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IMMOBILIZATION OF ENZYME BY PLASMA-INITIATED POLYMERIZATION †

Yoshihito OSADA*, Yasuhiro IINO, and Yu IRIYAMA
Department of Chemistry, College of Liberal Arts & Sciences,
Ibaraki University, Mito 310

Invertase was immobilized by the plasma-initiated polymerization, exposing the glow-discharge plasma for 60-90 sec onto the aqueous vinyl monomers, followed by the postpolymerization at room temperature. Obtained enzymes in the pellet and the film exhibited high activities for a long period of time without any leakage of the enzyme.

An increasing effort is being made to understand the theoretical as well as practical aspects of enzyme immobilization. The methods used to obtain active insoluble enzymes are classified into four main groups[1]: Chemical attachment to an insoluble support, Crosslinking with a bifunctional reactant, Physical adsorption on a inert support, and inclusion in porous materials, the cavities of which are surrounded by walls which do not allow the outflow of enzyme, but yet permit the entry of low molecular weight substrate. The last one[2-5] is of particular interest ,since it often duplicates situations exsisting within subcellular compartments.

A novel immobilization method of the enzyme presented in this article falls within the last of these categories and consists in the entrapment of the enzyme protein in the extremely high molecular weight polymer prepared by the plasma-initiated polymerization.

We have previously reported that a cold plasma produced by an electric discharge can initiate the polymerization of vinyl monomers[6-8], or crystalline cyclic ethers[8,10] to give linear very high molecular weight polymers, or highly crystalline poly(oxymethylene)s. Later, the inorganic cyclic compounds such as phosphazene[11] and siloxane[12] crystals were also found to undergo ring-opening polymerization by the plasma exposure. The principle of the enzyme immobilization by the plasma-initiated polymerization is based on the exceptionally high polymerizability of vinyl monomers at low temperature against the plasma in the presence of water.[13] In addition, the plasma is essentially the surface reaction, and a host of chemically active species such as electrons, ions and radicals in the gas plasma cannot penetrate into the medium more than a few microns in nature. These characteristics are different from other radiation methods and should be convenient particularly for the entrapment of bioactive substances including enzymes.

Invertase entrapped was of commercial origin from yeast(Wako Chemical, stabilized with 50% glycerine) and used without purification. 2-Hydroxyethyl metha-

† (study of plasma-initiated polymerization V)

crylate(HEMA), acrylamide(AAM), and methacrylic acid(MAA) were used as monomers entrapping the enzyme. An appropriate amount of N,N-methylene-bis-(acrylamide) was added in the cases of the polymerization of AAM and MAA to give the crosslinked polymer gel, but no crosslinking agent was used in the case of HEMA because of the spontaneous crosslinking tendency of the monomer.

Two types of immobilization were performed by the plasma-initiated polymerization: One is the polymerization of the aqueous monomer containing invertase in the ampule, 10 mm internal diameter, and 200 mm long, to give a pellet type immobilized enzyme. The method of the polymerization is essentially the same as previously described.[6,7,14] The ampule with the monomer solution was degassed by repeated freezing and thawing while under a vacuum of 10^{-2}-10^{-3}torr, inserted between a pair of parallel electrodes connected to a radio-frequency plasma generator which operates at 13.56 MHz(Fig.1A). A glow discharge was then initiated in the vapor phase for 60-90 sec of duration, and allowed to postpolymerize at 25°C. The sponge-like polymer containing the invertase was sliced with a thickness c.a. 3mm, immersed in the buffer solution of pH4.6 for more than a week to remove any unreacted monomer and provided as a sample to study the enzyme activity.

The other type of the polymer-entrapped invertase was the thin film prepared by use of the plasma reactor shown in Fig.1(B). The monomer-enzyme solution was casted on a slide glass(75mmx25mm), placed on the electrode, and exposed the plasma for a prescribed time. The solution was then postpolymerized in the reactor at room temperature. Water was circulated through the electrode to avoid any increase in temperature of the solution during the plasma exposure. The solid polymers were formed within a few hours after the plasma exposure for both cases of the pellet and the film, however another 16-20 hours were allowed to postpolymerize to complete the polymerization.

Immobilization conditions and appearances of the immobilized enzymes of both types are summarized in Table 1. The activities of the enzyme were studied in the 25 ml Erlenmeyer's flask with stirring at 25°C.

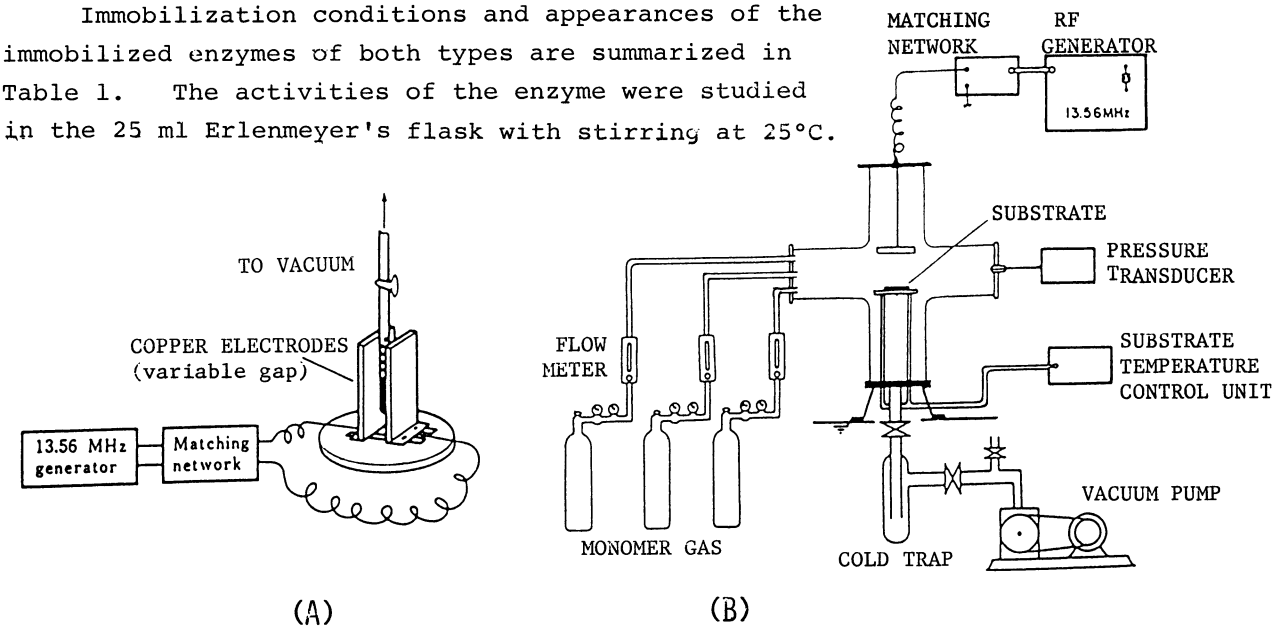

Fig.1 Schematics of apparatus for plasma-initiated polymerization
(A) for pellet type, (B) for thin film type(no monomer gas was used)

3 ml of aliquot was assayed by the change of the optical rotation in the acetic acid-acetate buffer solution at pH 4.6. As shown in Fig. 2(A) and (B), every samples obtained exhibited activities, whereupon the invertase entrapped in poly(AAM) showed higher activity than other two. This might be related to the higher swellability of poly(AAM) gel than other polymer gels. The relative activities of the invertase entrapped in the poly(AAM) were found to be 62, 40 and 21% for P-3, P-4 and F-2, respectively calculating from the amount of the enzyme used. From these data it is evident that it is possible to obtain insoluble enzymes, the activity of which can be varied within a wide range.

TABLE I IMMOBILIZED ENZYMES PREPARED BY PLASMA-INITIATED POLYMERIZATION

	EXP. NO	CONDITION		PLASMA (SEC)	POST-POLYM'N (h)	SAMPLE(THICKNESS)
PELLET TYPE	P-1	HEMA	1ml	60	20	0.25g,NO SWELL
		INVERTASE	1ml			
	P-2	HEMA	1ml	60	24	0.1g,SLIGHTLY SWELL
		INVERTASE	1ml			
		WATER	1ml			
	P-3	AAM	1.2g	60	24	0.3g,SWELL
		MBAA	0.01g			
		INVERTASE	0.1ml			
		WATER	2.8ml			
	P-4	AAM	1.2g	60	24	0.3g,SWELL
		MBAA	0.001g			
		INVERTASE	0.1ml			
		WATER	2.8ml			
FILM TYPE	F-1	HEMA	1.5ml	90	20	0.13g (150μ, DRY)
		INVERTASE	1.5ml			
		WATER	1.5ml			
	F-2*	AAM	0.5g	90	20	0.036g (170μ, DRY)
		MBAA	0.007g			
		INVERTASE	0.7ml			
		WATER	1.3ml			
	F-3	MAA	0.5ml	60	20	0.023g (300μ,DRY)
		MBAA	0.006g			
		INVERTASE	0.25ml			
		WATER	0.5ml			

PLASMA: 100 W, POSTPOLYMERIZATION 25°C
*CASTED ON A CROSSLINKED POLY(METHACRYLIC ACID) MEMBRANE(THICKNESS 30μ)

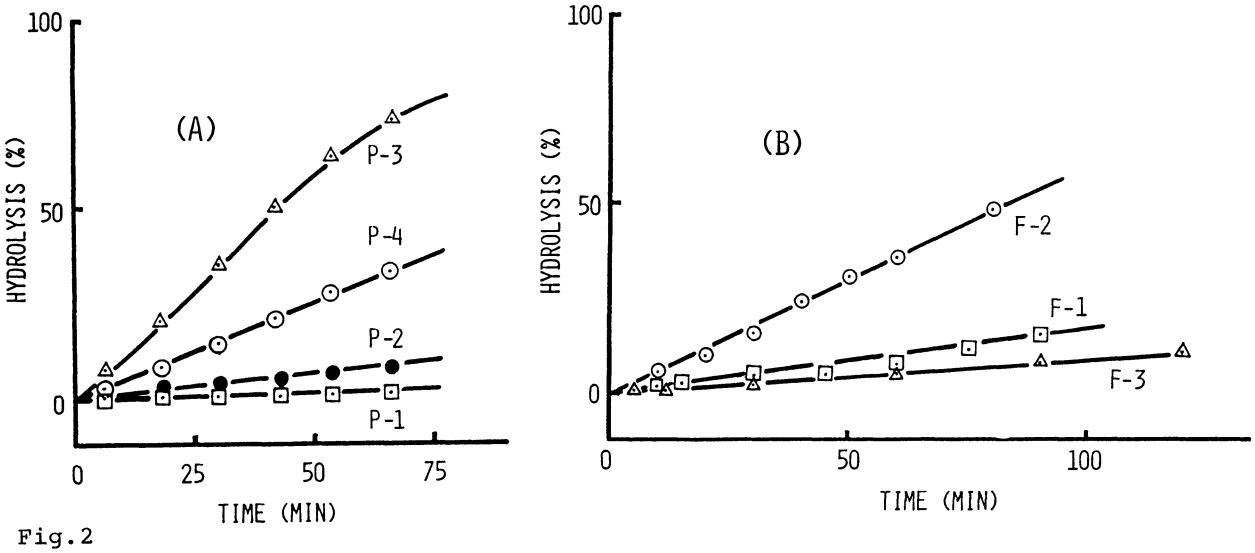

Fig.2
Percent hydrolysis versus time relationship for the invertase immobilized by the plasma-initiated polymerization. (A): pellet type, (B): film type saccharose; 10 wt%, 10 ml, pH: 4.6

Interesting feature here is that no leakage of the invertase occurred when the invertase is immobilized by the plasma-initiated polymerization. No absorption at 270 nm due to the enzyme or no activity was observed at all in the supernatant solution in which the polymer-occluded enzyme was immersed for more than two months. It was

found from the viscosity measurement that poly(AAM) and poly(MAA) obtained in this manner have molecular weights higher than 10^7 g/mol. Therefore, this important result might be related to the high entanglement of the extremely long macromolecular chains which allow the enzyme to remain entrapped in the networks.

A sponge-like immobilized enzyme from poly(HEMA) (P-1) was employed in a continueous system: a solution of the substrate was fed to the column containing the biological catalyst, where the reaction occurs at the outlet a solution was obtained containing the products and the unreacted substrate. Air adsorbed to the enzyme particles was carefully removed by pressing the particles gently in the column, since it prevents the desirable contact of the substrate with the enzyme. The efficiency of the catalyst depended on the degree of packing of the column (mg particle/ml) and on the linear velocity of the liquid. As shown in Fig.3, the degree of conversion of the substrate into products was a function of the flow rate, whereupon the conversion x was expressed as $x = 1 - e^{-k/F}$, where k is the apparent rate constant (min^{-1}) and F is the flux (ml/min cm^2) for the given column length.

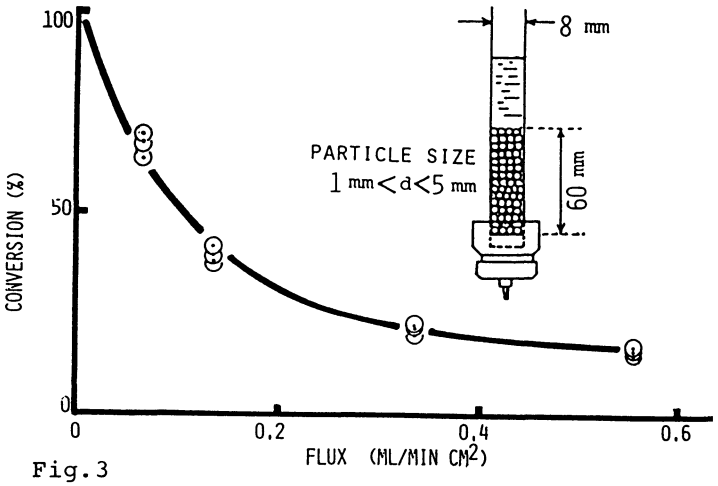

Fig.3

Effect of flowrate on percent conversion of hydrolysis of saccharose by the invertase entrapped in the poly(HEMA) packed in the column.
immobilized invertase: 0.7 g
saccharose: 10 wt %, pH: 4.6

The long term stability of the invertase in the particle has been tested on "resting" the enzyme in the column in the absence of the substrate at room temperature. So far the invertase immobilized is providing almost the same activity after 3 months "resting", whereas the native invertase in solution was quite unstable denaturating, and lost the activity within a month.

The technique of the entrapment of enzymes by the plasma-initiated polymerization is simple and can potentially be applied to any proteins and cells in principle.

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