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FLUID IMMOBILIZED CELLULASE

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SUMMARY

Fluid immobilized cellulase was prepared using polyethyleneglycols and hexamethylene diisocyanate, and its properties studied. The cellulase activity of the immobilized enzymes varied with monomer composition and molecular weight of polyethyleneglycols. The enzyme activity was affected by the viscosity of the carrier. A solid substrate (cellulose powder) can be hydrolyzed with the fluid immobilized enzyme.

INTRODUCTION

Enzymes immobilized to insoluble polymers are gaining importance in many industrial and biomedical applications. The technique has been developed to circumvent many difficulties associated with use of soluble enzymes (Weetal and Suzuki, 1974; Messing, 1975), and the main recent development has been in suitable carriers for enzymes. Enzymes have been usually immobilized in solid carriers which are relatively rigid. If enzymes are immobilized on a fluid carrier, the field of the use of immobilized enzymes could be extended. Though soft gels such as agarose and polyacrylamide have been used as carrier, their state was of flexible rather than fluid (Porath, 1974; Parikh et al., 1974; La Porte et al., 1977; Schnaar and Lee, 1975). In this work, a fluid immobilized enzymes were prepared and its properties were studied.

MATERIALS AND METHODS

Materials Cellulase (1×10^4 units/g) was obtained from Yakult Biochemicals Co., Ltd. Hexamethylene diisocyanate (HD) and polyethyleneglycols (PEG 200, 300, 400, and 600) were obtained from Wako Pure Chemical Industries Ltd.

Methods Preparation of fluid immobilized enzyme HD and PEG were mixed at room temperature for 10 min and then charged into a test tube containing enzyme solution (1.0 %, w/v). The fluid immobilized enzyme was obtained by shaking the test tube at room temperature for 20 min.

Enzyme activity of fluid immobilized enzyme The durability of the immobilized enzymes was examined by repetition of batch enzyme reaction (40 °C, 1 hr), measuring the enzyme activity (%) remaining in the repeated batch enzyme reaction of the immobilized and native enzymes at each batch enzyme reaction using 1.0 % w/v carboxyethylcellulose sodium (CMC) solution, pH 4.5. Glucose was determined with a glucose-specific reagent, "GOD-PODLK", obtained from Nagase Sangyo Co., Ltd.

Hydrolysis of cellulose powder with fluid immobilized enzyme The immobilized enzyme (5 g) and cellulose powder (1.0 g, 200 - 300 mesh, Tokyo Roshi Co., Ltd.) were suspended in 0.1 M acetate buffer solution (10 ml, pH 4.5) and then reacted at 40 °C. The glucose formed was determined. Glucose yield (%) was expressed as the ratio of glucose to cellulose powder.

RESULTS AND DISCUSSION

Variation of enzyme activity with repeated batch enzyme reaction The enzyme activity of the immobilized enzyme was effectively constant with up to twelve consecutive batch enzyme reactions, indicating that the enzymes do not

leak from the carrier. The immobilized enzymes prepared by the present method were effectively fluids, in which enzyme reactions were mainly carried out on the surface of the carrier. This feature results from the fluidty of the polymer chains resulting from the reactions of HD and PEG, in which the ends of the polymer chains are bound with the enzymes and the chains are not cross-linked. The fluid immobilized enzymes can be introduced into narrow spaces and contacted with substrate by the deformation of the carrier. Accordingly, such immobilized enzymes are suitable for the enzyme reactions of a water-insoluble substrate such as cellulose. The trapping site of the enzymes in the immobilized enzymes changes with the deformation of the carrier, so that the surface trapping the enzymes is not fixed.

Effect of monomer (HD, PEG) composition on enzyme activity

The effect of monomer composition on the enzyme activity of the immobilized enzymes was examined. The relationship between enzyme activity and composition is shown in Fig. 1. The enzyme activity increased, reached a maximum, and then decreased with increasing PEG component. The maximum of the enzyme activity was observed at a 1:1 composition, indicating that the end of the HD molecule reacts stoichiometrically with the enzyme molecule. Polyurethane is usually formed by the reaction of polyisocyanates with compounds containing active hydrogen such as glycols or polyglycols. In the present method, one hydroxyl group of PEG was reacted with one isocyanate group of HD, and the other isocyanate group of HD was reacted with the enzyme to form the polymer chains (PEG-HD-enzyme), in which the reaction of PEG-HD with the enzymes was carried out in aqueous solution containing the enzymes. In general, it is known that order of the reactivity of isocyanates with other compounds is $RNH_2 > R_2NH > RCH_2OH > H_2O > RCOOH$ (Saunders, 1962). Therefore, the reaction of the enzymes

having amino groups with PEG-HD proceeds preferentially even in aqueous solution. It is, of course, probable that part of the isocyanate group of PEG-HD is reacted with water. In the case of the composition rich in HD, two hydroxyl groups of PEG would be reacted with HD to form polyurethane. In fact, the enzyme activity of the immobilized enzymes prepared from the mixture rich in HD was lower than that from a 1:1 composition as seen in Fig. 1. Immobilized enzymes prepared from HD alone gave some enzyme activities(Fig.1), suggesting that HD itself reacted with the enzyme to form cross-linked enzymes.

Relationship between enzyme activity and viscosity Effect of the viscosity and molecular weight of PEG on the enzyme activity of the immobilized enzymes was examined. Fig. 2 shows the relationship between enzyme activity and viscosity or average molecular weight of PEG. It is thought that the reactivity of PEG with HD and the nature of the immobilized enzymes vary with the viscosity of the carrier. As the molecular weight of PEG increases, the viscosity at 25 °C increased as shown. This suggests that the feature of the enzyme reaction with the fluid immobilized enzymes is affected by the viscosity of the carrier of the immobilized enzymes though the viscosity of PEG increases slightly by the reaction of HD with the enzymes. The immobilized enzymes of low viscosity appeared to be suitable for the hydrolysis of CMC which is a substrate of relatively high molecular weight. This means that enzyme reactions with the fluid immobilized enzymes are considerably affected as the mobility of the enzyme molecule trapped on the surface of the carrier varies with the fluidty of the carrier.

Hydrolysis of cellulose powder The hydrolysis of cellulose powder with the fluid immobilized enzymes obtained by the present method was examined. Fig.4 shows the relationship between glucose yield and hydrolysis time, indicat-

ing that solid substrates such as cellulose powder can be effectively hydrolyzed by the fluid immobilized enzymes. It is known that the hydrolysis of solid cellulose with immobilized enzymes is difficult. The result in Fig.3 indicates that since the surface of the carrier of the immobilized enzymes is fluid, the accessibility of the enzymes to cellulose is increased and the cellulose can be hydrolyzed. The fluid immobilized enzymes can easily invade into the narrow space (cracks) in cellulose.

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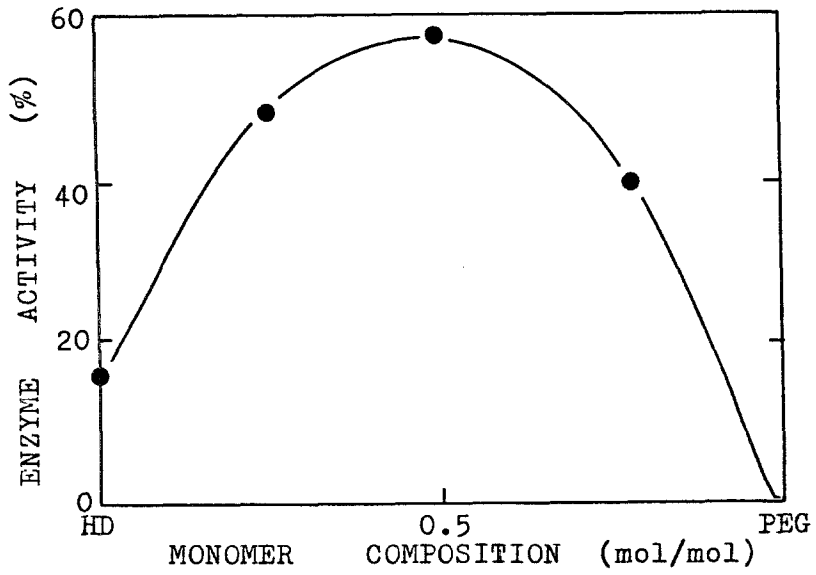

Fig. 1. Effect of monomer composition on enzyme activity.

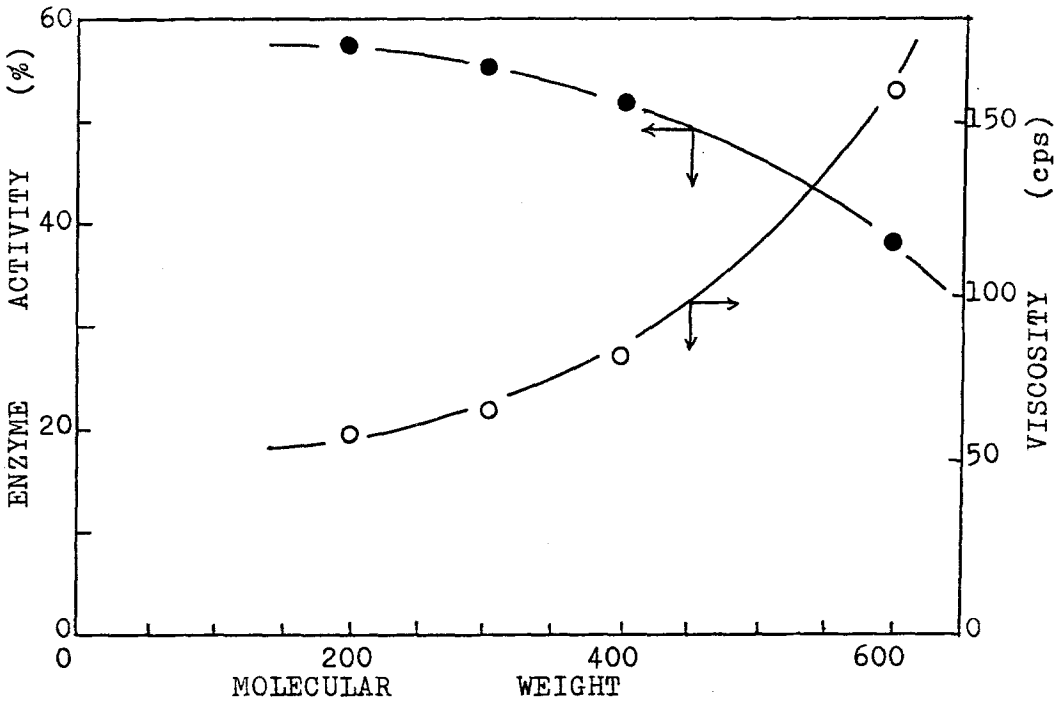

Fig.2. Effect of viscosity and molecular weight of PEG on enzyme activity.

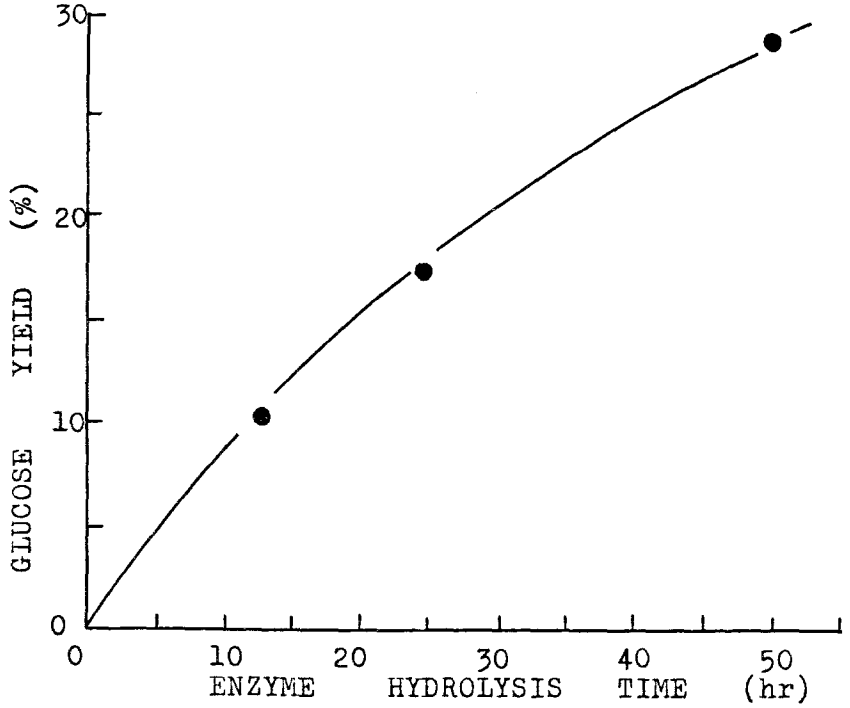

Fig.3. Enzyme hydrolysis of cellulose powder with the immobilized enzymes from HD and PEG 200.