PRELIMINARY NOTES

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Castor bean lipase: Specificity of action

Hydrolysis of long chain triglycerides catalysed by castor bean lipase (glycerol-ester hydrolase, EC 3.1.1.3) is known to proceed to completion, the end products being fatty acids and glycerol. As to the question of the positional specificity of the enzyme, however, no agreement has been reached. SAVARY *et al.*[2] reported that castor bean lipase was not specific for the position in the glyceride molecule of the ester bond, while ORY *et al.*[3] obtained results indicating that the enzyme catalysed the hydrolysis of the fatty acids from the 1- and 3-position only. The appearance of fatty acids from the 2-position was a result of an isomerization of the 2-monoglyceride to 1-monoglyceride favoured by the acid pH of the incubation medium. The evidence for this latter conclusion, however, was indirect, based on the finding that castor bean lipase does not catalyse the hydrolyses of secondary esters such as 2,3-butanedioleate and 2-hexyl oleate[3].

These experiments, the preliminary results of which are reported here, were undertaken in an attempt to resolve the apparent discrepancies in results obtained up to now.

Castor bean lipase was prepared as described[3]. [^{3}H]Glycerol-labeled triolein and 1,3-di-*O*-dodecyl-glyceryl-2-[1-^{14}C]oleate were synthesised as described[4]. Racemic 1,2-diolein was a synthetic product[5]. In general, 128 μmoles labelled substrate (tri- or diolein) were added with 16 ml of a 0.015 M phosphate buffer, pH 6.8, and 16 mg of the castor bean lipase preparation. After sonication at 20 000 kcycles for 1 min to obtain a homogeneous emulsion, the reaction was started by the addition of 16 ml of 0.2 M sodium acetate buffer, pH 4.0. 4-ml samples were withdrawn after appropriate times of reaction and rapidly mixed with 12 ml of equal proportions of ethanol, diethylether and heptane[4]. The upper phase was removed and the lower phase re-extracted with two additional preequilibrated upper phases. The lipids contained in the upper phase were separated on thin-layer chromatography using silicic acid, the appropriate spots visualized by exposure to iodine and their radioactivity determined by liquid scintillation counting[4]. The free glycerol of the lower phase was also determined by radioactivity[4]. In the experiments in which 1,2-diolein was used as substrate, the thin-layer chromatographic plates after development were charred with a sulfuric–nitric acid reagent and the spots measured using a densitometer.

In agreement with previous experience[1] a rapid and complete hydrolysis of the triolein was obtained. Little di- and monoglyceride appeared in the reaction mixture and free glycerol appeared very early in the reaction. The rate of disappearance of triolein was about 2 times the appearance of free glycerol. The pH optimum for triolein disappearance and glycerol appearance was around 4.2 and the curves parallel, indicating that these two activities were catalysed by the same enzyme.

Separation on thin-layer chromatography indicated that 1,3-diglyceride was present in the reaction mixture, making up 30–40% of the total diglyceride fraction. 1,2-Diolein when used as substrate was rapidly hydrolysed by the enzyme with the

appearance of 1,3-diglyceride in the reaction mixture. 1,2-Diolein, carried through the whole procedure in the absence of enzyme, did not indicate any isomerization to 1,3-diolein. No attempts were undertaken to separate the monoglyceride fraction into its isomers.

Incubation of the 2-[1-^{14}C]oleoyl 1,3-diether in solution in triolein gave only insignificant amounts of free radioactive oleic acid in the reaction mixture ($<1\%$).

The appearance of the 1,3-diglyceride in the reaction mixture during the course of the hydrolysis of tri- as well as 1,2-diolein by castor bean lipase is a new finding. It is parallelled by the report by SEMERIVA *et al.*[6] that acyl migration occurrs during lipolysis in 1,2-diglycerides with the lipase from *Rhizopus arrhizus*.

The findings of the present experiment would indicate that the isomerization of 1,2-diolein is catalysed by castor bean lipase. Such a course of reaction would explain the earlier interpretation of the results obtained as to the specificity of the enzyme[3]. Castor bean lipase could be specific for the primary ester bonds of the triglyceride but still produce a rapid liberation of the secondary fatty acid after its isomerization to the 1,3-position. Attempts to separate the hydrolytic and isomerase activities so far have been unsuccessful.

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