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Choice of Temperature for Safflower Oil Hydrolysis Catalyzed by *Candida rugosa* Lipase

A. B. Maidina, A. B. Belova, A. V. Levashov, and N. L. Klyachko

Chemical Enzymology Department

e-mail: klyachko@enzyme.chem.msu.ru

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Abstract—This work studies safflower oil hydrolysis catalyzed by *Candida rugosa* lipase as a function of temperature in an oil-in-water emulsion stabilized by the surfactant sodium deoxycholate. The choice of temperature for this reaction is dictated by the effects of temperature not only on the catalytic activity and stability of the enzyme but also on the state of the reaction medium (emulsion), whose quality substantially affects both the kinetic parameters of lipase and the product (linoleic acid) yield. For example, although the highest initial rate of the enzymatic reaction is observed at 40°C and the enzyme is virtually not inactivated during incubation (45°C), the highest reaction yield is observed at 30°C and decreases upon temperature elevation because of a change in the emulsion quality.

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Lipase (triglyceride hydrolase, EC 3.1.1.3)—an enzyme catalyzing triglyceride hydrolysis to monoglycerides and fatty acids—is contained in animals [1], plants [2], and microorganisms [3–5]. Lipase is a unique biocatalyst with a wide substrate specificity. Now it is widely used in organic synthesis [6, 7], medicine [8–10], and the paper and pulp industry [12] (see also reviews in [13, 14]).

Lipase substrates are water-insoluble triglycerides, whereas the enzyme is well soluble in water; in other words, reactions should occur at interfaces. The major specific feature of many lipases is their ability to surface activation in the presence of aggregated substrate molecules (oil droplets) [15–17].

Vegetable oils are known to differ in their fatty acid compositions. Safflower oil is unique; it contains up to 80% valuable polyunsaturated linoleic acid (*cis*-,*cis*-9,12-octadecadienoic acid) [18]. Enzymatic synthesis of free fatty acids from triglycerides is a very promising process. Enzymatic processes are attractive for the following reasons: (i) double bonds of unsaturated fatty acids are not oxidized, and (ii) lipase-catalyzed processes do not negatively affect the environment [19].

Apart from the ordinary parameters of enzymatic reactions, the properties of the reaction medium, in particular, the emulsion quality, are important for lipases. Here, we studied in the pH-stat mode the parameters of lipase-catalyzed enzymatic hydrolysis of safflower oil in an oil-in-water emulsion stabilized by the surfactant sodium deoxycholate at various temperatures. In so doing, our goal was to choose the optimum temperature for the reaction rate and product yield. The temperature effect was studied on both the enzyme and the reaction medium, namely, the emulsion.

EXPERIMENTAL

Materials and methods. Commercially available *Alcaligenes*, *Burkholderia*, and *Candida rugosa* lipases (EC 3.1.1.3) purchased from Sigma were used. Safflower (*Carthamus tinctori*) oil produced in the Peoples Republic of China was used as the substrate. Sodium deoxycholate, sodium chloride, and sodium hydroxide (high purity grade) were purchased from DIA-M.

The acid evolved during hydrolysis was titrated with 25 mM sodium hydroxide in an automated mode using a pH-stat (Radiometer TTT80/ABU80) with a temperature-controlled cell.

Ultrasonication in an ultrasonic bath (Bransonic 1510E-MTH, 150 W, 50–60 Hz) was used to additionally stabilize an oil-in-water emulsion.

Preparation of a stable emulsion. To 5 or 10 mL of 20–50 mM NaCl solution in water (pH 7.5–9.5), added were 50–300 μL of a 20% stock solution of sodium deoxycholate (the required optimal surfactant concentration was determined in a special experiment from the enzyme activity versus surfactant concentration dependence) and 60–100 μL of safflower oil. The emulsion was ultrasonicated two times for 10 min each with careful stirring between.

Rate measurements and yield determinations for the enzymatic reaction. To the prepared emulsion, 100 μL of the 10-mg/mL stock enzyme solution was added (in the case of an insoluble immobilized lipase sample, the enzyme in a concentration of 2 mg/5 mL was added directly to the pH-stat cell), and the acid evolved was automatically titrated with sodium hydroxide at the fixed pH optimal for each lipase.

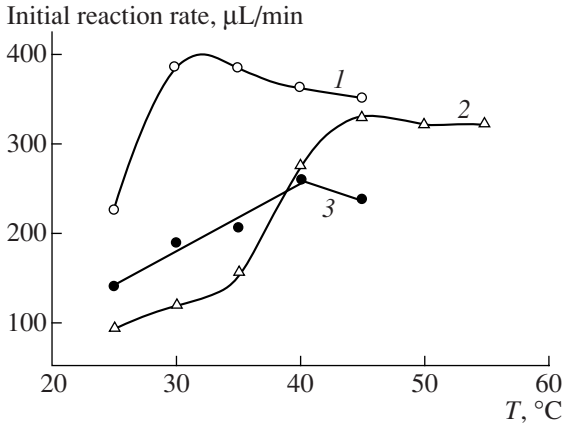

Fig. 1. Initial reaction rate vs. temperature for safflower oil hydrolysis catalyzed by lipases from (*1*) *Alcaligenes*, (*2*) *Burkholderia*, and (*3*) *Candida rugosa*. Experiment parameters: enzyme concentration, mg/mL: (*1*, *3*) 1 and (*3*) 2; sodium deoxycholate concentration: 1.2%; pH 9.5; and 50 mM NaCl.

Activity and stability of the enzyme and the product yield as functions of temperature. To determine the rate of the enzymatic reaction as a function of temperature, measurements were carried out at the required temperature in the pH-stat cell. To determine the stability (residual activity) of the enzyme, it was incubated at 20–55°C. Aliquots were sampled in certain time intervals, lipase was added, and the amount of acid evolved was measured in the pH-stat under standard conditions at 30°C. The stability of the emulsion and the effect of its quality on the enzyme activity were determined at 30 and 45°C by means of incubating the emulsion at these temperatures. Aliquots were sampled in certain time intervals, lipase was added, and the amount of acid evolved was measured in the pH-stat under standard conditions at 30°C.

RESULTS AND DISCUSSION

Temperature is known to influence the rate of both chemical and enzymatic reactions. Figure 1 shows the initial rates of safflower oil hydrolysis catalyzed by various lipases. In all cases, a rise in the reaction temperature increases the reaction rate until a certain limit is reached, which can be followed by a decrease in the rate, as shown for *Alcaligenes* and *Candida rugosa* lipases. For *Burkholderia* lipase, there is a seeming temperature independence of the reaction rate. Various processes (enzyme inactivation, transition to the diffusion-controlled regime, and others) can be responsible for this trend. Arise in temperature does not considerably affect the linolic acid yield in safflower oil hydrolysis. *Candida rugosa* lipase is the exclusion (Fig. 2). Along with the decrease in the reaction rate above 30°C (Fig. 2a), the product yield decreases considerably (Fig. 2b). For example, the yield of safflower oil hydrolysis catalyzed by *Candida rugosa* lipase at 45°C less than one half the yield observed at 30°C (Fig. 2b). Recall that the existence of an interface is crucial for the functioning of lipase [15–17]. In our case, the interface is stabilized by the surfactant sodium deoxycholate. What is responsible for such a considerable decrease in yield, enzyme inactivation or emulsion quality? To answer this question, we incubated both the enzyme and the emulsion at 45°C for 45 min, the reaction time until complete exhaustion occurred at which the product amount was determined (Fig. 2b). Figure 3 displays the results. The preincubation of the enzyme (*1*) virtually did not change either the initial rate of safflower oil hydrolysis catalyzed by *Candida rugosa* lipase (Fig. 3a) or the reaction yield (Fig. 3b). At the same time, the preincubation of the emulsion (*2*) affected both the initial reaction rate (Fig. 3a) and the reaction yield (Fig. 3b); both parameters of the process catalyzed by *Candida rugosa* lipase decreased considerably.

To summarize, the choice of the reaction temperature is of fundamental importance for *Candida rugosa*

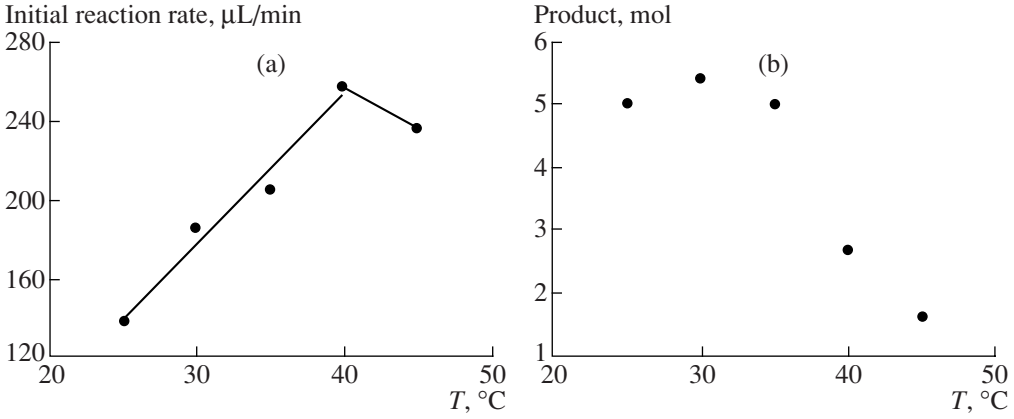

Fig. 2. (a) Initial reaction rate and (b) product concentration vs. temperature for safflower oil hydrolysis catalyzed by *Candida rugosa* lipase. For the experiment parameters, see the legend to Fig. 1.

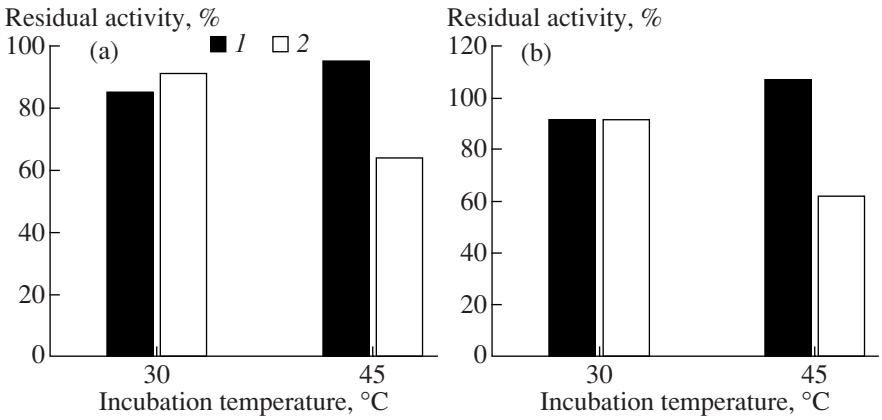

Fig. 3. (a) Residual enzyme activity and (b) product yield for safflower oil hydrolysis catalyzed by *Candida rugosa* lipase measured under standard conditions at 30°C after (*1*) enzyme or (2) emulsion was incubated at 30 and 45°C for 45 min. For the experiment parameters, see the legend to Fig. 1.

lipase not only as a factor influencing the enzyme activity and stability but also as a factor influencing the state of the reaction medium and/or interface.

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