A STUDY OF SOME PROPERTIES OF THE LIPASE FROM THE SEEDS OF *Nigella damascena*

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In a preceding communication [1], it was shown that the seeds of *Nigella damascena* L. (love-in-a-mist) family Ranunculaceae juss. contain a highly active alkaline lipase. The present paper gives the results of an investigation of some properties of the lipase isolated from the seeds of this plant.

The enzymatic activity of the *N. damascena* lipase increases in proportion to the time of incubation for a period of 90 min in the hydrolysis of olive and sunflower seed oils. According to the results of Yu. D. Gavrichenkova et al., [2], the maximum rate of lipolysis for the lipase from soya beans is observed at 60-80 min. In view of these facts, we studied the activity of the lipase of the seeds of *Nigella damascena* L. at 60 minutes' incubation.

For each enzyme, a pH zone in which it exhibits its maximum activity is characteristic. Thus, the lipase from the castor oil plant has its maximum activity at pH 4.7-5.0, that from the pancreatic gland at 8-9, that from wheat germ at 8-9 [3], and that from cotton seeds at 8-9.5 [4]. Some plants have more than one lipase and exhibit activities both in acid and in alkaline media [2, 4].

To determine the optimum pH at which the hydrolysis of olive oil by the lipase that we isolated takes place most vigorously, the activity of the enzyme was tested in the pH range from 2.2 to 11 (Fig. 1).

As can be seen from Fig. 1, the lipase of this plant is active at pH 6-10, with an optimum at pH 8. While exhibiting lipolytic action mainly under alkaline conditions, the lipase from the seeds of *N. damascena* is nevertheless extremely stable in the pH range from 3 to 12 with an exposure time of 30 minutes. When a solution of the enzyme was kept under these conditions for 60 and 120 min, the zone of 100% stability contracted to pH 7-10, while at a pH below 7 the activity of the lipase fell and at pH 3 it was lost completely.

In an investigation of the temperature optimum of the lipase from the seeds of *N. damascena* it was established that the lipolytic action of the enzyme is shown at temperatures from 10 to 70°C, with a temperature optimum at 37-40°C (Fig. 2). At 80°C the lipolytic activity of the preparation disappears completely.

For the lipase from wheat germ, the temperature optimum is at about 38°C [5], for that from soya beans 37-43°C [2], from milk 30-37°C [3], and from microorganisms ("seiken" lipase) 38-43°C [6].

The results of a study of temperature stability showed that the activity of the lipase of the plant under consideration depends on the time of exposure and the temperature. The preparation showed high stability at 40°C, retaining its initial activity for 2 h. Exposure at 50°C for 30 minutes decreased the lipolytic activity by 4%, at 60°C by 11%, at 70°C by 35%, and at 80°C the activity was lost completely. For the lipases isolated from other sources, likewise, considerable loss of activity at 70°C and complete loss at 80°C have been reported [7].

Under the conditions of the pH and temperature optima, the lipase from *N. damascena*

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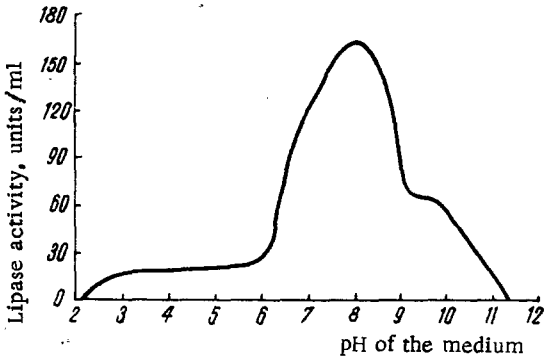

Fig. 1

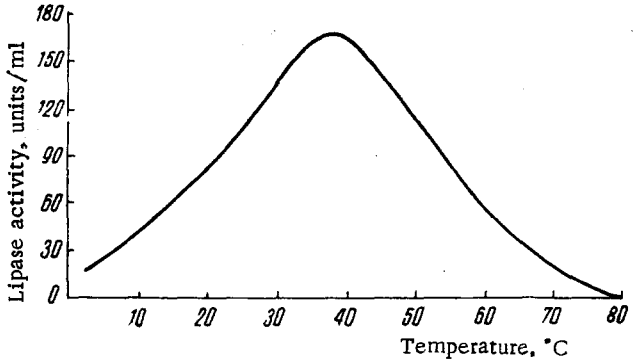

Fig. 2

Fig. 1. Influence of the pH of the medium on the activity of the lipase of the seeds of *N. damascena*.

Fig. 2. Influence of the temperature on the activity of the lipase from *N. damascena*.

seeds catalyzes the hydrolysis of triglycerides (tributyrin), and of emulsified fats and oils (Fig. 3) but has no effect on such monoesters as ethyl acetate, ethyl oleate, and isopropyl myristate, which are readily cleaved by the lipases isolated from the seeds of the cotton plant, poppy, flax, sunflower, wheat germ, animal tissue, and other sources [4, 5, 8-10].

The substrates best cleaved by the *N. damascena* lipase are tributyrin, and the oils of maize, sunflower, cotton, apricot, olive, and linseed, and also lard. The "seiken" lipase recommended by the Japanese firm "Nagase" as a medicinal agent has a better hydrolytic action on lard and on cotton seed, sunflower seed, and olive oils [6].

The specific action of the lipase from the seeds of *N. damascena* on various substrates was compared with that of pancreatin, which is a complex of several enzymes from the pancreatic glands of slaughtered cattle, and with the lipase from porcine pancreatic gland (see Fig. 3). The lipase we have isolated cleaves oils used in food (sunflowerseed, cottonseed, and maize oils) and also lard more actively that a medicinal preparation of pancreatin. With respect to the substrates lard and maize and linseed oils, its hydrolytic action is considerably higher than that of pancreatin and the lipase from porcine pancreatic gland.

It can be seen from Fig. 3 that the lipase from porcine pancreatic glands as a purified preparation is more active than pancreatin with respect to the majority of the substrates studied. However, on groundnut oil and lard it has a weaker action than pancreatin. This

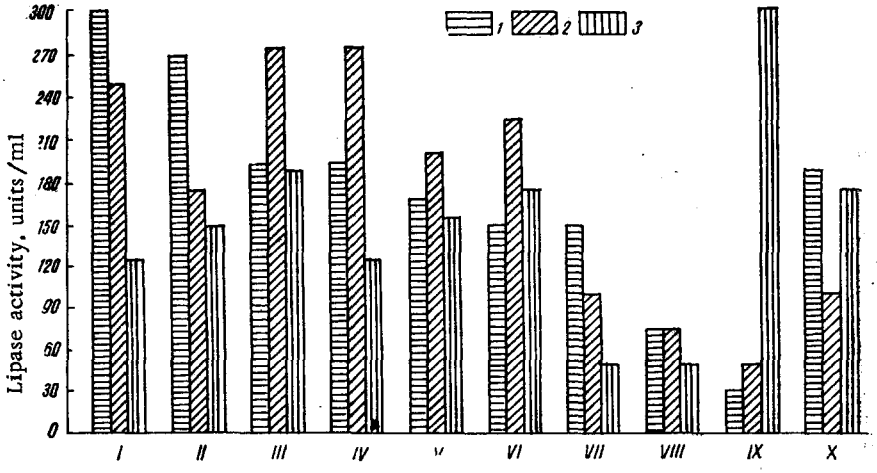

Fig. 3. Hydrolysis of some substrates by lipases of different origins: 1) lipase from the seeds of *N. damascena*; 2) lipase from porcine pancreatic gland, product of the Olaine factory; 3) pancreatin. I) Tributyrin; II) maize oil; III) sunflower-seed oil; IV) cottonseed; V) apricot oil; VI) olive oil; VII) linseed oil; VIII) castor oil; IX) groundnut oil; X) lard.

can be explained by the loss or destruction of some specific properties of factors on purification, but this requires a special study.

EXPERIMENTAL

Enzymatic Materials. The seeds of *Nigella damascena* L., 1972 crop, grown in the "Lozovskoi" [Soukloz state farm], Khar'kov oblast, were steeped in water and left to germinate at 37°C for a day. The seeds after having been pressed out and comminuted on rolls, were defatted with cold acetone (−10°C), and dried in a stream of air, and from the acetone powder so obtained the lipase was isolated by a 30-min extraction with 0.025 M ammonia in a ratio of 1:10. The investigations were performed with freeze-dried enzyme material.

Substrates. As substrates we used olive, maize, sunflowerseed, cottonseed, apricot, linseed, castor, and groundnut oils, lard, and tributyrin emulsified with 2% poly (vinyl alcohol) in a ratio of 2:3, and also ethyl oleate, ethyl acetate, and isopropyl myristate.

Determination of Lipase Activity. The lipase activity was determined titrimetrically [7]. As the unit of activity we took the number of micromoles of fatty acid formed from the substrate under the action of the lipase.

The heat stability of the enzyme was established by keeping a solution of the lipase at a predetermined temperature for 30, 60, and 120 min. Then the temperature of the solution was lowered to 37°C and the lipase activity was determined in accordance with the method given.

The pH stability of the enzyme was found by keeping a solution under predetermined pH conditions for 30, 60, and 120 min. Then the pH was brought to 8 and the lipase activity was determined by the prescribed method.

In the study of the influence of the pH of the medium and of the temperature on the activity of the preparation, and also its pH and temperature stabilities, olive oil was used as the substrate.

SUMMARY

1. A lipase isolated from the seeds of *Nigella damascena* L. showed a pH optimum of 8, a temperature optimum of 37-40°C, a pH stability with an exposure of 30 min in the pH range from 3 to 12 and with exposures of 60 and 120 min in the pH range from 7 to 10. Complete inactivation of the lipase takes place at 80°C.

2. The lipase hydrolyzes a number of plant oils and animal fats well, but has no action on fatty acid esters, ethyl oleate, ethyl acetate, and isopropyl myristate.

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