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THE SYNTHESIS OF γ-L-GLUTAMYL-L-CYSTEINE USING AN IMMOBILIZED DERIVATIVE OF γ-GLUTAMYLCYSTEINE SYNTHETASE

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An immobilized derivative of γ-glutamylcysteine synthetase was prepared by chemically combining γ-glutamylcysteine synthetase with Sepharose 4B. The immobilized enzyme had a similar pH-activity profile to that of the native one and was much more stable to heat than the native enzyme. The immobilized enzyme retained its full activity after storage at least for 5 months at 4°C, while the native enzyme lost its activity within 2 weeks under the same conditions.

Concerned in the γ-glutamyl cycle[1] in vivo and having a glutathione-like activity in vitro, γ-L-glutamyl-L-cysteine is a biochemically interesting substance. Chemical synthesis of γ-L-glutamyl-L-cysteine has been attained via complicated processes as selective protection of functional groups of constituent amino acids, and particular attentions have been paid to avoid racemization. If the γ-glutamylcysteine synthetase[2] which is rich in rat kidney can be effectively used for the biochemical synthesis of γ-L-glutamyl-L-cysteine, the complicated problems mentioned above may be fully solved. The present paper describes the successful use of immobilized γ-glutamylcysteine synthetase and some characteristics of the enzyme preparations.

γ-Glutamylcysteine synthetase(9.6 mg), prepared according to the method of Meister et al[3,4], was allowed to react in a borate buffer(0.05 M, pH 8.2) at 4° C for 24 hrs with Sepharose 4B[5](30 ml) which had been activated with cyanogen bromide, to give an immobilized enzyme. The protein content(2.7 mg/30 ml Sepharose gel) bound in Sepharose 4B was determined by the method of Lowry et al[6]. The specific activity(48 units/mg) of immobilized enzyme was deter-

mined to be 70 % compared with that(69 units/mg) of the native enzyme by the method of Orlowski and Meister[3]. Both the immobilized and native enzymes had the same optimum pH, around pH 8.4(Fig. 1). As shown in Fig. 2, the heat-stability of the enzyme was extremely improved by the immobilization.

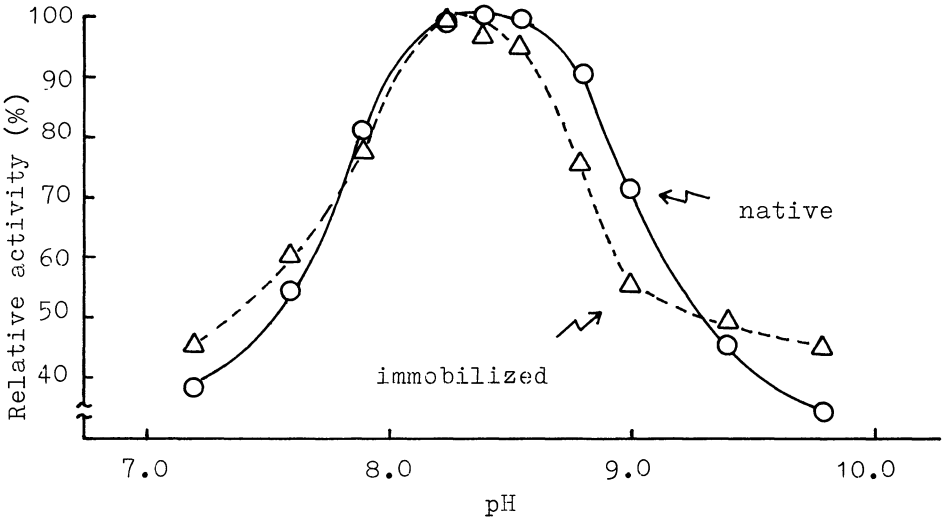

Fig. 1 Enzymatic activity of native and immobilized γ-glutamyl-cysteine synthetase as a function of pH. The maximum values for native and immobilized γ-glutamylcysteine synthetase are both taken as 100 %.

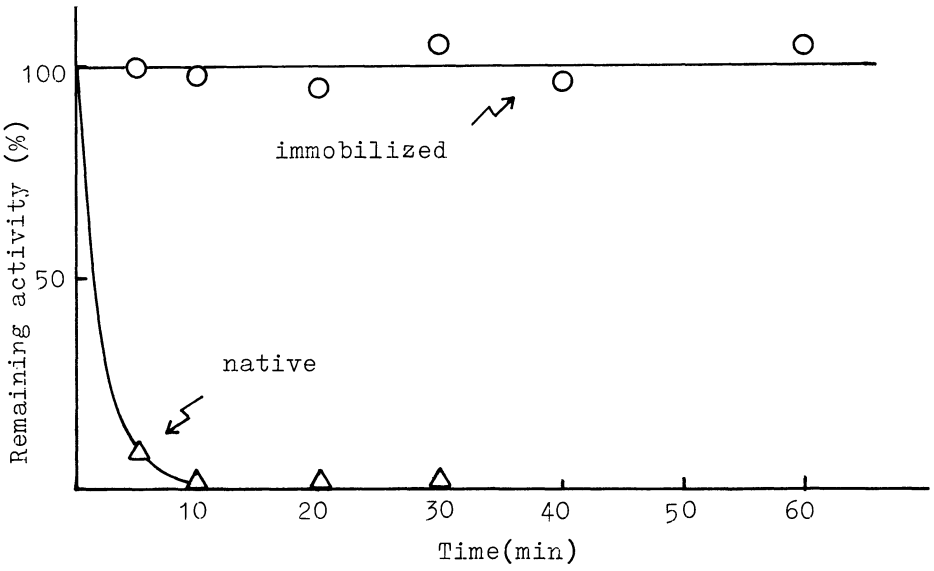

Fig. 2 Heat stability of immobilized and native γ-glutamyl-cysteine synthetase. Each sample was heated in 0.1 M Tris-HCl buffer (pH 8.2) at 54°C then assayed under the standard conditions at 37°C.

The synthesis of γ-L-glutamyl-L-cysteine was tried using the immobilized preparation. The immobilized enzyme(protein content: 0.7 mg) was added to 10 ml of Tris-HCl buffer(0.1 M, pH 8.2) containing 10 mM L-glutamic acid, 10 mM L-cysteine, 15 mM ATP, 15 mM dithiothreitol, 2 mM EDTA, and 10 mM $MgCl_2$ and stirred at 37°C for up to 50 hrs under atmosphere of nitrogen. At intervals, aliquots of the reaction mixture were withdrawn to analyze the amount of the synthesized peptide and concomitant decrease in the amount of glutamic acid using a high performance liquid chromatography. As shown in Fig. 3, the time course of the γ-L-glutamyl-L-cysteine synthesis reached to a plateau when 75 % of the L-glutamic acid was converted to the peptide. The product was isolated from the reaction mixture by gel filtration on a column of Sephadex G-10. The synthesized peptide was identified chromatographically using a high performance liquid chromatography by comparing with an authentic specimen of γ-L-glutamyl-L-cysteine chemically synthesized.

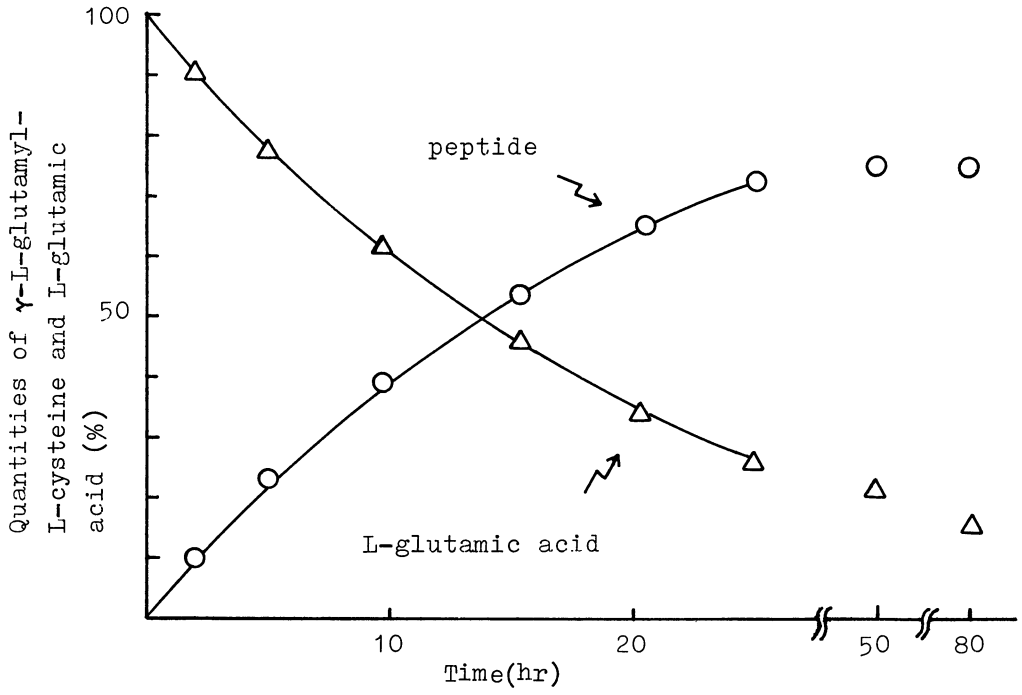

Fig. 3 Formation of γ-L-glutamyl-L-cysteine and concomitant decrease of L-glutamic acid during coupling reaction of L-glutamic acid with L-cysteine by use of immobilized γ-glutamylcysteine synthetase as determined by a high performance liquid chromatography.

When the immobilized enzyme was stored in a imidazole buffer(0.01 M, pH 8.2) at 4°C, it retained its full activity for more than 5 months including several times usage for the synthesis(around 300 hrs in total) at 37°C during the time. On the other hand, the native enzyme was almost inactivated within 2 weeks in the imidazole buffer at 4°C.

Thus, by immobilization γ-glutamylcysteine synthetase was stabilized enough for the repeated use to synthesize γ-L-glutamyl-L-cysteine in high yield.

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