression analysis has been shown that MeHXK3 gene may play an important role for hexose phosphorylation during OES (organized embryogenic structures) and FEC (friable embryogenic callus) development of cassava. In this study, the enzymatic properties of MeHXK3 protein to the phosphorylation of glucose and fructose were explored. The optimum pH for the enzyme activity of MeHXK3 was ~8.5. The maximum reaction rate (Vmax) of MeHXK3 to glucose (25.50 nmol/mg pr/min) and fructose (25.33 nmol/mg pr/min) were similar, while the Km value of MeHXK3 for fructose (2.136 mM) was higher than for glucose (0.2176 mM). These results suggest that MeHXK3 probably mainly phosphorylates glucose.
Keywords: hexokinase, cassava, hexose, phosphorylation.

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1. INTRODUCTION

Hexoses (glucose and fructose) are the original substrates for most of the metabolic pathways and organic materials found in plants [1]. Hexokinases (HXKs), which can catalytic the phosphorylation of hexoses, and play important roles in responses to abiotic or biotic stresses, pollen germination, germination, stomatal closure, and programmed cell death [2-6]. Cassava is an important source of food for the tropical people. It has been reported that HXKs play a pivotal regulatory role during cassava starch synthesis in tuber roots [7]. Our previous studies

have demonstrated that *MeHXK2* gene might be a key gene for hexose phosphorylation in cassava plants during tuber root development, while *MeHXK3* gene was lowly expressed at tuber roots during the accumulation of starch [8].We have found that *MeHXK3* gene might play an important role for hexose phosphorylation during OES and FEC development (unpublish). In this study, the enzymatic properties of MeHXK3 protein to the phosphorylation of glucose and fructose were explored.

2. MATERIALS AND METHODS

The hexokinase-deficient yeast strain YSH7.4-3C was kindly provided by Prof. Stefan Hohmann (University of Gothenburg). In thw previous studies, the full-length c DNAs of *Me HXK3* gene was sub cloned into the yeast shuttle vector pDR195, and the yeast functional complementation experiment has demonstrated that *MeHXK3* has a hexose phosphorylation function. In this research, the YSH74-3C yeast cells, transformed with pDR195–MeHXK3, were grown in 25 ml of SD liquid medium (no uracil) for 72 h to approximate 5×10^7 cell per ml. The yeast cells were spun down for 5 min at 5000 rpm; and the total proteins were extracted using a yeast total protein extraction kit (Sangon, Shanghai, China) according to the manufacturer's instructions. The extracted total proteins were quantified by Bradford assay kit (Sangon, Shanghai, China), which were used as crude enzymes for enzymatic property analyses of MeHXK3. A HXK assay kit (Solarbio Science and Technology) was used to measure the *K*m values for fructose and glucose and the pH-dependence of the hexose phosphorylation activity of MeHXK3.

3. RESULTS

3.1. Hexose phosphorylation activity of MeHXK3 to fructose

To analyze the enzymatic properties of the MeHXK3 protein, the total proteins were extracted from the yeast cells carrying *MeHXK3* gene. The results showed that the extracted crude enzyme from the cells carrying pDR195–MeHXK3 has fructose phosphorylation activity; and its activity was increased with the concentration of fructose addition, and the maximum reaction rate (*V*max) was 25.33 nmol/mg pr/min (Fig. 1). The enzyme activity to fructose concentration was in accordance with Michaelis–Menten kinetics, and the Km value was 2.136 mM (Fig. 2).

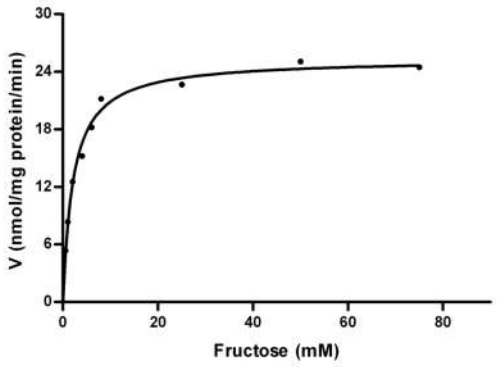

Fig. 1 Effect of fructose concentration on hexokinase activity in the extracted proteins from the yeast cells carrying pDR195-MeHXK3

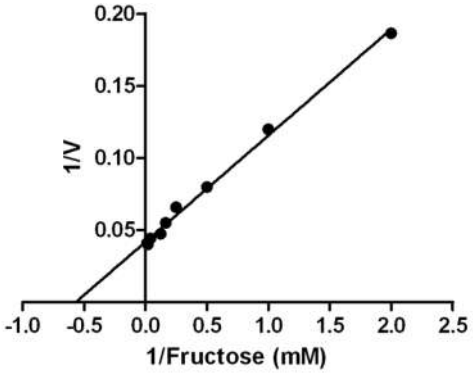

Fig. 2 The graphical determination of the *K*m for fructose by Lineweaver-Burk plot

3.2. Hexose phosphorylation activity of MeHXK3 to glucose

In order to explore the hexose phosphorylation activity of MeHXK3 to glucose, the glucose phosphorylation activity in the extracted crude enzyme from the yeast cells carrying pDR195–MeHXK3 was measured. The results showed that the enzyme activity of MeHXK3 was increased with the concentration of glucose addition, and the maximum reaction rate (*V*max) was 25.50 nmol/mg pr/min (Fig. 3). The enzyme activity to glucose concentration was in accordance with Michaelis–Menten kinetics, and the *K*m value was 0.2176 mM (Fig. 4).

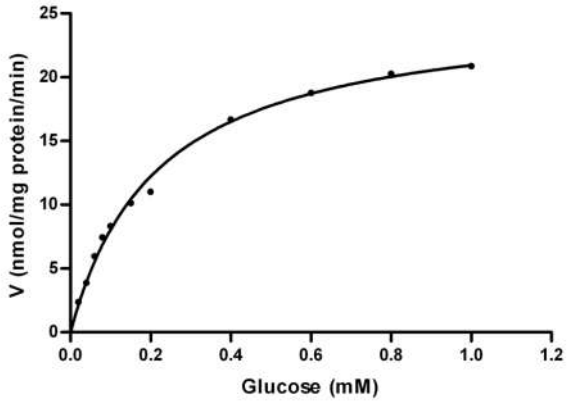

Fig. 3 Effect of glucose concentration on hexokinase activity in the extracted proteins from the yeast cells carrying pDR195-MeHXK3

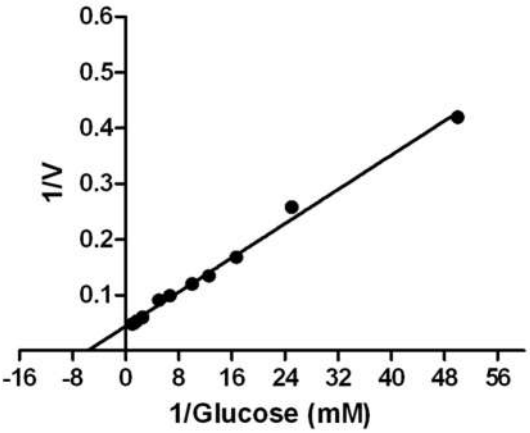

Fig. 4 The graphical determination of the *K*m for glucose by Lineweaver-Burk plot

3.3. The activity of MeHXK3 at different pHs

The enzyme activitities of MeHXK3 at different pH values (pH 6.0–9.0) were assayed. The results showed that the enzyme activities of MeHXK3 were gradually increased from pH 6.0 to pH 8.5, and the optimum pH of MeHXK3 was 8.5 (Fig. 5).

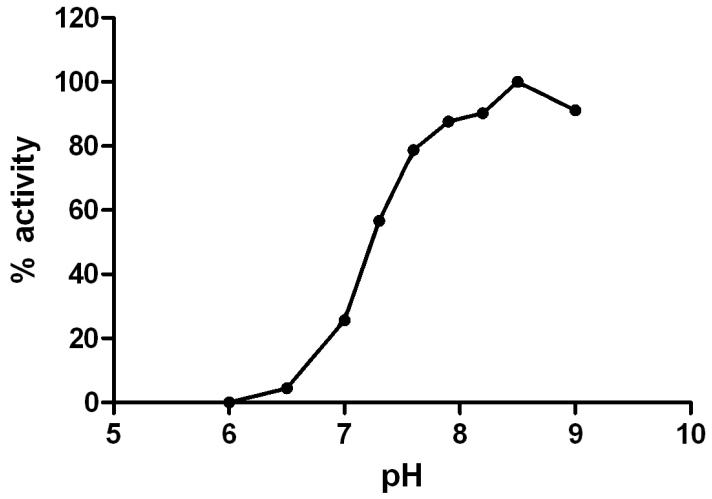

Fig. 5 The hexose phosphorylation activities of MeHXK3 at different pHs

4. CONCLUSION

This study showed that the optimum pH for the enzyme activity of MeHXK3 was ~8.5. The maximum reaction rate (Vmax) of MeHXK3 to glucose (25.50 nmol/mg pr/min) and fructose (25.33 nmol/mg pr/min) were similar, while *K*m value of MeHXK3 for fructose (2.136 mM) was higher than for glucose (0.2176 mM). These result suggest that MeHXK3 probably mainly phosphorylates glucose.

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