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百合鳞茎淀粉磷酸化酶分离纯化及酶学性质研究

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Research on Starch Phosphorylase Purification and Enzymatic Properties in Bulbs of Lilium

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摘要 从兰州百合（Lilium davidii var. unicolor）鳞茎中分离纯化淀粉磷酸化酶（Starch Phosphorylase，SP）并研究其酶学性质。结果表明：选用30% ~ 60%硫酸铵分级沉淀，SP 纯化倍数为14.78，回收率为23%，纯化的SP 亚基分子量约62.5 kD；SP 的最适反应体系缓冲液为pH 5.0 的柠檬酸—柠檬酸钠缓冲液，最适反应温度为30 ℃，且体系中不适合加入酚类抑制剂聚乙烯吡咯烷酮（PVP）；SP 不耐强酸，在中性和弱碱性条件下活性稳定，pH < 5 时活性较弱；在合成方向上，SP 对葡萄糖－1－磷酸（G-1-P）的Km值为2.84 mmol • L-1；1 mmol • L-1 Mg2+、Ca2+对SP 活性有极显著的促进作用，K+、Zn2+也有一定的促进作用；大部分离子在高浓度（> 10 mmol • L-1）下抑制SP 活性，但Na+随着浓度的升高，对SP 活性的促进作用增强；10 mmol • L-1 的抗坏血酸可将SP 活性提高30%。

关键词： 百合 兰州百合 鳞茎 淀粉磷酸化酶 纯化 酶学性质

Abstract： Starch phosphorylase（SP）in bulbs of Lilium davidii var. unicolor was purified and its enzymatic properties were studied. The results indicated that SP activity was improved 14.78 fold by 30% －60% ammonium sulfate fractionation with a final yield of 23% and a subunit of 62.5 kD. The optimal reaction buffer and temperature were citric acid-sodium citrate buffer（pH 5.0）and 30 ℃, respectively. However, SP was sensible to strong acid and it was not suitable for adding phenolic inhibitors（PVP）to the reaction system. The activity was stable under neutral and alkaline conditions, while decreased under pH < 5. In the synthetic direction, Km value for G-1-P was 2.84 mmol • L-1. Furthermore, 1 mmol • L-1 Mg2+ and Ca2+ promoted SP activity significantly, K+ and Zn2+ also played a promoting role. Most ions with high concentration（> 10 mmol • L-1）could inhibit SP activity, whereas Na+ enhanced the activity with its concentration increasing. Besides, the enzyme activity increased by 30% while adding 10 mmol • L-1 ascorbic acid to the reaction system.

Keywords： lily, Lilium davidii var. unicolor, bulb, starch phosphorylase, purify, enzymatic properties

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