Note

Synthesis of C₁₈-Fatty Acid Esters in Organic Solvent by Lipase from *Candida cylindracea*

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Although it is well known that lipases synthesize esters from fatty acids or amino acids in organic solvents,¹⁻⁷⁾ this reaction mechanism has yet to be clarified in many respects. Thus, we have done an experiment on the synthetic reaction of ester from fatty acids that had the same numbers of carbons, and sterols with a similar structure.

In our current experiment, we used four kinds of C₁₈-fatty acids (oleic acid, stearic acid, isostearic acid, and 12-hydroxystearic acid) as similar fatty acids and three kinds of sterols (ergosterol, stigmasterol, and cholesterol). Cyclohexane was selected after it was confirmed that cyclohexane was capable of dissolving all these substances.

Five mmoles of fatty acid, 5 mmol of sterol, and 10 μ l of 0.05 M phosphate buffer (pH 7.0) were added to 50 ml of cyclohexane. To the above reaction mixture, 0.4 g of powdered lipase MY (Meito Sangyo, Nagoya) was added and left to react, while being stirred, at 37°C for 72 hrs. After the reaction was over, 5 ml of a solution (a mixture of isopropyl alcohol, *n*-butyl alcohol, and 2 N sulfuric acid in a ratio of 40:10:1, v/v/v) was added to a reaction solution and well mixed.

To the above mixture, 3 ml of *n*-heptane and 2 ml of distilled water were added and strongly shaken before non-reacted, liberated fatty acids were transferred to the *n*-heptane layer. After this solution was allowed to stand, a solution thus obtained was titrated with 0.01 N potassium hydroxide using 1% thymol blue ethanol as an indicator. A solution for stopping the reaction was added beforehand to the substrate as a control and incubated for 72 hrs. The amount of ester synthesized was calculated in accordance with the amount of fatty acids consumed, and confirmed by thin-layer chromatography (TLC) compared with that of a control.

Figure 1 shows the results of esterization with C₁₈-fatty acids and sterols after 72 hrs, which suggested that C₁₈-fatty acids had strongly reacted with cholesterol. However,

Fig. 1. Products of Ester Synthesis by Lipase.

A, mixtures (oleic acid, cholesterol, lipase MY); B, cholesterol ester after 72 hr; C, mixtures (stearic acid, cholesterol, lipase MY); D, stearyl ester after 72 hr; E, mixtures (isostearic acid, cholesterol, lipase MY); F, isostearyl ester after 72 hr; G, mixtures (12-hydroxystearic acid, cholesterol, lipase MY); H, 12-hydroxystearyl ester after 72 hr; I, mixtures (ergosterol, oleic acid, lipase MY); J, ergosterol ester after 72 hr; K, mixtures (stigmasterol, oleic acid, lipase MY); L, stigmasterol ester after 72 hr. Solvent, benzene; 25% H₂SO₄ sprayed.

Fig. 2. Changes in Rate of Ester Synthesis by Lipase (Solvent, cyclohexane).

Conditions: fatty acid, 5 mmol; sterol, 5 mmol; lipase MY, 0.4 g; cyclohexane, 50 ml; phosphate buffer, 50 mmol (pH = 7.0) 10 μ l; temperature, 37°C.

▲—▲, cholesterol + stearic acid; △—△, cholesterol + isostearic acid; ●—●, cholesterol + oleic acid; ○—○, cholesterol + 12-hydroxystearic acid.

the degree of reaction with other sterols and C₁₈-fatty acids was traced. A big difference among the three kinds of sterols (namely, stigmasterol, ergosterol, and cholesterol) was the presence of a side chain of either a 24 β -CH₃ or 24 α -C₂H₅ group and that of double bonding of C₂₂- and C₂₃-positions. It is conceivable from this fact that the esterization with C₁₈-fatty acids is influenced by the side chains of the sterol.

We did an experiment on the passage of time to measure the degree of four kinds of ester reactions with 5 mmol of cholesterol. The results are shown in Fig. 2. The results

Fig. 3. Lineweaver-Burk Plot of Ester Synthesis by Lipase.

Reaction time; 3 hr (oleic acid 0.1 M).

indicate that all of fatty acids including stearic acid, oleic acid, isostearic acid, and 12-hydroxystearic acid have reacted with 5 mmol of cholesterol.

Oleic acid and stearic acid reached the maximum level of synthesis, the degree of esterization being as high as 80%, but it took 50 hrs for 12-hydroxystearic acid to reach the maximum level with the degree of 70%, and approximately 48 hrs for isostearic acid to reach the maximum level even though the degree was as low as 30%. It was considered from this that either the side chain or hydroxy group of fatty acids affected the synthesis. As for the

reaction of fatty acids with cholesterol, a single compound was synthesized by oleic acid, stearic acid, and isostearic acid, but these were two compounds from 12-hydroxystearic acid.

Although the results we have obtained have yet to be analyzed these compounds in detail, it is presumable that a lactone other than cholesteryl ester was synthesized.⁸⁾ The apparent K_m of the oleic acid against 0.1 M of cholesterol was 0.3 M (Fig. 3).

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