ENZYMATIC SYNTHESIS OF γ-AMINOBUTYRIC ACID USING IMMOBILIZED L-GLUTAMATE DECARBOXYLASE

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γ-Aminobutyric acid (GAMA) is an important metabolite of nerve tissues and is widely used in medicine. In industry, GAMA is obtained by the hydrolysis of pyrrolidone [1]. Considerable interest is presented by a biotechnological method of obtaining GAMA by the α-decarboxylation of L-glutamic acid. The production of GAMA by this method has been described previously with the use as decarboxylation biocatalyst of the water-soluble enzyme L-glutamate decarboxylase (GDC) or bacterial cells [2-6]. We have investigated the process of obtaining GAMA through the decarboxylation of L-glutamic acid by immobilized GDC.

The production and main properties of immobilized GDC have been described elsewhere. The enzyme was immobilized by the addition of a partially purified preparation of GDC from *Escherichia coli* to Silochrome CX-2,5 modified by the introduction of bromoacetamide groups. Decarboxylation was carried out in a thermostated (37°C) laboratory minireactor connected with a pH-stat apparatus. The reactor was charged with 50 mg of immobilized GDC having an activity of 400 U/g (U — μmole/min) and 25 ml of a 0.05 M solution of L-glutamic acid in which pyridoxal phosphate had previously been dissolved ($5 \cdot 10^{-5}$ M) and the pH has been brought to a 4.6 with the aid of 0.1 N NaOH. On decarboxylation, the equivalent amount of OH^- ions is liberated. A constant pH of 4.6 was maintained by titration with 0.2 N HCl with the aid of the pH-stat. By simultaneously recording the consumption of titrant it was possible to follow the degree of conversion and the yield of product. After decarboxylation of the first portion of the L-glutamate solution, a minipump was switched on and the solution of the substrate was fed at a constant rate of 10^{-5} mole/min with the simultaneous removal of part of the reaction mixture in such a way that the total volume remained constant. Under these conditions a constant rate of decarboxylation of $9.5 \cdot 10^{-6}$ mole/min was maintained, which corresponds to a 95% conversion of the substrate. The solution of the product was concentrated in vacuum and the GAMA was precipitated with ethanol. After reprecitation from water with ethanol, a crystalline product was obtained with mp 200-201°C, which corresponds to the literature figure. Yield 85%. Thin-layer chromatography on Silufol plates on the ethanol—water (7:3) solvent system showed the identity of the GAMA obtained with a known sample (R_f 0.38) and the absence of contamination by L-glutanic acid (R_f 0.66).

The immobilized GDC can be used as biocatalyst repeatedly and the synthesis can be performed continuously. The process takes place at a low concentration of L-glutamic acid, which permits the inhibition by the substrate that is characteristic for GDC to be avoided.

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