Immobilization of β-Galactosidase in Collodion Microcapsules

One of the more flexible and efficient techniques of enzyme immobilization is the microencapsulation procedure developed by Chang.[1] Nylon microcapsules can be made by the interfacial polymerization method, while collodion microcapsules can be made by interfacial precipitation. While the nylon microcapsules have thinner membranes (~100 Å), the interfacial polymerization procedure often leads to denaturation of the enzyme. This was found to be the case by Østergaard and Martiny[2] in their study of the microencapsulation of β-galactosidase (E.C. 3.2.1.23) from *E. coli*. In the present investigation, we have microencapsulated this enzyme by the milder interfacial precipitation method for collodion microcapsules and show that the enzyme maintains high catalytic activity as measured by the conversion of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) (Sigma Corp.) in packed beds. To encapsulate the enzyme, 75 mg of *E. coli* β-galactosidase (Worthington Biochemical) were dissolved in 2.5 ml of a Tris-buffered hemoglobin solution made by dissolving 1 g of hemoglobin (Sigma) and 200 mg Tris base (Worthington) in 10 ml of distilled water. The enzyme solution was then placed in a 150 ml beaker with 25 ml of an organic solution made by mixing 100 ml of water-saturated ether (U.S.P.) and 1 ml of Span 85 (City Chemical Co.). The solutions are immediately stirred using a Fischer Jumbo magnetic stirrer at a speed setting of 7 with a 4 cm stirring bar. After 5 sec of stirring, 25 ml of a cellulose nitrate solution (made by evaporating collodion (U.S.P., MCB Corp.) to 20% of its original weight and replacing its original volume with ether) is added and stirring is continued for 1 min. The solution is then kept at 4°C for 1 hr. After settling, the supernatant is removed and 30 ml of *n*-butyl benzoate (MCB Corp.) containing 0.3 ml of Span 85 is added to the microcapsules and stirred at a speed of 5 for 30 sec. The resulting suspension is allowed to stand uncovered in crushed ice for 1 hr. After settling, the supernatant is again removed and 25 ml of a dispersing solution containing 12.5 ml of Tween 20 (Sigma) and 12.5 ml of H_2O is added to the microcapsules. The suspension is stirred for 30 sec at a speed setting of 10. The stirring rate is slowed to a setting of 5 and 225 ml of water are slowly added. This suspension is again allowed to settle, and the microcapsules are washed repeatedly with a 0.9 wt % NaCl solution (approximately 100 ml at a time). This is done until no trace of enzyme (measured by ONPG conversion) is detectable in the wash (~5 washings are required). Approximately 1 ml of microcapsules is obtained using this procedure, and approximately 49 mg of the enzyme is unbound. Thus, a concentration of enzyme corresponding to 26 mg/ml is finally encapsulated. This is almost exactly the concentration of enzyme initially dissolved in the hemoglobin (30 mg/ml). The encapsulated enzyme exhibited a value of k_p = 6.02 μmol/mg enzyme·min for ONPG, equal to the turnover number for the free enzyme, indicating that no appreciable enzyme activity was lost during encapsulation.[3] This turnover number was measured in a 0.1*M* phosphate buffer (pH 6.6) at 25°C.

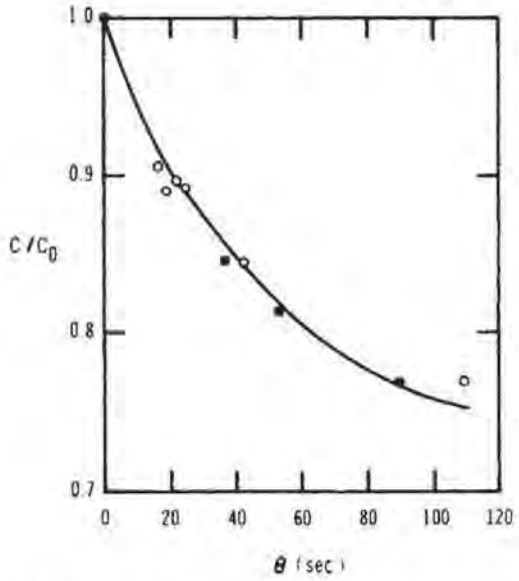

Fig. 1. Ratio of outlet to inlet ONPG concentration in a 1.6 cm diameter column containing suspended microcapsules: (○) 0.4 cm column, (■) 0.9 cm column. Buffer: 0.1*M* phosphate, pH 6.6, temperature 25°C.

The 1 ml of microcapsules was mixed thoroughly with 25 ml of washed Sepharose 4B gel (Pharmacia), and the slurry poured into a 1.6 cm diameter column (Pharmacia, Model K-16) at the same time that the phosphate buffer was being added. The gel settled quickly with an even distribution of microcapsules. The size of the bed could be controlled by the amount of slurry originally added. A 0.625*M* ONPG solution in the buffer (as described above) was then pumped through the top of the column at various flow rates (0.5–3 ml/min). The amount of product (*o*-nitrophenyl) formed was measured spectrophotometrically at 420 nm using a Hitachi Model 102 spectrophotometer. The results are shown in Figure 1 where the ratio (C/C_0) of the outlet substrate concentration to the inlet concentration is plotted as a function of residence time $\theta = z/v$ where z is the column length and v is the fluid velocity. Column lengths were short (0.4 and 0.9 cm), and with a residence time of ~100 sec, approximately 23% conversion was obtained. This is a high conversion, considering that only 0.006 to 0.01 ml of microcapsules were used in these experiments.

This shows that the collodion microencapsulation procedure is a suitable technique for the insolubilization of β-galactosidase. Even though the membrane thickness of collodion microcapsules is greater than that of nylon microcapsules (400 Å when compared to 100 Å),[1] the results of these experiments indicate that the membrane is still permeable to large molecules such as ONPG (mol wt = 301). Furthermore, the encapsulation procedure is milder than the interfacial polymerization method leading to nylon microcapsules, resulting in little enzyme deactivation.

References

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