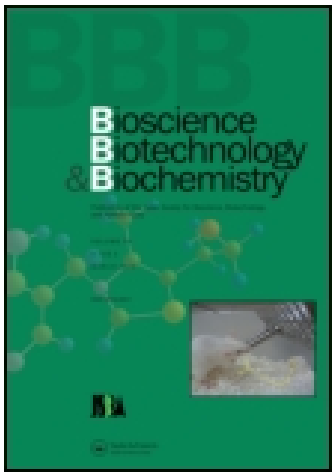

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Positional Specificity and Stereoselectivity of a Lipase Preparation from Oat Seeds Acting on 1,2,3-Trihexanoylglycerol

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Note

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Oat seed lipase was extracted with 0.01 M calcium chloride solution containing 0.5% Triton X-100 and precipitated with ammonium sulfate. The precipitate was dissolved in phosphate buffer at pH 6.0 and the supernatant was used as the lipase preparation. The lipase was very selective in the ester positions of 1,2,3-trihexanoylglycerol, hydrolyzing *sn*-3 most quickly, *sn*-1 moderately, and *sn*-2 hardly at all.

Key words: oat; lipase; positional specificity; stereoselectivity; triglyceride

Oat lipase is located in the outer pericarp layers of the groats and can be extracted with distilled water by suspending the groats in it.[1] Peers[1] reported that purified oat lipase hydrolyzed only one ester bond of 1,2,3-tributanoylglycerol (TBG), leaving a dibutanoylglycerol (DBG) as a reaction product, and that the DBG isolated from the reaction mixture was the 1,3-isomer, predicting that the lipase was completely specific for the 2-position of TBG. Recently, the positional specificity of oat seed lipase was reexamined.[2] The crude lipase solution prepared from the groats catalyzed the hydrolysis of both primary and secondary esters of long-chain fatty acid in triacylglycerol. Oat seeds were also used as a source of lipase to hydrolyze vegetable oils such as soybean oil[3] and rice bran oil.[4]

In this note, our attention was focused on the reexamination of the positional specificity of oat lipase for the 2-position, because such a lipase has not been reported otherwise, except that lipases from *Geotrichum candidum*,[5] *Geotrichum* sp.,[6] and *Candida antarctica*[7] showed a preference for the 2-position. As a result, the stereoselectivity for triacylglycerol of oat lipase has been found instead.

Hulled oat seeds obtained from a commercial source were pulverized with an autocrusher. The resultant meal was defatted with diethyl ether for 3 h using a Soxhlet extractor. Fifty grams of the defatted meal were stirred in 500 ml of 0.01 M calcium chloride solution containing 0.5% Triton X-100 at 4°C for 24 h to extract lipase, and centrifuged at 10,000 rpm for 30 min with cooling. To the supernatant, solid ammonium sulfate was added to give 60% saturation and the solution was stirred gently at 4°C for 6 h. The precipitate was collected by centrifugation, suspended in 10 mM phosphate buffer at pH 6.0, and dialyzed against the same buffer. The insoluble solid was removed by filtration with Hyflosupercel and the filtrate was used as the lipase preparation.

The activity of oat lipase was measured at 37°C by the polyvinyl alcohol method with TBG (pH 8.5) and olive oil (pH 7.4) as the substrate as described previously.[8] One lipase unit was defined as the amount of the enzyme that released 1 μmol of fatty acid from the substrate per min. The lipase amount extracted with the calcium chloride and surfactant solution was 26.2 and 21.2 units per g of the defatted meal, when measured with TBG and olive oil, respectively. The recovery of lipase was 66.8 and 59.0% after the purification, using TBG and olive oil as the substrate for the measurement, respectively.

The positional specificity and stereoselectivity of the lipase preparation was examined by high-pressure liquid chromatography (HPLC) using a chiral column containing (*R*)-(+)-1-(1-naphthyl)ethylamine polymeric phase.[9,10] The enzymatic hydrolysis of 1,2,3-trihexanoylglycerol (THG) was done at 37°C with stirring (1000 rpm) for 5 min in the mixture consisting of 5 mg of THG (99%, Sigma), 1 ml of Tris–HCl buffer at pH 8.5, and about 10 units of the lipase preparation, which was measured with TBG as the substrate, and stopped by adding 2 ml of diethyl ether. The isomers of dihexanoylglycerol (DHG) extracted in the ether layer was converted into their 3,5-dinitrophenylurethane (DNPU) derivatives. The derivatives were purified by TLC on silica gel and analyzed on a chiral column (25 cm × 4.6 mm i.d., YMC-Pack A-K03) at room temperature isocratically, using *n*-hexane–1,2-dichloroethane–ethanol (40 : 30 : 1 by volume) as the mobile phase at a flow rate of 0.5 ml/min.

THG was used in the study for two reasons: (1) dibutanoylglycerols of high purity (99%) could not be purchased as authentic compounds; (2) the oat lipase preparation had only a small activity for 1,2,3-trioleoylglycerol (TOG), so it was impossible to measure exactly its positional specificity and stereoselectivity.

Figure shows the results obtained from authentic DHG (99%, Sigma) and from the THG hydrolysate catalyzed by the oat lipase. Isomeric 1,3-DHG and enantiomeric *sn*-1,2- and *sn*-2,3-DHGs were eluted in that order (Fig. A). The DNPU derivative of the DHGs produced by the lipase was divided into two peaks (Fig. B); however, the former peak had likely a very small shoulder in the front side, which was not recognized as a peak by the integrator used. As judged by the retention times, the former peak is *sn*-1,2-DHG and the latter peak *sn*-2,3-DHG, although all peaks were eluted about 0.5 min faster in Fig. B.

The oat lipase is considered to be almost completely 1,3-specific, because the peak of 1,3-DHG hardly appeared on the HPLC chromatograms. PSI (positional specificity index) value was calculated as 100 by the following equation[11,12]:

$$\mathrm{PSI} = \frac{1,2\mathrm{DHG} + 2,3\mathrm{DHG} - 2 \times 1,3\mathrm{DHG}}{1,2\mathrm{DHG} + 2,3\mathrm{DHG} + 2 \times 1,3\mathrm{DHG}} \times 100$$

Furthermore, the lipase showed a very important property, the stereoselectivity for triacylglycerol, that is, preferred the *sn*-3 position of THG, producing mainly *sn*-1,2-DHG. The enantiomeric

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Abbreviations: DBG, dibutanoylglycerol; DHG, dihexanoylglycerol; DNPU, 3,5-dinitrophenylurethane; HPLC, high-pressure liquid chromatography; TBG, 1,2,3-tributanoylglycerol; THG, 1,2,3-trihexanoylglycerol; TLC, thin-layer chromatography; TOG, 1,2,3-trioleoylglycerol.

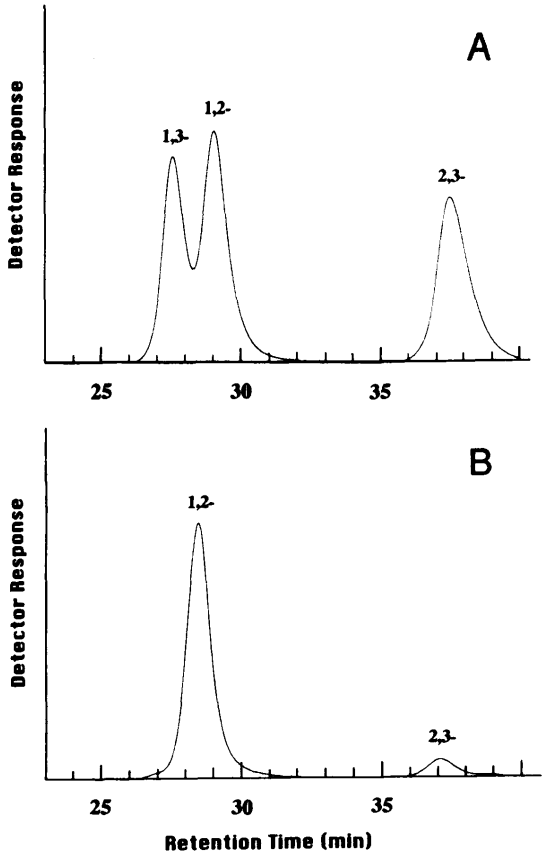

Fig. HPLC Chromatograms of Authentic Dihexanoylglycerol and the Dihexanoylglycerol Released from 1,2,3-Trihexanoylglycerol by the Catalysis of Oat lipase.

Dihexanoylglycerol was converted to the 3,5-dinitrophenylurethane derivative and analyzed by the chiral phase HPLC. Details are described in the text. Detector response means absorbance at 254 nm. A, authentic dihexanoylglycerol; B, oat lipase hydrolysate.

excess of *sn*-1,2-DHG (S) was estimated to be 80.6%.

These results are different from those reported by Piazza *et al.*[2] It is likely that the crude lipase preparation used by these authors contained different lipases with respect to positional specificity, while the preparation used for our study was as pure as to result in positional and stereoselectivity.

Rogalska *et al.*[7] identified the positional and stereoselectivity of 25 lipases of animal and microbial origin, using TOG and trioctanoylglycerol as the substrate. Most of them showed a preference for the *sn*-1 position, while *Fusarium solani* cutinase, and dog and rabbit gastric lipases had a preference for the *sn*-3 position. The oat lipase, as well as *Carica papaya* latex lipase,[13] is an example of the latter case in the plant world.

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