Chemistry of Natural Compounds, Vol. 31, No. 3, 1995

PROPERTIES OF TWO LIPASES FROM THE FUNGUS *Mucor miehei*

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UDC 577.153.2

The influence of the temperature and the pH on the activity and stability of two lipases (A and B) from the fungus Mucor miehei *has been studied. It has been shown that the temperature and the pH optimum are between 55 and 45°C and 8.7-8.8 and 8.2-8.3, respectively, for A and B. The study of the thermal and pH stabilities of the enzymes has shown that lipase A is stable at 50°C for 5 h, while lipase B loses its activity completely under these conditions. Both lipases are stable between pH 5.0 and 8.5. Both enzymes actively hydrolyze various natural oils and exhibit position specificity with respect to α-ester bonds.*

At the present time, microbial lipases are being widely used in many sectors of the national economy [1-3]. The use of lipases in industry is due to such properties as substrate specificity, stability, pH and temperature dependence, and a capacity for catalyzing synthetic and transesterification reactions of esters in organic solvents and in supercritical liquid media. In view of this, interest is rising in the lipases isolated from these sources. Thus, the properties of the majority of lipases isolated and purified from *Rhizopus, Rhizomucor, Geotrichum, Aspergillus, and Penicillium* have been studied [4-6].

We have previously isolated and purified two lipases (A and B) from the fungus *Mucor miehei*. In the present paper we give the results of a study of the properties of the purified enzymes.

It is known that the temperature has a substantial influence on the activity of an enzyme — influencing, on the one hand, the rate of enzymatic reactions and, on the other hand, the stability of the enzyme. In order to establish the temperature optimum of the action of the lipases in the homogeneous state, hydrolysis of the substrate was carried out in the temperature interval from 20 to 90°C. We used 0.001% solutions of the enzymes. The optimum temperatures for the action of the enzymes were 55 and 45°C, respectively, for A and B. At a temperature of 60°C the rate of hydrolysis (particularly for the lipase B) fell sharply, and at 70°C there was no hydrolysis of the substrate by either of the enzymes.

The thermal stabilities of the two enzymes were determined in phosphate buffer solution, which was kept at various temperatures for 10, 20, and 30 min and from 1 to 6 h. After incubation for 6 hours at 40°C, enzyme A retained its initial activity completely, while enzyme B was 25-30% inactivated. At 50°C with an incubation time greater than 5 h, more than 60% of the activity of enzyme A was retained and more than 45% of that of enzyme B (Fig. 1). A further rise in the temperature led to falls in the activities of both lipases. As compared with other lipases of *Mucor* [7, 8] the lipase A that we had obtained from the fungus *Mucor miehei* was more resistant to high temperatures.

The pH of the extraction mixture is extremely important for the action of enzymes. The results of a study of the influence of the pH are given in Fig. 2. The lipases differed with respect to their optimum action pH values: 8.7-8.8 and 8.2-8.3, respectively, for A and B. Both enzymes were stable in the pH range from 5 to 7. At other values of the pH, lipase A lost more than 50% of its activity, while lipase B was relatively stable under these conditions.

It is known that surface-active agents (SAAs) exhibit dissimilar effects on the activity and stability of different lipases of microbial origin [9, 10]. We have studied the influence of such SAAs as Tweens and salts of bile acids on the activity of the lipases of *Mucor miehei* UzLT-3. The addition of all the types of Tween tested to the reaction mixture in a concentration of 0.001-0.0002% had no appreciable influence on the activities of the two enzymes but when the concentration of Tweens-20, -60, and -80 was raised the activity of the enzymes fell considerably, and it disappeared completely at a concentration of 0.01%.

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Translated from Khimiya Prirodnykh Soedinenii, No. 3, pp. 449-453, May-June, 1995. Original article submitted August 15, 1994.

0009-3130/95/3103-0372$12.50

TABLE 1. Substrate Specificities of Lipases A and B

Substrates	Activity in relation to olive oil, %	
	A	B
Olive oil	100	100
Tributyrin	125	107
Cottonseed oil	113	98
Soybean oil	98	86
Sunflowerseed oil	108	100
Sesame oil	77	53

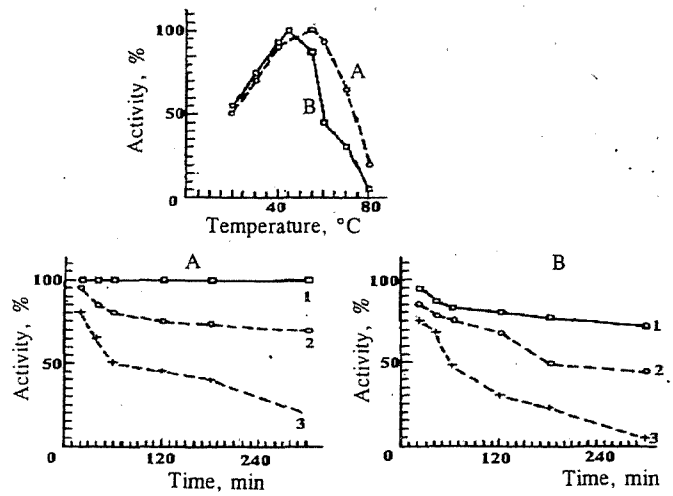

Fig. 1. Influence of the temperature on the activity and stability of the two lipases: *1*) 40°C; *2*) 50°C; and *3*) 60°C.

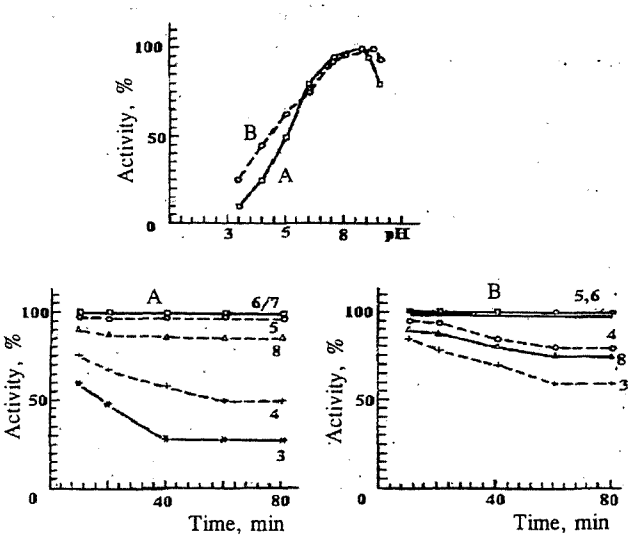

Fig. 2. Influence on the pH of the activity and stability of the two lipases.

The Tweens — esters of fatty acids with polyhydric alcohols — apparently affect lipase activity in the manner of competitive inhibition by substrate analogues.

On studying the influence of certain salts of bile acids on the activity of the enzymes, we established that they were resistant to the action of high concentrations of the substances investigated, and also to medicinal bile, the active principle of

TABLE 2. Hydrolysis of Synthetic Substrates by Lipases A and B

Substrate and fatty-acid composition	Lipase activity, mole-%	
	A	B
1,3-Dioleoyl-2-palmitoyl-glycerol (18:1–16:0–18:1)		
Palmitic acid (16:0)	11.7	17.4
Oleic acid (18:1)	88.3	82.6
1,3-Dipalmitoyl-2-oleoyl-glycerol (16:0–18:1–16:0)		
Palmitic acid (16:0)	89.1	92.3
Oleic acid (18:1)	10.9	7.7

which consists of bile acids. The inertness of the lipases to salts of bile acids and also to medicinal bile enables them to be recommended for use in medical practice as a healing or prophylactic drug.

Results on the substrate specificities of the individual lipases are given in Table 1. All the substrates used were intensively hydrolyzed by both lipases. Tributyrin and cottonseed and sunflowerseed oils were hydrolyzed very rapidly, but the hydrolysis of cottonseed oil by lipase B was slower than that of olive oil. Soybean oil was also hydrolyzed better by lipase A, the sesame oil was 77 and 53% hydrolyzed in comparison with olive oil by lipases A and B, respectively.

The results of the hydrolysis of such synthetic substrates as 1,3-dioleoyl-2-palmitate and 1,3-dipalmitoyl-2-oleate showed that in both cases the lipase liberated a large amount of fatty acids (82.6-92.3%) in the first position, which exceeds the indices of lipolysis for a nonspecific lipase [11] (Table 2). It must also be mentioned that an unsaturated fatty acid (18:1) was liberated more intensitively than a saturated one (16:0), particularly on hydrolysis by lipase B.

EXPERIMENTAL

In our study of the influence of the temperature on lipase activity, we used a reaction mixture with the following composition: 4.5 ml of phosphate buffer, pH 8.7; 2.5 ml of a 40% emulsion of olive oil in a 2% solution of polyvinyl alcohol; and 1 ml of enzyme solution. To determine their thermal stabilities, the proteins were dissolved in 20 mM phosphate buffer, and incubation was carried out at 40, 50, and 60°C. After predetermined intervals of time, the residual lipase activity was determined in aliquots. As the lipase activity we took the amount of enzyme liberating 1 μmole of oleic acid after hydrolysis for 1 h. The amount of oleic acid was established titrimetrically (titration was performed with a 50 mM solution of KOH using phenolphthalein as indicator).

The influence of the pH on the activities and stabilities of the lipases was studied with the use of phosphate-citrate buffers having pH values of from 3 to 9. The pH stabilities of the enzymes were tested by keeping them in buffer solutions with the given pH values for different times. Activities were determined in a reaction mixture with the composition given above. Incubation was carried out at 55°C for 1 h.

In a study of the influence of surface-active agents (SAAs) on lipase activity we used such SAAs as Tweens-20, -60, and -80 and salts of bile acids — sodium taurocholate and sodium deoxycholate — and also medicinal bile. Various concentrations of the Tweens (0.001-0.01%) and of the bile acid salts (0.005-0.02%) were added to the reaction medium. The lipase activities of the enzymes were determined after incubation at 55°C for 1 h.

Substrate Specificity. We also used such natural substrates as olive, cottonseed, soybean, sunflowerseed, and sesame oils, and also tributyrin. All the substrates were emulsified with 2% polyvinyl alcohol in a ratio of 1:1.5 and were added to the reaction medium in the same volume (2.5 ml). The lipase activities of the enzymes were calculated in relation to olive oil.

The position specificities of the individual lipases were determined with the use of synthetic triacylglycerols — 1,3-dioleoyl-2-palmitoylglycerol (18:1-16:0-18:1) and 1,3-dipalmitoyl-2-oleoylglycerol (16:0-18:1-16:0). Hydrolysis was conducted at pH 8.7 and 55°C. The amount of hydrolysis products was determined on a Hewlett-Packard 583OA gas–liquid chromatograph fitted with an ionizing detector. Steel column (0.3 × 180 cm) filled with 10 of PEGS, temperature of the sample-injection block 250°C, temperature of the column thermostat 300°C, rate of flow of carrier gas, helium, 18 ml/min.

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