

SUCROSE PHOSPHORYLASE FROM *PSEUDOMONAS SACCHAROPHILA* AND SUCROSE SYNTHESIS BY PHOSPHATE PROCESS

S. Wijeratna and K. Balasubramaniam

(Dept. of Biochemistry, Faculty of Medicine,
University of Colombo, Colombo 8)

Pseudomonas saccharophila was grown in sucrose phosphate medium. Its growth reached the late log phase in 20 h when the inoculum was 30 h old. Tween 20 (0.2%) reduced the time taken to reach the late log phase to 10 h but the total growth as determined by turbidity measurements was halved. This limitation in growth was not observed when the concentration of sucrose in the incubation medium was increased from 0.2% to 2%. Tween 20 also doubled the activity of sucrose phosphorylase.

On purification of sucrose phosphorylase by salt precipitation, the highest specific activity (30.4 units/mg protein) was shown in the 50-60% ammonium sulphate fraction. Further purification of this fraction by hydrophobic interaction chromatography increased the specific activity to 48.4 units/mg proteins. The degree of purification was 103 fold and recovery was 34%.

The sucrose phosphorylase preparation in solution was more stable at 40°C than at 29°C or -2°C. The loss of activity at 29°C and -2°C were 28% and 99% after 15 days. Its temperature optimum was 30°C while its pH optimum was 7.0. The enzyme had a K_m of $4.2 \times 10^{-3}M$ for sucrose.

SECTION E

The ammonium sulphate fractionated sucrose phosphorylase was used for sucrose synthesis. Fifty five percent of the glucose-1-phosphate which was used as substrate along with fructose was converted to sucrose. However, on altering the equilibrium by the removal of phosphate, sucrose yield based on G-1-P increased to 98%.

This work was supported by NARESA RGB/82/20 and University of Colombo.