Purification of Ethyl Docosahexaenoate through Selective Alcoholysis with Immobilized Rhizomucor miehei Lipase

Kazuaki MARUYAMA*1, Yuji SHIMADA*2, Takashi Baba*1, Tomoaki OoGuri*1, Akio SUGIHARA*2, Yoshio TOMINAGA*2 and Shigeru MORIYAMA*1

*1 Central Research Institute, Maruha Corporation
(16-2 Wadai, Tsukuba-shi, Ibaraki-ken 300-4295)
*2 Osaka Municipal Technical Research Institute
(1-6-50 Morinomiya, Joto-ku, Osaka 536-8553)

Abstract: Ethyl docosahexaenoate (E-DHA) was efficiently enriched by selective alcoholysis of ethyl esters from tuna oil with lauryl alcohol (LauOH) using immobilized Rhizomucor miehei lipase. We thus attempted the development of an enzymatic process applicable to the industrial purification of E-DHA. The amount of LauOH was found the most important factor in the alcoholysis, and a larger amount of LauOH was effective for the enrichment of E-DHA. In this study, the amount was fixed at 7 molar equivalents for ethyl esters originating from tuna oil (E-DHA55; E-DHA content, 54.6 mol%). A substrate mixture of E-DHA55/LauOH was introduced into a column packed with 8.0 g of immobilized Rhizomucor lipase (22~63 mm) at 30°C and a flow rate of 10 mL/h (8.3 g/h). E-DHA content increased to 87 mol% with 58% alcoholysis. Even after 150 d, E-DHA content increased to 85 mol%, although alcoholysis decreased to 48%. The half life of the lipase was determined as 150 d based on decrease in alcoholysis in the batch reaction. The reaction mixture flowing from the column was applied to film distillation, and unreacted ethyl esters were recovered in 82% yield. The ethyl ester fraction was contaminated with 2.4 wt% LauOH and 6.3 wt% lauryl esters, and lauryl esters could be completely removed by urea adduct fractionation. Through a series of purifications, E-DHA content was raised to 88 wt% in 52% yield of the initial content in E-DHA55.

Key words: ethyl docosahexaenoate, selective alcoholysis, immobilized enzyme, lipase, Rhizomucor miehei, tuna oil, molecular distillation, urea adduct fractionation

1 Introduction

Docosahexaenoic acid (22:6 n-3, DHA) competes with arachidonic acid (20:4 n-6) and plays a role in the prevention of a number of human diseases, including cardiovascular disease\(^{10-13}\), inflammation\(^{14-16}\), and cancer\(^{17,18}\). It has also been reported that DHA shows important functions in the brain\(^{19-21}\) and retina\(^{22,23}\), and accelerates the growth of preterm infants\(^{24,25}\). For this reason, tuna oil containing DHA has been used as a health food and an ingredient of baby milk\(^{26,27}\). In addition, the useful physiological activities lead to an increasing attention for the medical application of ethyl docosahexaenoate (E-DHA).

Several methods of purifying DHA have been proposed, such as high-performance liquid chromatography\(^{28}\) and silver ion-exchange column chromatography\(^{29}\). However, these methods have not been adopted for industrial purification because of high cost. In addition to these methods, enzymatic methods have been also reported: selective esterification to free fatty acid\(^{30-32}\) and selective alcoholysis to ethyl ester\(^{33,34}\). Out of these, selective alcoholysis will probably be suitable for purifying DHA as a pharmaceutical substance, because the desired form is ethyl ester. We reported that a long-chain fatty alcohol was strongly recognized by a lipase, but its fatty acid esters were recognized very weakly. In particular, lauryl alcohol (LauOH) was effective as a substrate in selective esterification because of
liquid state at 30°C. The alcohol was also superior as a substrate for alcoholysis of fatty acid ethyl esters, and E-DHA was efficiently enriched in the ethyl ester fraction by selective alcoholysis of ethyl esters originating from tuna oil with *Rhizomucor miehei* lipase. However, industrial application of the enzymatic reaction requires continuous reaction with immobilized lipase and separation of unalcoholylzed ethyl esters from the reaction mixture.

This paper deals with a continuous flow reaction with a fixed-bed bioreactor and separation of E-DHA-rich ethyl ester fraction from the reaction mixture by a combination of molecular distillation and urea adduct fractionation.

### 2 Materials and Methods

**2.1 Materials**

Fatty acid ethyl esters of which E-DHA content was 54.6 mol% (57.2 wt% ; E-DHA55) was prepared by molecular distillation of ethyl esters originating from tuna oil. The saponification value of E-DHA55 was 170 mg KOH/g (3.03 mmol/g), and the contents of main fatty acid ethyl esters other than E-DHA were as follows: ethyl stearate, 2.8 mol%; ethyl oleate (E-OA), 3.9 mol%; ethyl eicosamonoenoate, 2.3 mol%; ethyl arachidonate (E-AA), 2.8 mol%, ethyl eicosapentaenoate (E-EPA), 11.6 mol%; ethyl n-6 docosapentaenoate (E-DPAn-6), 3.9 mol%; ethyl n-3 docosapentaenoate (E-DPAn-3), 2.5 mol%. Immobilized *Rhizomucor miehei* lipase (Lipozyme IM60) was purchased from Novo Nordisk ( Bagsvaerd, Denmark). LauOH (Conol 20 P; New Japan Chemical Co. Ltd., Osaka) was of industrial grade.

**2.2 Selective Alcoholysis**

Batch reaction was performed as follows; a mixture of E-DHA 55, LauOH, and immobilized *Rhizomucor lipase* was incubated with shaking (140 oscillations/min) at 30°C in a screw-capped vessel. Flow reaction was conducted at 30°C with a column packed with 8.0 g immobilized lipase (22 × 63 mm). The extent of alcoholysis was calculated from the molar ratio of lauryl esters to the sum of ethyl and lauryl esters, which were determined by gas chromatography as described below.

**2.3 Analysis**

Ethyl and lauryl esters of fatty acids were analyzed with a Hewlett-Packard 5890 gas chromatograph (Avondale, PA, USA) connected to a DB-5 capillary column (0.25 mm × 10 m; J & W Scientific, Folsom, CA, USA) as described elsewhere. Quantitative analysis of ethyl and lauryl esters of fatty acids was carried out based on the peak area of each fatty acid ester.

**2.4 Distillation**

Dehydration was carried out at 80°C and 660 Pa before applying to molecular distillation. Separation of LauOH, ethyl esters, and lauryl esters was performed by film distillation with a molecular distillation apparatus (Wiprene type 2-03; Shinko Pantec Co. Ltd., Hyogo).

**2.5 Urea Adduct Fractionation**

Urea adduct fractionation was performed as described previously. In brief, a mixture of ethyl esters/lauryl esters (400 g) was dissolved at 50°C in a solution of 400 g urea, 2 L methanol, and 50 mL water, and the temperature was gradually decreased to 5°C with stirring. After removing the precipitate, the volume of the filtrate was reduced to ca. 700 mL with an evaporator, and 300 mL of 0.2 N HCl was then added. The oil layer (ethyl ester fraction) was dehydrated after washing with 250 mL water three times.

### 3 Results

**3.1 Effect of LauOH Amount on Enzymatic Alcoholysis of E-DHA**

E-DHA55 was alcohololyzed with various amounts of LauOH using 4% immobilized *Rhizomucor* lipase, because the amount of LauOH was the most important factor for the enrichment of E-DHA (Table 1). The alcoholysis extent was raised by a larger amount of LauOH and longer reaction period. E-DHA content in the ethyl ester fraction did not increase, even though the alcoholysis extent was raised by extending the reaction period. However, the content was increased by raising the alcoholysis extent with a larger amount of LauOH : The contents of E-DHA reached 87 and 90 mol% using 7 and 15 molar equivalents of LauOH for ethyl esters in E-DHA55, respectively. These results shows that the fatty acid selectivity was increased by a larger amount of LauOH. Meanwhile, a larger amount of LauOH decreased the ethyl ester content in the reaction mixture ; the ethyl ester content was 4.7 wt% with 15 molar equivalents of LauOH, although 9.9 wt% with 7 molar equivalents. In addition, the reaction scale
Table 1 Effect of Amount of Lauryl Alcohol on Alcoholysis of E-DHA55 with Immobilized *Rhizomucor miehei* Lipase.

<table>
<thead>
<tr>
<th>E-DHA55/LauOH (mol/mol)</th>
<th>Reaction time (h)</th>
<th>Alcoholysis (%)</th>
<th>Ethyl ester content* (wt%)</th>
<th>E-DHA content* (mol%)</th>
<th>Recovery of E-DHA* (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:3</td>
<td>20</td>
<td>42.7</td>
<td>21.3</td>
<td>83.5</td>
<td>87.6</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>56.1</td>
<td>16.3</td>
<td>82.1</td>
<td>66.0</td>
</tr>
<tr>
<td>1:5</td>
<td>20</td>
<td>49.3</td>
<td>13.3</td>
<td>86.2</td>
<td>80.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>61.9</td>
<td>10.0</td>
<td>85.1</td>
<td>59.4</td>
</tr>
<tr>
<td>1:7</td>
<td>20</td>
<td>50.8</td>
<td>9.9</td>
<td>87.1</td>
<td>78.5</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>67.2</td>
<td>6.6</td>
<td>86.6</td>
<td>52.0</td>
</tr>
<tr>
<td>1:10</td>
<td>20</td>
<td>54.5</td>
<td>6.9</td>
<td>89.0</td>
<td>74.2</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>67.5</td>
<td>4.9</td>
<td>87.0</td>
<td>51.8</td>
</tr>
<tr>
<td>1:15</td>
<td>20</td>
<td>55.7</td>
<td>4.7</td>
<td>90.0</td>
<td>73.0</td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>68.9</td>
<td>3.3</td>
<td>89.1</td>
<td>50.8</td>
</tr>
</tbody>
</table>

A mixture of 30 g E-DHA55/LauOH and 1.2 g immobilized lipase was shaken at 30°C for 20 and 40 h. The amount of LauOH was 3 to 15 molar equivalents for ethyl esters in E-DHA55. Ethyl docosahexaenoate (E-DHA) content in E-DHA55 was 54.6 mol%.

*Ethyl ester content in the reaction mixture.

*E-DHA content in the ethyl ester fraction.

*E-DHA amount recovered in the ethyl ester fraction relative to the initial E-DHA content.

becomes large as the amount of LauOH increases. Hence, the amount of LauOH was fixed at 7 molar equivalents for ethyl esters in E-DHA55 in this study.

Alcoholysis of E-DHA55 with LauOH was performed under the following conditions: A mixture of 30 g E-DHA55/LauOH (1:7, mol/mol) and 1.2 g immobilized lipase was shaken at 30°C. A typical time course is shown in Fig. 1. The alcoholysis extent increased rapidly until 5 h, and gradually thereafter. The contents of E-DPAn-3 and E-OA in the ethyl ester fraction decreased rapidly, and the contents of E-EPA and E-AA decreased after 1-h lag. The lipase acted on E-DHA and E-DPAn-6 very weakly, and their contents increased up to 10 h with increasing the alcoholysis extent. The contents of E-OA and E-DPAn-3 increased a little after 5 and 17 h, respectively (Fig. 1 B). This increase was caused by continuing alcoholyses of E-DHA and E-DPAn-6 even after the cessation of the conversion of E-OA and E-DPAn-3 to their lauryl esters (Fig. 1 C).

3.2 Effect of Flow Rate on Alcoholysis of E-DHA55

In general, an effective reaction can be obtained by a batch reaction, because the substrates contact efficiently with the enzyme. However, when the batch reaction with the immobilized lipase was performed in a reactor with an impeller, the carrier of lipase was destroyed by several times of repetition. To eliminate the problem, we attempted the flow reaction using a fixed-bed bioreactor. Effect of the flow rate on the alcoholysis of E-DHA55 was first investigated using a column packed with 8.0 g immobilized lipase (22 × 63 mm) (Table 2). E-DHA content in the ethyl ester fraction increased with the decrease of flow rate, and reached a constant value (87 mol%) at a flow rate of 11.5 g/h (13.9 mL/h). Meanwhile, the recovery of E-DHA decreased as the flow rate decreased. It was therefore found that 10-15 mL/h of flow rate was effective to increase E-DHA content with a good yield. Similar experiments with columns of 15 and 30 mm diameters were next performed, indicating that the column size did not affect the alcoholysis extent and enrichment of E-DHA. The reaction mixture obtained by feeding the substrate at 11.5 g/h corresponds to that obtained by 17.4 h batch reaction with 4% immobilized lipase. The time course of the batch reaction (Fig. 1 B) showed that the E-DHA content in the ethyl ester fraction reached a constant value after 15 h. These results indicated
Fig. 1 Time Course of Selective Alcoholysis of E-DHA 55 with Lauryl Alcohol Using Immobilized Rhizomucor miehei Lipase. The reaction was conducted as described in the text.

A: Alcoholysis extent. B: Ethyl ester content in the ethyl ester fraction. Ethyl ester content was expressed relative to the initial content which was described in Materials and Methods section. ○, Ethyl oleate; ▲, ethyl n-3 docosapentaenoate; □, ethyl eicosapentaenoate; ●, ethyl arachidonate; △, ethyl n-6 docosapentaenoate; ◆, ethyl docosahexaenoate. C: Amount of lauryl ester. The amount of each fatty acid lauryl ester was expressed as the alcoholysis extent of the fatty acid; the molar ratio of a lauryl ester to the sum of ethyl and lauryl esters. ○, Lauryl oleate; ▲, lauryl n-3 docosapentaenoate; □, lauryl eicosapentaenoate; ●, lauryl arachidonate; △, lauryl n-6 docosapentaenoate; ◆, lauryl docosahexaenoate.

that the efficiency of the flow reaction was almost the same as that of the batch reaction.

3.3 Stability of Immobilized Enzyme

A substrate, E-DHA55/LauOH (1:7, mol/mol), was introduced into a column packed with 8.0 g immobilized lipase at 10 mL/h (8.3 g/h) at 30°C for 150 d. Table 3 shows the alcoholysis extent, the content of ethyl ester, and the recovery of E-DHA. The alcoholysis extent decreased from 58 to 48% after 150 d. The time course of the batch reaction showed that 58 and 48% of alcoholysis were achieved by 24- and 12-h reactions, respectively (Fig. 1 A). Therefore, the half life of immobilized enzyme was found 150 d under the conditions employed. In addition, E-DHA content in the ethyl ester fraction decreased slightly after 150 d (85 mol%), and the E-EPA content increased to 4.7 mol%. These values also coincided with those of 12-h batch reaction. The results show that the immobilized lipase can be used for at least 150 d in the fixed-bed bioreactor.

3.4 Large-Scale Separation of E-DHA-Rich Ethyl Esters from Reaction Mixture

The reaction mixture includes LauOH, ethyl esters, and lauryl esters. Because their molecular weights are different, the ethyl esters will be separated by distillation. Thus we attempted to purify the ethyl esters from the reaction mixture by film distillation (Table 4). The reaction mixture flowing from the above column after 50-100 d was collected (the alcoholysis extent, 55.4%), and 8.0 kg of the mixture was evaporated at 80°C and 660 Pa under nitrogen stream to remove ethanol and water. The dehydrated mixture was distilled at 100°C and 27 Pa to remove LauOH, and 5.61 kg distillate 1-1 was obtained: LauOH content, 99.3 wt%. The residue was then distilled at 190°C and 2.7 Pa to recover almost all the ethyl esters. The distillate 1-2 (1.07 kg) was composed of 19.2 wt% LauOH, 60.8 wt% ethyl ester, and 19.9 wt% lauryl ester. E-DHA content in the ethyl ester fraction was 87.5 wt%, and the recovery of E-
Table 2 Effect of Flow Rate on Alcoholysis of E-DHA55 with Immobilized Rhizomucor Lipase.

<table>
<thead>
<tr>
<th>Flow rate (g/h)</th>
<th>Alcoholysis (%)</th>
<th>E-DHA content (mol%)</th>
<th>Recovery of E-DHA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7.0</td>
<td>58.5</td>
<td>87.7</td>
<td>66.7</td>
</tr>
<tr>
<td>11.5</td>
<td>53.6</td>
<td>87.3</td>
<td>74.2</td>
</tr>
<tr>
<td>24.0</td>
<td>42.3</td>
<td>82.3</td>
<td>87.0</td>
</tr>
<tr>
<td>40.0</td>
<td>37.1</td>
<td>79.1</td>
<td>91.1</td>
</tr>
</tbody>
</table>

A mixture of E-DHA55/LauOH (1:7, mol/mol; 0.83 g/mL) was fed at 30°C into a column packed with the immobilized lipase at different flow rates. See Table 1.

DHA was determined as 88.9%. The distillation of the distillate 1-2 at 110°C and 27 Pa separated 199 g distillate 2-1 of which LauOH content was 91.9 wt%. To remove lauryl esters, the final distillation was performed at 180°C and 27 Pa. The resulting distillate 2-2 (650 g) contained 91.3 wt% ethyl ester, and was contaminated with 2.4 wt% LauOH and 6.3 wt% lauryl ester. The recoveries of ethyl esters and E-DHA were 91.1 and 91.4%, respectively. Urea adduct fractionation of distillate 2-2 resulted in the complete removal of lauryl esters, and 549 g ethyl ester-rich fraction was obtained (ethyl ester content, 98.5 wt%). This fact shows that lauryl esters of polysaturated fatty acids form complexes with urea as well as those of saturated and monoenoic fatty acids.

The final product obtained by enzymatic alcoholysis, distillation and urea adduct fractionation contained LauOH (1.5 wt%), E-AA (0.8 wt%), E-EPA (3.0 wt%), E-DPAn-3 (5.9 wt%), and E-DHA (88.2 wt%). E-DHA content in the ethyl ester fraction was raised from 57.2 to 89.5 wt% with a 52.4% recovery of the initial content of E-DHA55. These results show that a combination of film distillation and urea adduct fractionation is very effective for the separation of ethyl esters from the reaction mixture.

4 Discussion

We have described a large-scale purification of E-DHA through selective alcoholysis with immobilized Rhizomucor lipase, distillation, and urea adduct fractionation. The process proposed in this study can be expected as a new industrial purification method of E-DHA from the following outstanding characteristics. i) Enzymatic alcoholysis increases E-DHA purity to nearly 90 wt% with 70% recovery. ii) The immobilized lipase can be used for 150 d in a fixed-bed reactor. iii) Any organic solvents are not necessary except for methanol in a process of urea adduct fractionation. iv) Ethyl ester fraction is recovered from the enzyme reaction mixture in a high yield by conventional methods; film distillation and urea adduct fractionation. v) Unreacted LauOH recovered by distillation can be recycled. On the other hand, enzymatic alcoholysis included a drawback that Rhizomucor lipase acted on E-DPAn-6 very

Table 3 Stability of Immobilized Rhizomucor Lipase in Continuous Flow Alcoholysis of E-DHA55.

<table>
<thead>
<tr>
<th>Operation period (d)</th>
<th>Alcoholesis (%)</th>
<th>Composition (mol%)</th>
<th>Recovery of E-DHA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>20:5n-3</td>
<td>22:5n-6</td>
</tr>
<tr>
<td>1</td>
<td>57.8</td>
<td>2.7</td>
<td>6.1</td>
</tr>
<tr>
<td>10</td>
<td>57.9</td>
<td>2.8</td>
<td>6.1</td>
</tr>
<tr>
<td>20</td>
<td>57.0</td>
<td>2.9</td>
<td>6.0</td>
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<tr>
<td>40</td>
<td>56.1</td>
<td>3.1</td>
<td>6.1</td>
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<tr>
<td>70</td>
<td>55.5</td>
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<td>6.0</td>
</tr>
<tr>
<td>100</td>
<td>52.2</td>
<td>3.5</td>
<td>6.0</td>
</tr>
<tr>
<td>150</td>
<td>48.4</td>
<td>4.7</td>
<td>5.9</td>
</tr>
</tbody>
</table>

Continuous flow reaction was performed at 30°C by feeding a mixture of E-DHA55/LauOH (1:7, mol/mol) into a column packed with 8.0 g immobilized Rhizomucor lipase at a flow rate of 10 mL/h.

*Ethyl ester composition in the ethyl ester fraction.
Table 4  Large-Scale Purification E-DHA from E-DHA55.

<table>
<thead>
<tr>
<th>Step</th>
<th>Weight (g)</th>
<th>Composition (wt%)</th>
<th>Amount of E-DHA (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>LauOH</td>
<td>EtEst³</td>
</tr>
<tr>
<td>Substrate mixture³</td>
<td>8000</td>
<td>79.8</td>
<td>20.2</td>
</tr>
<tr>
<td>Reaction mixture⁴</td>
<td>8000</td>
<td>73.6</td>
<td>9.1</td>
</tr>
<tr>
<td>Distillation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distillate 1-1</td>
<td>5610</td>
<td>99.3</td>
<td>0.7</td>
</tr>
<tr>
<td>Distillate 1-2</td>
<td>1070</td>
<td>19.2</td>
<td>60.8</td>
</tr>
<tr>
<td>Residue 1</td>
<td>1000</td>
<td>ND</td>
<td>5.0</td>
</tr>
<tr>
<td>Distillate 2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Distillate 2-1</td>
<td>199</td>
<td>91.9</td>
<td>8.1</td>
</tr>
<tr>
<td>Distillate 2-2</td>
<td>650</td>
<td>2.4</td>
<td>91.3</td>
</tr>
<tr>
<td>Residue 2</td>
<td>167</td>
<td>ND</td>
<td>12.0</td>
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<tr>
<td>Urea fractionation</td>
<td>549</td>
<td>1.5</td>
<td>98.5</td>
</tr>
</tbody>
</table>

³Ethyl ester.
⁴Lauryl ester.
⁵Calculated on the basis of the composition of substrate mixture.
⁶Ethanol content was calculated to be 1.56 wt% from the alcoholysis extent.
⁷Not detected.

When a lipase is applied to oil processing and purification of a desired fatty acid, the fatty acid specificity becomes one of the most important factors. The activity of lipase on DPA has not been reported yet. The time course of alcoholysis of E-DHA55 (Fig. 1B and C) showed that the activity of the lipase was in the order of OA > DPAn-3 > EPA > AA > DHA, DPAn-6. Though the chain length and number of unsaturated bonds of DPAn-3 are the same as those of DPAn-6, the lipase acted on DPAn-3 strongly, and on DPAn-6 very weakly. The unsaturated bonds which are nearest to the carboxyl ends of DPAn-3 and DPAn-6 are present at Δ7 and Δ4, respectively. In addition, we observed Candida rugosa and Rhizopus delemar lipases acted on α-linolenic acid (18:3 n-3) strongly, and on γ-linolenic acid (18:3 n-6) very weakly20,21). These results implied that the unsaturated bond near the carboxyl end decreased the catalytic activity of lipase. The fatty acid specificity of lipase is determined by the position of unsaturated bonds in addition to the chain length and the number of unsaturated bonds.

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固有化 Rhizomucor miehei リバーゼによる選択的
アルコリシス反応を利用したドコサヘキサエン酸エチルの精製

丸山 一 輝*1 ・ 島田 裕 司*2 ・ 馬場 貴 司*3 ・ 大 栗 智 昭*4
杉 原 敏 雄*2 ・ 富 永 嘉 男*2 ・ 森 山 茂*2
*1 マルハ(株)中央研究所 (〒300-4295 兵庫県つくば市台和台16-2)
*2 大阪市立工業研究所 (〒536-8553 大阪府大阪市城東区森之宮1-6-50)

ドコサヘキサエン酸エチル (E-DHA) の大量精製方法の確立を目的とし、固有化 Rhizomucor miehei リバーゼでマグロ由来のエチルエステル (E-DHA 55; E-DHA 含量、55 mol%) をラウリアルコール (LauOH) でアルコリシスした。固有化リバーゼを充填したカラム (8.0 g 22×63 mm) に E-DHA/LauOH (1:7、mol/mol) の混液を
30℃、10 mL/h の流速で流した。その後、エステル交換率は 58% に達し、エチルエステル画分の E-DHA 含量は 87 mol% まで上昇した。この固有化酵素リアクターを 150 日間連続運転すると、エステル交換率は 48% まで低めたものの、E-DHA 含量はほとんど低下できず 85 mol% まで上昇していた。カラムから分離した分画液中のエチルエステルは薄膜蒸留により収率よく回収できた (82%), この画分には 24 wt% の LauOH と 63 wt% のラウリアルコールが混在していた。このうち、ラウリアルコールは尿素包括により完全除去できた。一連の精製操作により、E-DHA 55 に含まれていた E-DHA の 52% が回収でき、E-DHA 含量を 88 wt% まで高めたことができた。

（連絡者：島田裕司） Vol.49, No.8, 793 (2000)

アクリアル酸とアクリアル酸 n-ヘキシル、
2- エチルヘキシルおよび n-デデキシルコテロマーの合成と
多類性界面活性剤としての性質

吉村 倫一*1 ・ 小出 善文*1 ・ 正 泉 禾 秀 元*1 ・ 江 角 邦 男*2
*1 熊本大学工学部物質生命化学科 (〒860-8555 熊本県熊本市黒髮 2-39-1)
*2 東京理科大学理学部応用化学科 (〒162-8601 東京都新宿区神楽坂 1-3)

アクセリアル酸とアクリアル酸アルキルコテロマーの多類性界面活性剤 (xRnA-yAA, x, y 及び m はそれぞれアルキル鎖の数、親水基の数及びアルキル鎖長を意味する) を選択移動剤に 2-アミノエチルチオール塩酸塩を用いて、
アクリアル酸とアクリアル酸 n-ヘキシル、2-エチルヘキシル及び n-デデキシルのコテロメジションにより合成し、
界面化学的性質について検討した。xRnA-yAA, xRnA-yAA 及び xRnA-yAA 水溶液の表面張力は、それぞれ 28
32, 27 30 及び 38 45 mN m-1 であった。臨界ミセル濃度 (cmc) は、アルキル鎖長及び鎖長の增加に伴って
減少した。300 ppm の Ca2+ 存在下では、2.9 RnA-2.3 AA, 2.8 RnA-2.5 AA 及び 2.7 RnA-2.9 AA は、それぞれ
24, 28 及び 33 mN m-1 の表面張力であった。短いアルキル鎖を有する 2.9 RnA-2.3 AA は高い泡沫安定性を示し
たが、分岐鎖を有する 2.8 RnA-2.5 AA は低い安定性を示した。xRnA-yAA, xRnA-yAA 及び xRnA-yAA 水溶液
とトルエンとの界面張力は、それぞれ 11 13, 8 12 及び 10 15 mN m-1 であり、cmc は一般的な界面活性剤ド
デカサン酸ナトリウムに比べて 1/3 1/4 となった。トルエンの乳化は、コテロマーより水溶液とより混ぜることによっ
て形成し、特に 2 3 のアルキル鎖を有するコテロマーに高い安定な水中油滴型のエマルジョンが認められた。

（連絡者：吉村倫一） Vol.49, No.8, 801 (2000)