Utilization of Rice Hull Ash as a Support Material for Immobilization of *Candida cylindracea* Lipase

Jitawan Tantrakulsiri, Narumon Jeyashoke and Kanit Krisanangkura*

School of Bioresources and Technology, King Mongkut's Institute of Technology Thonburi, Bangkok, Thailand

ABSTRACT: Rice hull ash was heated in a muffle furnace at 700°C for 2 h and metallic oxides were leached with 10% sulfuric acid. The acid-activated ash was then examined for immobilization of *Candida cylindracea* lipase. Immobilization was carried out by direct addition of the enzyme solution to the activated ash suspended in hexane. The immobilized lipase retained 30% of its hydrolytic activity, but thermal stability was greatly increased. Half-lives of the immobilized enzyme at 50, 60, and 70°C were 45, 17, and 4 min, respectively. Optimal pH of the immobilized enzyme was 7.2. The apparent K_m and V_{max} for olive oil were 41 mM and 99.5 μmol/h-mg solid, respectively.

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KEY WORDS: *Candida cylindracea* lipase, enzyme immobilization, enzyme in organic solvent, kinetic properties, rice hull ash, thermal stability.

The lipase (EC 3.1.1.3) from the microorganism *Candida cylindracea* is a positional nonspecific enzyme (1) with great potential for industrial production of fatty acids and glycerol (2). Fifteen units of lipase per milliequivalent of oil can hydrolyze 95–98% of tallow, coconut oil, and olive oil in 72 h (2). The enzyme has been shown to discriminate against polyunsaturated fatty acids. Thus, it has been used to concentrate γ-linolenic acid of evening primrose oil and borage oil (3) and docosahexaenoic acid of fish oil (4). Also, *C. cylindracea* lipase works well in many organic solvents (5–8). When an enzyme is used in an organic solvent, it is beneficial to deposit the enzyme on a porous support so that it is spread over a large surface area. The type of support material has much influence on the activity of the immobilized enzyme, and this has been reviewed by Malcata *et al.* (9). Also, Haas *et al.* (7) have reported that Amberlite XAD-7 is a better support than Celite 545, polypropylene powder, and controlled-pore glass for *C. cylindracea* lipase.

In this study, we report that rice hull ash (RHA) may be developed for use as a support material for immobilization of *C. cylindracea* lipase. Some cottage industries in Thailand normally burn rice hulls for energy. The ash mainly consists of silicates (10). X-ray diffraction shows that it is an amorphous silica in the form of Opal CT (11). Furthermore, the infrared spectrum of rice hull silica is similar in many respects to that of silicic acid (12). Thus it has been evaluated as an alternative adsorbent in vegetable oil industries (11–13). However, attempts to use RHA as a porous support for immobilization of lipase have not been reported.

EXPERIMENTAL PROCEDURES

Preparation of porous RHA. Rice hull was donated (Samaki Rice Mill, Ayutthaya,Thailand) and burnt to ash in open air. The partially combusted ash was sieved and the particle sizes between 63–106 μm were collected. Activated RHA was prepared from this partially combusted ash as described by Proctor and Palaniappan (10) with some modifications. The muffle furnace (Model 818, Eurotherm, Darington, England) temperature was maintained at 700°C for 2 h, and metallic oxides were leached with 10% sulfuric acid for 5 h.

Immobilization of enzyme. Lipase from *C. cylindracea,* Type VII (700–1500 units/mg solid), was purchased from Sigma Chemical Co. (St. Louis, MO). Immobilization of lipase on RHA was carried out in an ice bath. Three milligrams of the enzyme were dissolved in 1 mL of 0.1 M phosphate buffer. Fifty μL of the enzyme solution were taken up with a 100-μL syringe and added slowly to the magnetically stirred suspension of 1.0 gram RHA in 5 mL of water-saturated hexane over a period of 1 min. The tip of the syringe needle was immersed into the suspension (slightly above the magnetic bar) so that the lipase would be adsorbed immediately by the RHA.

Hydrolytic activity of lipase. Olive oil (Sigma Chemical Co.), 0.2 mL, was added to the immobilized enzyme suspension, and the mixture was magnetically stirred at 37°C for 30 min. The enzyme-liberated free fatty acid was determined spectrophotometrically as described by Kwon and Rhee (14).

RESULTS AND DISCUSSION

Effect of pH. Linfield *et al.* (2) have reported that the pH optimum of unimmobilized *C. cylindracea* lipase is between 6.0–6.5, but results in Figure 1 show that the pH optimum of RHA-immobilized lipase is 7.2, which is slightly higher than the free enzyme. The shift in pH optimum to a higher value by a negatively charged support is not unexpected (15,16). However, the optimal pH of *C. cylindracea* lipase was not changed when it was immobilized on silica gel (17).

*To whom correspondence should be addressed.

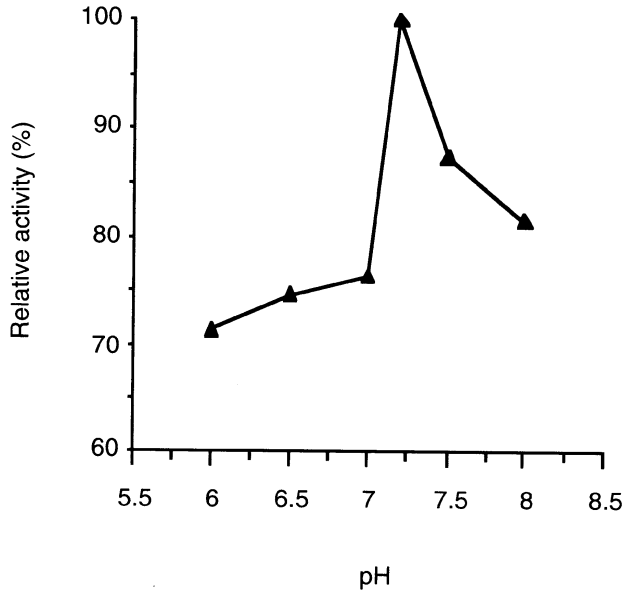

FIG. 1. The effect of pH on immobilized *Candida cylindracea* lipase activity.

Effect of water content. For hydrolytic activity, water is required not only for structural integrity of the enzyme but also as a substrate. In a study of the effect of water, additional buffer was added to the immobilized enzyme. Total water (water used for solubilizing the enzyme as well as added after immobilization) was then plotted against the hydrolytic activity. Figure 2 shows that 100 µL water was required for maximum activity of the enzyme. The water content represented approximately a 14-fold molar excess for complete hydrolysis of the olive oil. Hirata and Higuchi (5) and Reslow *et al.* (18) have reported that the amount of water required for optimal activity is a function of the amount of enzyme, polarity of the solvent, solid support, and type of reaction. For transesterification of tributyrin and octanol, catalyzed by *C. cylindracea* in *n*-hexane, 0.53% (vol/vol) of water was required for the optimal rate at 20 mg lipase powder/mL substrate solution. This is equivalent to 53 µL water/5 mL hexane or about half of the amount in this study. The difference is probably due to the type of reactions. Esterification releases water to the environment, and lower amounts of water would favor ester formation.

Thermal stability. Thermal stability of the immobilized lipase was studied in hexane suspension in tightly capped tubes, and the tubes were incubated at predetermined temperatures. The free enzyme, suspended in 0.1 M phosphate buffer pH 7.2, was tested in parallel. At the end of the incubation time, the enzyme mixture was cooled and residual lipase activity was measured at 37°C by addition of 0.2 mL olive oil. Figure 3 shows that hydrolytic activity of the immobilized lipase was reduced. The residual activity was about 30% of free enzyme. The hydrolytic activity was comparable to that immobilized on Kieselkuhr and alumina but slightly higher than those immobilized on silica gel and celite (19). However, the immobilized lipase was much less active than that immobilized on Accrurel (high-density polyethylene and polypropylene) (19). The greater rigidity of RHA would allow it to be operated at higher pressure. Also, RHA is widely available and almost costless. Its surface can be modified to different degrees of polarity, which can probably improve its quality as a support material (9,18). Thermal stability of the immobilized enzyme was greatly improved. Half-lives of the free enzyme at 50, 60, and 70°C were

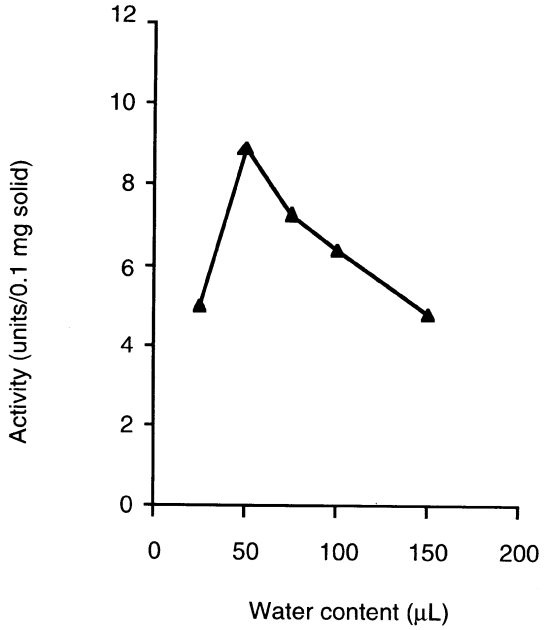

FIG. 2. The effect of water content on immobilized *Candida cylindracea* lipase activity.

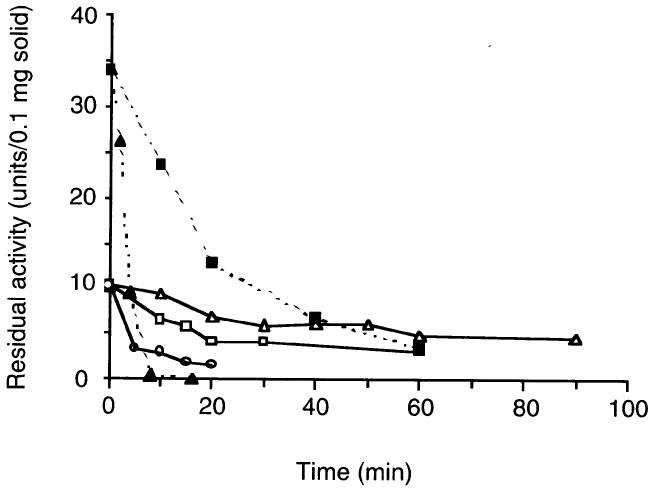

FIG. 3. Thermal stability of immobilized *Candida cylindracea* lipase activity. ■------■, free enzyme at 50°C; ▲------▲, free enzyme at 60°C; △------△, immobilized enzyme at 50°C; □------□, immobilized enzyme at 60°C; ○------○, immobilized enzyme at 70°C.

16, 3, and <1 min, respectively (the 70°C line is not shown), whereas half-lives of the immobilized enzyme increased to 45, 17, and 4 min, respectively. However, thermal stability of the immobilized enzyme is normally improved (20).

Kinetic properties. The Lineweaver-Burk double reciprocal plot of reaction rate (v) and substrate concentration [S] yielded a straight line whose x and y intercepts were –24.4 mL/g and 6.7×10^{-2} h/μmol (Fig. 4). These correspond to the K_m and V_{max} of 41 mM and 99.5 μmol/h-mg solid (for olive oil), respectively. The correlation coefficient was 0.996. The apparent K_m value was in good agreement with that reported by Tahoun (50 mM) for polyacrylamide-entrapped lipase (21). On the contrary, the apparent K_m of covalently bonded enzyme (bound to agarose) is only 20 mM (21). However, the V_{max} of this enzyme has not been reported.

The results of these preliminary studies indicate that RHA might be used as a porous support for *C. cylindracea* lipase. Immobilization of lipase can be carried out simply by direct deposition of the enzyme solution onto RHA suspended in an organic solvent. Although enzymic activity retained in the immobilized lipase (30%) was not impressive when it was compared to those in organic polymers, it was comparable to many inorganic supporting materials (19). Proper treatment of RHA would undoubtedly improve its quality for use as supporting material for enzyme immobilization.

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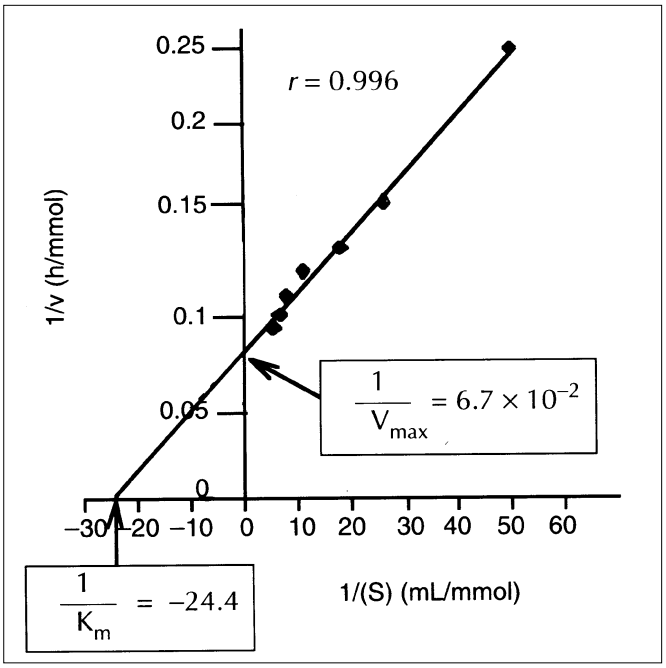

FIG. 4. Lineweaver-Burk plot of immobilized *Candida cylindracea* lipiase.

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