Polymeric Membranes for Lipase Immobilization

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Lipase triacylglycerol acylhydrolase, (E.C. 3.1.1.3) is an enzyme that is fully active on aggregated substrates and practically inactive on monodisperse systems. A lipase immobilized on polymeric membranes has been applied for sunflower oil hydrolysis. The influence of membrane properties on enzyme activity is studied. Membranes made of poly(vinyl chloride), collagen, cellulose acetate and polytetrafluoroethylene were used for adsorption of lipase. The porosity and hydrophobicity of membranes did not influence the lipase activity. The difference of the work of adhesion for the water/membrane system and oil/membrane system reflected the activity data, while work of adhesion for water or oil (done separately) did not. For oil hydrolysis to occur on the membrane surface, accessibility of two liquid phases is important, and the lower the difference of work of adhesion between water and oil, the greater the activity of immobilized lipase.

KEY WORDS: Immobilization, lipase, polymeric membranes.

The relatively recent availability of large quantities of enzyme preparations has broadened the search for industrial applications of enzymes and studies of continuous mode of operation using immobilized enzymes. Presently, lipases are employed in a variety of industrial processes. For example, the enzyme removes fat stains from fabrics, accelerates maturation of cheese, and degrades fat skin waste products (1). A Japanese company was reported to use a lipase to hydrolyze oils for producing soaps (2). The enzymatic hydrolysis of lipids is an interesting process as it could help overcome some of the drawbacks of a high-temperature process. Recently, membrane reactors with immobilized lipase have been reported for a successful olive oil (2–4) or sunflower oil (5,6), hydrolysis. A microporous membrane is the enzyme's carrier and separates the bulk phases. A large surface area of contact between the two phases—oil and water—is provided. The advantages of this approach include a stable interface, ease of scale-up, and elimination of emulsification problems.

It is well known that enzymatic properties change after immobilization; this is believed to be caused by changes in the enzyme itself, or by the carrier's properties. This work reports the results of studies on an immobilized lipase on membranes made of various polymers and submits a discussion on the influence of the membrane properties on the enzyme activity.

EXPERIMENTAL PROCEDURES

Enzyme and membranes. Lipase Type VII from *Candida cylindracea*, 690 U/mg powder was purchased from Sigma Chemical Co. (St. Louis, MO). Polytetrafluoroethylene (PTFE) membranes were prepared at The Technical University of Szczecin (5). Polyethylene (PE) (7) and collagen (COL) (8) membranes were prepared at The Technical University of Wrocław. Cellulose acetate (CA) membranes were prepared in our laboratory according to the procedure described previously (9) by adding 0.1% of glycerol (w/w) to the casting solution. Poly(vinyl chloride) (PVC) membranes were also made in our laboratory according to the method of Smith and Playne (10) by adding diethylenetriamine to the casting solution.

Immobilization of lipase. A solution of lipase (1 mg/mL, 95 mL) in tris-HCl buffer, pH 8, was ultrafiltered through the membrane, and then the enzyme that was adsorbed on the membrane surface was cross-linked with glutaraldehyde. A solution of 8% glutaraldehyde in tris-HCl buffer was ultrafiltered through the membrane and the excess of the enzyme and glutaraldehyde was washed out with water. The amount of enzymatic protein adsorbed on the membrane surface was calculated from the difference between the concentrations before and after the enzyme immobilization.

Activity measurements. The experiments were performed in a diffusion cell where oil and water phases were separated by the membrane under investigation. The detailed description of the apparatus had been reported (6). The membrane area was 19.63 cm^2, and the volume of each compartment was 125 cm^3. The activity of lipase immobilized on membranes was measured under conditions where the water phase was buffered with tris-HCl at pH 8, the oil phase was sunflower oil, and the temperature was 37°C. The contents of two compartments were removed after 24 hr of contact with the enzymatic membrane, and determination of free fatty acids was done in both.

Contact angle measurements. The wetting characteristics of the membrane studied were accomplished by contact angle measurements using the sessile drop method (11). The test consists of depositing a drop of liquid on the freshly cleaned, or freshly wetted, membrane surface and calculating the contact angle from the microscopic measurements of the drop image height and length. The determination was performed within 1–2 seconds after depositing the drop, and each measurement was repeated thirty times to give a mean value.

Porosity measurements. Porosity was evaluated using olive oil as a medium to penetrate the membrane. The membrane was immersed for 24 hr in olive oil, and then hung for 24 hr to allow the excess oil to run off. The weight of the membrane, dried and wetted, was determined, as was the membrane's thickness, and the porosity was calculated as percent of membrane total volume.

Analysis. The amount of free fatty acids (FFA) liberated by immobilized lipase was measured in oil and water phases by the method of Kwon and Rhee (12), using linoleic acid as a standard. FFA were extracted from the water phase with ethyl ether. One unit of lipase activity was defined as the amount of enzyme that liberated 1 μmol of FFA per min under standard conditions. The protein concentration was determined using the method of Lowry *et al.* (13).

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RESULTS AND DISCUSSION

The membranes used for immobilization of lipase differed considerably in their wetting properties as measured for both water and oil, as well as in porosity (Table 1). All of the membranes studied showed a better wettability with oil than with water. Thus, we used olive oil to determine their porosity. Porosity was calculated according to the following equation:

$$P = \frac{m_1 - m_2}{0.85\ \Pi r^2 h}$$

where r was membrane radius; h, membrane thickness; m_1, weight of membrane impregnated with olive oil; m_2, weight of dry membrane; and 0.85, specific gravity of olive oil.

TABLE 1

Membrane Characteristics

Membrane material	Contact angle		Oil sorption	Porosity
	Oil/membrane	Water/membrane		
	θ_{MO}	θ_{MW}	g/m²	%
PTFE	51	91	31.6	92.9
PE	29	65	0.2	0.3
COL	19	45	3.1	54.4
CA	15	50	27.8	40.8
PVC	39	67	5.3	18.0

The results obtained for immobilization of the lipase on these membranes are summarized in Table 2. The method of immobilization applied in this work produced the enzyme membranes with a lipase deposit on one of the flat membrane surfaces. The activity measurements for each membrane type were done when the enzyme was in direct contact with water or oil phases.

TABLE 2

Immobilization of Lipase on Various Membranes

Membrane material	Adsorbed protein	Activity when enzyme is on			
		water side		oil side	
	mg/m²	U/m²	U/mg	U/m²	U/mg
PTFE	24	288	12	428	17
PE	24	263	11	326	13
COL	253	280	1	290	1
CA	26	266	10	374	14
PVC	104	270	3	312	3

The amount of enzyme protein adsorbed on various membrane types differed considerably, although this was not reflected in the observed lipase activities expressed as the membrane area activity. Adsorption of the enzymatic protein alters its catalytic ability; the calculated values of specific activity ranged between 1 U/mg and 17 U/mg. Clearly, the membrane material can have a direct effect on the enzymatic activity. this could be due to an influence on an enzyme molecule, or it could be an effect of reactant transport between the phases. Kimura *et al.* (14) found that hydrophobic gels were better carriers for lipases than were hydrophilic gels. It was found that the water contact angle is a significant measure of cell hydrophobicity, and that the contact angle has a well-defined physical meaning in terms of the thermodynamics of interfacial processes (15,16). Hydrophobicity of the membranes under investigation has been evaluated by measuring the contact angle of water and oil (Table 1) and calculating the work of adhesion between water or oil and the membrane surface (Table 3). The work of adhesion was calculated from the contact angle values on each membrane type, from the following equation (17):

$$W_\sigma = (1 + \cos\theta)$$

where σ is the surface tension of the liquid and θ is the contact angle. The surface tension values were (18): Water, 72.58 mN/m; oil, 37.30 mN/m; and water-oil interface, 15.60 mN/m. No relationship could be found between lipase activity and parameters of membrane hydrophobicity.

TABLE 3

Work of Adhesion for Water and Oil

Membrane material	WA for water mJ/m²	WA for oil mJ/m²
PTFE	71.1	60.8
PE	103.1	69.8
COL	124.1	72.2
CA	119.1	73.5
PVC	100.2	66.4

In the three-phase system, water/oil/membrane, we introduced another parameter, Bartell's adhesion constant, k, calculated from the following equation:

$$k = \frac{\sigma_w \cos\theta_{mw} - \sigma_o \cos\theta_{mo}}{\sigma_{wo}}$$

where σ_W and σ_O are surface tensions and θ_{MW} and θ_{MO} are contact angles for water and oil, respectively.

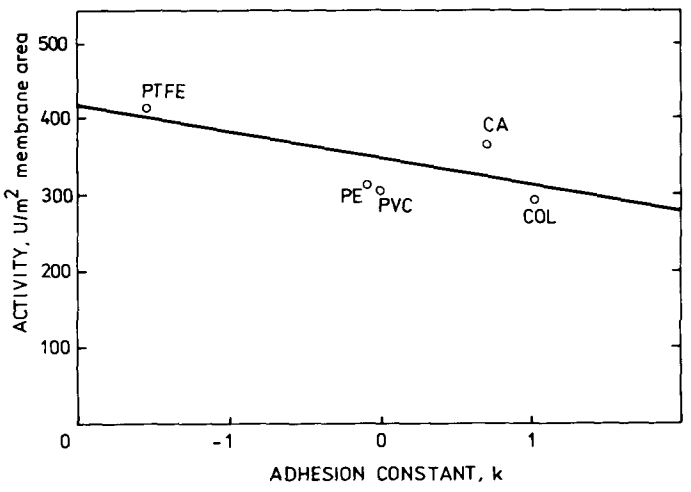

FIG. 1. Dependence of lipase activity on the adhesion constant.

A plot of the activity vs the calculated values of k is shown in Figure 1. In the range between −1 and +1, k has precise physical meaning, and it is equal to the cos

θ_{ow}. The correlation coefficient was found to be –0.74. The adhesion constant, k, is proportional to the difference of the work of adhesion for the water/membrane and oil/membrane systems, the latter reflecting the activity data, while the adhesion work for water or oil (done separately), did not. For oil hydrolysis to occur on the membrane surface, accessibility of the two liquid phases is important, and the lower the difference of the work of adhesion between the water and oil, the greater the activity of the immobilized lipase.

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