Short Paper

Activities of Glycogen Degrading Enzymes in Ascidian Muscle

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It has been shown that storage of ascidian muscle leads to a marked increase in glucose, suggesting amylolytic pathway as a main route for glycogen degradation.¹⁾ On the other hand, the absence of glucose 1-phosphate (G1P) raised questions about the existence of glycolytic pathway. To give further information on previous work needs study on the activities of some amylolytic and glycolytic enzymes, such as α -amylase (EC 3.2.1.1), α -glucosidase (EC 3.2.1.20), hexokinase (HK, EC 2.7.1.1), phosphorylase (EC 2.4.1.1), and phosphoglucomutase (PGM, EC 5.4.2.2). In this paper, the authors report on the activities of these enzymes at pH 6.4 (pH of muscle) and at their optimum pHs.

Four live specimens of ascidian *Halocynthia roretzi* cultured in Miyagi Prefecture were obtained from the Tokyo Central Wholesale Market in May 1992. After removal of the tunic and evisceration, the muscles were washed with tap water, cut into small pieces, mixed, and withdrawn for the extraction of crude enzyme. The preparation of crude enzyme and the determination of the activities of HK, phosphorylase, and PGM were carried out according to the methods reported by Yamanaka.²⁾ The activities of α -amylase and α -glucosidase were determined by the method of Bergmeyer *et al.*³⁾ The protein content was determined by the Lowry method.⁴⁾

Fig. 1 shows the activities of enzymes at different pHs. PGM possessed noticeably high activity between pH 6.0 and 8.0, and its optimum pH was 7.5. Phosphorylase was observed at very low activity, when compared with other enzymes, and its optimum pH was 7.0. The observation of these activities indicates the existence of glycolytic pathway as a minor route for glycogen degradation in ascidian muscle. In comparison with the activities, it is justifiable to take into account the activities at muscular pH, which changed little before the muscle reached the stage of initial decomposition during storage.¹⁾ In this way, the activity of PGM was about 160 times higher than that of phosphorylase at pH 6.4. These findings can elucidate why G1P was not detected in ascidian muscle.

Fig. 1. The specific activities of enzymes extracted from ascidian muscle.

(HK: hexokinase, PGM: phosphoglucomutase)

With respect to the amylolytic pathway, scattered reports show that glycogen is degraded by α -amylase⁵⁾ and α -glucosidase⁶⁾ and, subsequently, glucose is converted to glucose 6-phosphate by HK. As shown in Fig. 1, the activity of α -amylase was relatively high and its optimum pH was 7.0. α -Glucosidase showed high activity in moderately acidic pH region, especially at pH 5.0. Contrastingly, the activity of HK was high in the buffer of moderately alkaline pH, optimum at pH 8.0, but it dropped substantially in the buffer of neutral or acidic pH. Considering the activities at pH 6.4, it is apparent that α -amylase and α -glucosidase were 110 and 2.7 times higher in activities than HK, respectively. These findings indicate that α -amylase and α -glucosidase are responsible for the accumulation of glucose in ascidian muscle during storage and that amylolytic pathway is the main route for glycogen degradation.

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