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A Novel Immobilized-Enzyme System Utilizing Microcapsules

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ABSTRACT

A novel immobilized-enzyme system that has glucoamylase on the surface of, and glucose oxidase within, polyurea microcapsules was developed. This system was found to carry out its sequential enzymatic reaction effectively. It was also demonstrated that the use of microcapsules was useful for this immobilized-enzyme system since a large amount of the second enzyme, glucose oxidase, was required for the sequential reaction to proceed efficiently. In addition, this system was found helpful for making the size of reaction batches smaller.

Index Entries: Immobilized-enzyme system; microencapsulation of glucose oxidase; sequential enzymatic reaction; adsorption of glucoamylase; polyurea microcapsules.

INTRODUCTION

One of the possible applications of microencapsulation is to immobilize enzymes, enabling a fairly large amount of one enzyme or several different enzymes to be incorporated into small containers without being subjected to any chemical modifications.

The research of microencapsulated enzymes was pioneered by Chang, who, in his first paper on the subject, enclosed microdroplets of urease solution within semipermeable polymer membranes for the purpose of utilizing the microcapsules as an artificial kidney (*1*). Since then, he has so far prepared many kinds of encapsulated enzymes and multienzyme systems and found them useful in the field of medical treatment (*2–8*).

However, the enzmes to be microencapsulated are restricted, inevitably, to those that can act on low-molecular-weight substrates, since the microcapsule membrane is semipermeable in nature. In this context, our new immobilized-enzyme system will be promising, since one enzyme capable of decomposing a high-molecular-weight substrate into a low-molecular-weight product is attached to the microcapsule surface, and another is entrapped within microcapsules so that it can act on the product flowing in from the outside. This sort of immobilized-enzyme system can be expected to work *in situ* and will make the application of microencapsulated enzymes wider in various fields of science and technology as bioreactors and, moreover, as a model of living cells.

This paper reports an attempt to make an immobilized-enzyme system in which glucoamylase is attached to the surface of, and glucose oxidase enclosed within, polyurea microcapsules.

METHODS

Preparation of Microcapsules Containing Glucose Oxidase

Polyurea microcapsules containing glucose oxidase were prepared by utilizing the interfacial polymerization reaction between tetraethylenepentamine in an aqueous phase and toluylenediisocyanate in an organic solvent. An equal volume of an aqueous mixed solution of 0.2*M* tetraethylenepentamine and 0.25*M* Na_2CO_2 was added to 5 mL of an aqueous 5% (w/v) polyvinylpyrrolidone K30 solution containing glucose oxidase (GOD, EC 1.1.3.4 from *Aspergillus niger*, 68 U/mg, Sigma) just before use. This solution was mechanically dispersed as fine droplets into 50 mL of cyclohexane containing 2% (v/v) sorbitan sesquioleate as an emulsifier. Without stopping stirring, 50 mL of 0.115*M* toluylenediisocyanate solution in chloroform was quickly added to the emulsion, and the stirring was continued for 10 min at room temperature. The microcapsules thus obtained were transferred into the aqueous phase with the aid of 50% (v/v) sorbitan monolaurate and washed several times with distilled water. These GOD-loaded microcapsules are designated hereafter as GOD_x-MC. The subscript x means the amount of GOD (mg/10 mL) added to the aqueous monomer solution in the preparation. The mean diameter of GOD_x-MC was found to be about 30 μm, regardless of the amount of GOD added. The values of K_m were 31.1, 30.1, 21.4, and 17.5 m*M* for free GOD, GOD_{16}-MC, GOD_{45}-MC, and GOD_{75}-MC, respectively.

Immobilization of Glucoamylase on GOD_x-MC

To 4 mL of GOD_x-MC suspension in 0.1 *M* acetate buffer solution (pH 4.47) was added an excess amount of glucoamylase (GA, EC 3.2.1.3 from *A. niger*, 14 U/mg, Boehringer) dissolved in the same buffer solution. The mixture was incubated for 30 min at 35°C and unadsorbed excess GA was removed by washing the microcapsules with buffer solution on the centrifuge. Although no chemical reaction was employed to immobilize GA on the microcapsule surface in fear that chemical reagents might cause adverse effects on the GOD already entrapped within the microcapsules, no desorption of GA be detected after several washings with the buffer solution. The GOD_x-MC with adsorbed GA on the surface are designated hereafter as GA–GOD_x-MC. The immobilization of GA caused a slight increase in the value of K_m for the enzyme.

Measurement of Sequential Enzymatic Reaction Rate

The GA is known to decompose soluble starch into D-glucose (*9*), which is in turn converted by GOD into glucono-δ-lactone (*10*), accompanying the concomitant consumption of oxygen dissolved in the medium. Therefore, the rate of oxygen consumption was monitored with a Galvani-type oxygen electrode (Ishikawa Seishakusho, Tokyo), and was used as a measure of the sequential enzymatic reaction. The measurement was carried out in the medium of 0.1*M* acetate buffer solution (pH 4.47) at 25°C, since it was found from preliminary experiments that GA and GOD fully displayed their enzymatic activities at this pH.

RESULTS AND DISCUSSION

The sequential enzymatic reaction used here is schematically shown in Fig. 1. Since the K_m value of GA for soluble starch is extremely small (less than 10^{-3} m*M*) and that of GOD for D-glucose is relatively large (about 30 m*M*), a considerably large amount of GOD seems necessary for the overall reaction to proceed efficiently. These circumstances are visualized in Fig. 2, in which the overall reaction rate is found to increase linearly with an increase in the amount of GA at a given amount of GOD. This suggests that the rate of D-glucose supply by GA is not enough for GOD to display its maximal ability under the condition employed here since the K_m value of GOD is relatively large.

On the other hand, when the amount of GA is fixed, the overall reaction rate increases and tends to level off with an increase in the amount of GOD. This tendency is more remarkable with low amounts of GA than high amounts, which means that high ratios of GOD to GA are favorable for the overall enzymatic reaction to proceed efficiently without accumulation of the intermediate substrate, D-glucose.

In view of this, the use of microcapsules in such an immobilized-enzyme system as employed here seems favorable in that a large amount

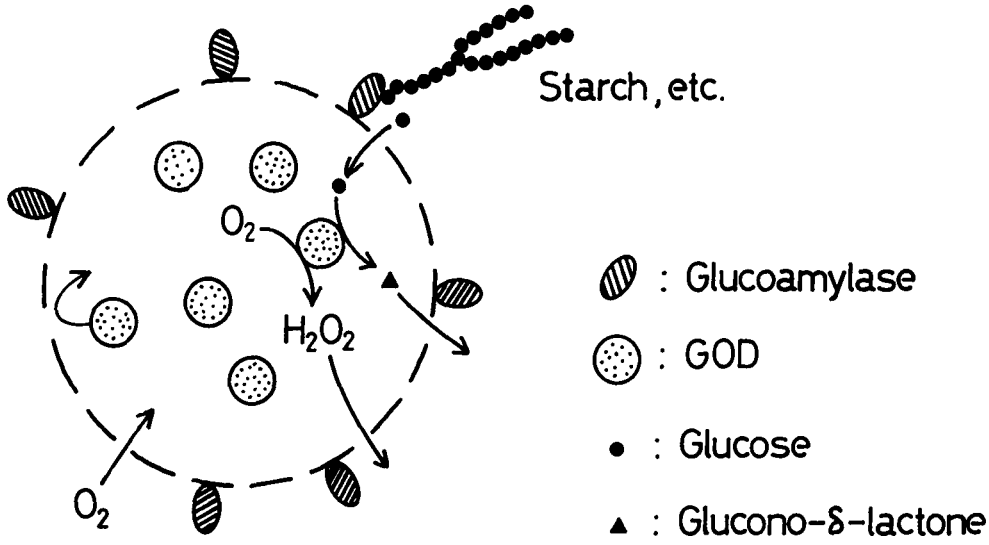

Fig. 1. Schematic representation of dually immobilized enzyme system utilizing microcapsule.

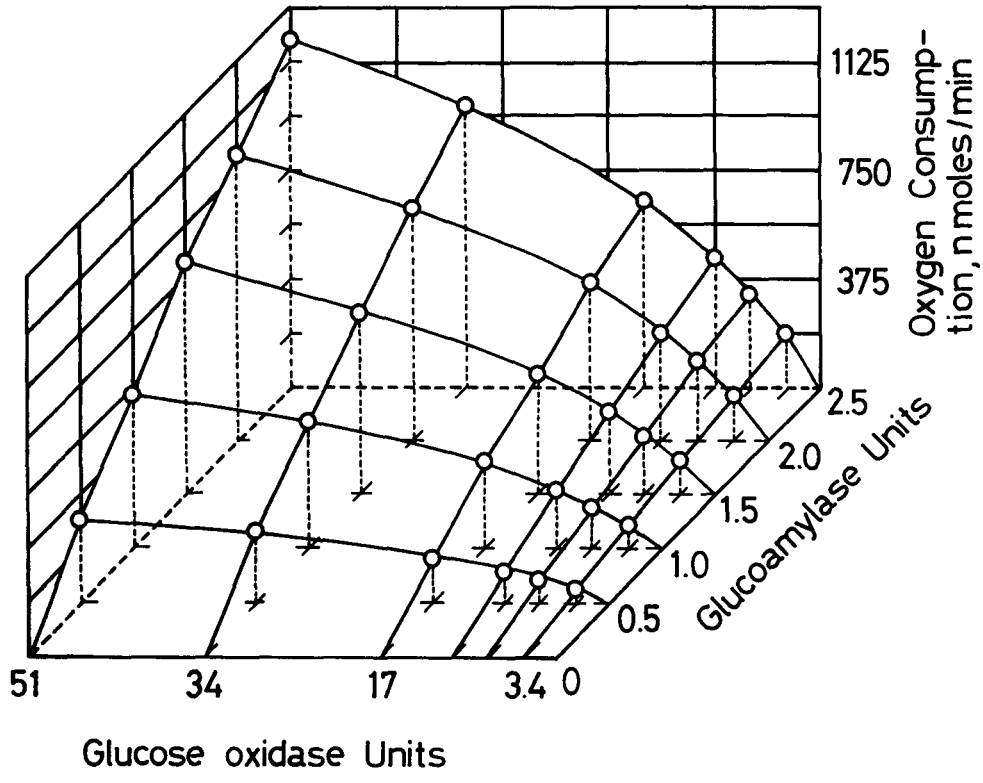

Fig. 2. Interrelation between the activities of free GOD and free GA in overall enzymatic reaction.

of one enzyme with a large K_m, like GOD, can be incorporated within microcapsules in the state of solution, which will make it possible to construct a system of an extremely low ratio of another enzyme with a small K_m, like GA, to the former.

In order to make sure that the immobilized-enzyme system GA–GOD_x-MC can actually perform its sequential enzymatic reaction, a given amount of GA–GOD_x-MC was added to a soluble starch solution, and the oxygen consumption was measured. Figure 3a indicates that oxygen is consumed by GA–GOD_{12}-MC after showing a lag phase of several minutes, which would correspond to the time necessary for the product, D-glucose, to accumulate in an amount large enough to be decomposed by GOD. It also shows that oxygen consumption increases with an increase in the amount of GA–GOD_{12}-MC added. The increment of overall enzymatic reaction rate was roughly in geometrical progression, with an increase in the amount of GA–GOD_{12}-MC (Fig. 3b), which would confirm that the immobilized-enzyme system, GA–GOD_{12}-MC, could really simulate the sequential enzymatic reaction by GA and GOD in free solution, since the geometrical progression in the overall reaction rate could be traced by utilizing Fig. 2 at a fixed ratio in units of GOD to GA.

In Fig. 4 is shown the oxygen consumption induced by the addition of the same number of GA–GOD_x-MC. Here, the amount of GA ad-

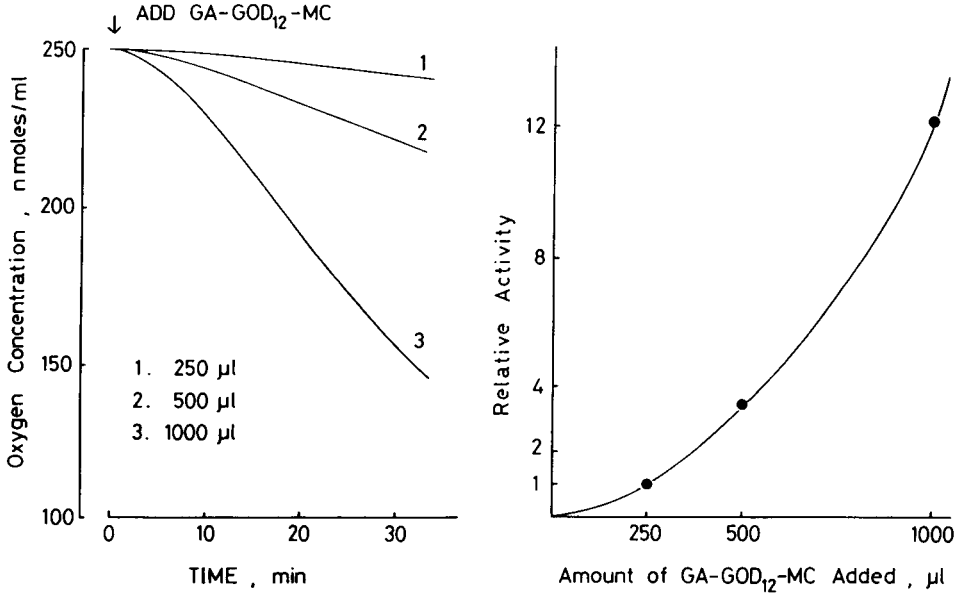

Fig. 3. Effect of the amount of GA-GOD_{12}-MC on the overall enzymatic reaction. (a) Oxygen consumption was traced along the time elapsed. (b) The ordinate means the activity relative to that obtained by addition of 250 µL of GA-GOD_{12}-MC suspension. Here, the activity was evaluated by the maximal velocity of oxygen consumption calculated using the data in (a).

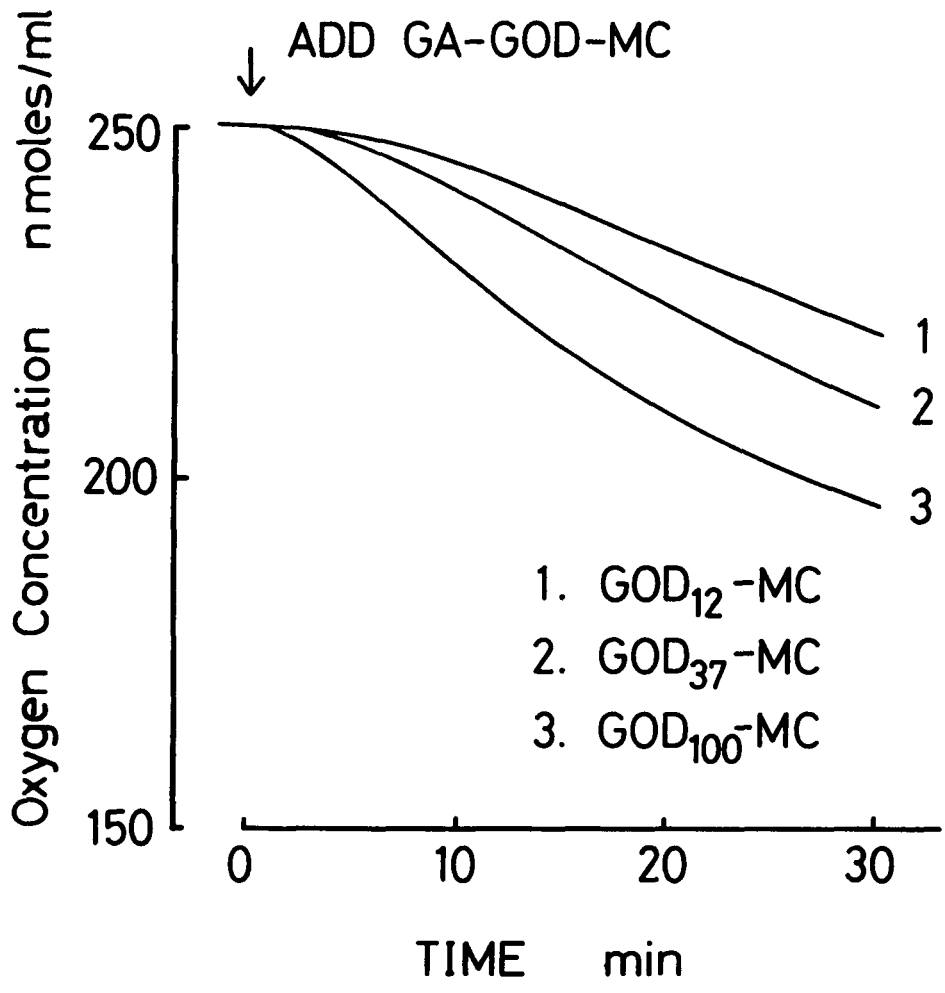

Fig. 4. Effect of the amount of GOD entrapped in microcapsules on the overall enzymatic reaction.

sorbed is identical for all GOD_x-MC, guaranteeing an equal supply of D-glucose to the encapsulated GOD. It reveals that the overall reaction proceeds more efficiently, and the lag time becomes shorter with increases in the amount of GOD entrapped within the microcapsules. These results again confirm that a relatively high ratio of GOD to GA is required for GA–GOD_x-MC to work efficiently without D-glucose accumulation.

The effect on the overall reaction rate of the amount of GOD entrapped in the microcapsules was examined under somewhat different conditions as follows: Each GOD_x-MC suspension was adjusted by varying the number of GOD_x-MC to have the identical maximal enzymatic reaction rate evaluated in the presence of excess amount of D-glucose. Then, each of the GOD_x-MC suspensions was mixed with a soluble starch solution. To this suspension was addd a given amount of GA dissolved in the medium, instead of making all of the molecules adsorb on

the GOD_x-MC, because the total surface areas of GOD_x-MC in suspension differed from each other, which, in turn, would result in the difference in the amount of GA adsorbed. Here, it was assumed that there was no difference in enzymatic activity between free and adsorbed GA because the values of K_m were approximately equal. Figure 5 indicates that the enzymatic reactions also proceed sequentially, and that the overall reaction is more efficient for the microcapsules with larger amounts of GOD entrapped than for those with smaller ones. The amount of D-glucose produced by GA is the same for each GOD_x-MC suspension, so that the amount of D-glucose passing through the microcapsule membrane

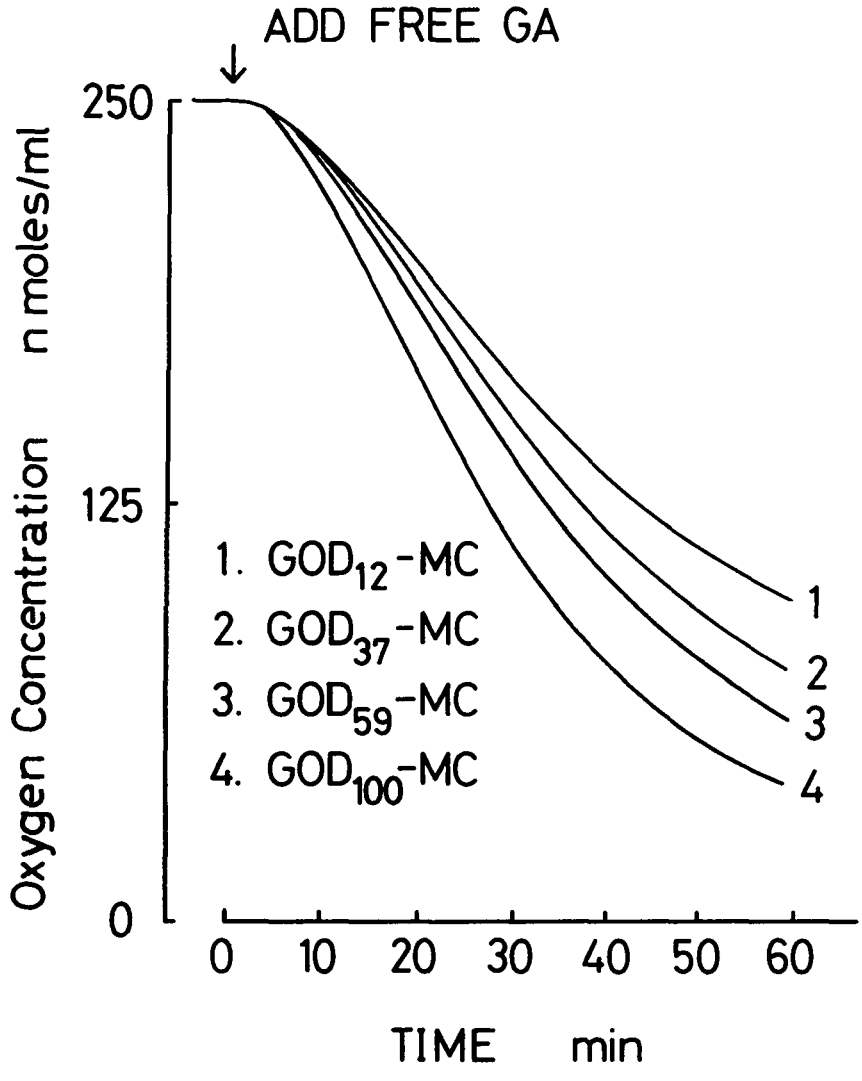

Fig. 5. Overall enzymatic reaction induced by addition of GOD_x-MC having different number but identical apparent maximal velocity of GOD's enzymatic reaction. Here, a given amount of GA solution was added to the medium.

will be larger for the GOD_x-MC containing smaller amounts of GOD than for those containing larger amounts, because the number of the former is larger than that of the latter. However, the sequential enzymatic reaction was more efficient for GOD_{100}-MC suspension. No plausible explanation for this result can be given at present, but it is assumed that the amount of effective GOD might differ from each other though the maximal velocity of enzymatic reaction for all GOD_x-MC was identical in the presence of excess amount of D-glucose (*11*).

At any rate, Fig. 5 discloses the possibility that this immobilized-enzyme system will be promising for constructing a smaller size of reaction batches with high efficiency of the sequential enzymatic reaction, since a smaller number of GOD_{100}-MC was required to give comparable efficiency.

ACKNOWLEDGMENT

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