Enzymatic Synthesis of New Hepoxilins and Trioxilins from Polyunsaturated Fatty Acids†

In-Gyu Lee,‡a Jung-Ung An,‡a,b Yoon-Joo Ko,c Jin-Byung Park,d and Deok-Kun Oh*a

‡I.-G. Lee and J.-U. An equally contributed to this work.

aDepartment of Bioscience and Biotechnology, Konkuk University, Seoul 05029, Republic of Korea, E-mail: deokkun@konkuk.ac.kr; Fax: +82-2-444-5518; Tel: +82-2-454-3118
bSynthetic Biology and Bioengineering Research Center, Korea Research Institute of Bioscience and Biotechnology (KIRIBB), Daejeon 34141, Republic of Korea.
cNational Center for Inter-University Research Facilities (NCIRF), Seoul National University, Seoul 08826, Republic of Korea.
dDepartment of Food Science and Engineering, Ewha Womans University, Seoul 03760, Republic of Korea.
Table of Contents

Supporting Tables
Table S1. Chemical names and classification of HXs and TrXs.................................................................................................................................................5
Table S2. Isolated yields, purities, and regression equations for calibration curves of PUFAs and HFAs, HXs, and TrXs obtained from PUFAs by ARA 15-LOX from B. thailandensis without and with EH from M. xanthus ...........................................................................................................................................6
Table S3. Specific activity of ARA 15-LOX from B. thailandensis and ARA 11-LOX, ARA 12-LOX, and EH from M. xanthus towards C20 and C22 PUFAs .....................................................................................................................................................................................................................................................7

Supporting Figures
Fig. S1  Nomenclature rules of HXs and TrXs ...........................................................................................................................................................................8
Fig. S2  Biosynthetic pathways of PUFAs into TrXs via HXs previously reported and identified in this study .............................................................................9
Fig. S3  Biosynthetic pathway of DGLA (21) into 13,14,15-TrXB₂(γ) (25) via 14,15-HXB₂(γ) (24) .................................................................................................10
Fig. S4 HPLC chromatograms for the conversion of 15-HpETE to 15-HETE with 50 mM HEPES buffer, E. coli cells without plasmid, and 15-LOX from B. thailandensis ........................................................................................................................................................................................................................11
Fig. S5 HPLC chromatograms for the standard and purified compounds of 15-HETE .....................................................................................................................................................................................................................................................12
Fig. S6  Peak areas of 15-HpETE and 15-HETE at the same concentrations .........................................................................................................................................................................................13
Fig. S7  HPLC profiles of metabolites from ARA by recombinant E. coli expressing ARA 15-LOX from B. thailandensis ....................................................................................................................................................................................................................................................14
Fig. S8  SDS-PAGE analysis of ARA 15-LOX expressed in recombinant E. coli ER2566 .................................................................................................................................................................................................................................................15
Fig. S9  Biotransformation of DGLA (21) into 14,15-HXB₂(γ) (24) by recombinant E. coli expressing ARA 15-LOX from B. thailandensis ....................................................................................................................................................................................................................................................16
Fig. S10 Increase of the ratio of HFAs to HXs by adding cysteine as a reducing agent .........................................................................................................................................................................................................................................................17
Fig. S11  LC-MS/MS analysis of the products obtained after biotransformation of ARA (1) by recombinant E. coli expressing ARA 15-LOX from B. thailandensis
thailandensis without and with EH form M. xanthus

Fig. S12  LC-MS/MS analysis of the products obtained after biotransformation of EPA (6) by recombinant E. coli expressing ARA 15-LOX from B. thailandensis without and with EH form M. xanthus

Fig. S13  LC-MS/MS analysis of the products obtained after biotransformation of DHA (11) by recombinant E. coli expressing ARA 15-LOX from B. thailandensis without and with EH form M. xanthus

Fig. S14  LC-MS/MS analysis of the products obtained after biotransformation of ADA (16) by recombinant E. coli expressing ARA 15-LOX from B. thailandensis without and with EH form M. xanthus

Fig. S15  LC-MS/MS analysis of the products obtained after biotransformation of DGLA (21) by recombinant E. coli expressing ARA 15-LOX from B. thailandensis without and with EH form M. xanthus

Fig. S16  NMR data of 14,15-HXB\(_3\) (4)

Fig. S17  2D NMR data of 14,15-HXB\(_3\) (4)

Fig. S18  NMR data of 14,15-HXB\(_4\) (9)

Fig. S19  2D NMR data of 14,15-HXB\(_4\) (9)

Fig. S20  NMR data of 16,17-HXB\(_5\) (14)

Fig. S21  2D NMR data of 16,17-HXB\(_5\) (14)

Fig. S22  NMR data of 16,17-HXB\(_3\) (19)

Fig. S23  2D NMR data of 16,17-HXB\(_3\) (19)

Fig. S24  NMR data of 14,15-HXB\(_3\)(\(\gamma\)) (24)

Fig. S25  2D NMR data of 14,15-HXB\(_3\)(\(\gamma\)) (24)

Fig. S26  SDS-PAGE analysis of ARA 15-LOX and EH expressed in recombinant E. coli ER2566

Fig. S27  NMR data of 13,14,15-TrXB\(_3\) (5)

Fig. S28  2D NMR data of 13,14,15-TrXB\(_3\) (5)
Fig. S29  NMR data of 13,14,15-TrXB₄ (10)............................................................................................................................................................................60
Fig. S30  2D NMR data of 13,14,15-TrXB₄ (10)......................................................................................................................................................................62
Fig. S31  NMR data of 15,16,17-TrXB₅ (15)............................................................................................................................................................................66
Fig. S32  2D NMR data of 15,16,17-TrXB₅ (15)......................................................................................................................................................................68
Fig. S33  NMR data of 15,16,17-TrXB₆ (20)............................................................................................................................................................................72
Fig. S34  2D NMR data of 15,16,17-TrXB₆ (20).................................................................................................................................................................74
Fig. S35  Effects of pH and temperature on the activity of ARA 15-LOX from B. thailandensis .................................................................78

References 79
## Supporting Tables

**Table S1.** Chemical names and classification of HXs and TrXs.

<table>
<thead>
<tr>
<th>Type</th>
<th>Type</th>
<th>Product</th>
<th>Chemical name</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
<td>HXA₃</td>
<td>8-Hydroxy-11,12-epoxyeicosa-5,9,14-tetraenoic acid</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HXA₄</td>
<td>8-Hydroxy-11,12-epoxyeicosa-5,10,14,17-tetraenoic acid</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HXA₅</td>
<td>10-Hydroxy-13,14-epoxydocosa-4,7,11,16,19-pentaenoic acid</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14,15-HXA₂</td>
<td>11-Hydroxy-14,15-epoxyeicosa-5,8,12-tetraenoic acid</td>
<td>4</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>HXB₁</td>
<td>10-Hydroxy-11,12-epoxyeicosa-5,8,14-tetraenoic acid</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HXB₂</td>
<td>10-Hydroxy-11,12-epoxyeicosa-5,8,14,17-tetraenoic acid</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HXB₃</td>
<td>12-Hydroxy-13,14-epoxydocosa-4,7,10,16,19-pentaenoic acid</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14,15-HXB₁</td>
<td>13-Hydroxy-14,15-epoxyeicosa-5,8,11-tetraenoic acid</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14,15-HXB₂</td>
<td>13-Hydroxy-14,15-epoxyeicosa-5,8,11,17-tetraenoic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16,17-HXB₂</td>
<td>15-Hydroxy-16,17-epoxydocosa-4,7,10,13,19-pentaenoic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>14,15-HXB₂(γ)</td>
<td>13-Hydroxy-14,15-epoxyeicosa-8,11-dienoic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16,17-HXB₂(γ)</td>
<td>15-Hydroxy-16,17-epoxydocosa-7,10,13,19-pentaenoic acid</td>
<td>This study</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>HXD₃</td>
<td>13-Hydroxy-11,12-epoxyeicosa-5,8,14-tetraenoic acid</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>HXE₃</td>
<td>15-Hydroxy-11,12-epoxyeicosa-5,8,13-tetraenoic acid</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>A</td>
<td>TrXA₃</td>
<td>8,11,12-Trihydroxyeicosa-5,9,14-tetraenoic acid</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TrXA₄</td>
<td>8,11,12-Trihydroxyeicosa-5,10,14,17-tetraenoic acid</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TrXA₅</td>
<td>10,13,14-Trihydroxydocosa-4,7,11,16,19-pentaenoic acid</td>
<td>3</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>TrXB₃</td>
<td>10,11,12-Trihydroxyeicosa-5,8,14-tetraenoic acid</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TrXB₄</td>
<td>10,11,12-Trihydroxyeicosa-5,8,14,17-tetraenoic acid</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>TrXB₅</td>
<td>12,13,14-Trihydroxydocosa-4,7,10,16,19-pentaenoic acid</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13,14,15-TrXB₁(5)</td>
<td>13,14,15-Trihydroxyeicosa-5,8,11-tetraenoic acid</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13,14,15-TrXB₁(10)</td>
<td>13,14,15-Trihydroxyeicosa-5,8,11,17-tetraenoic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15,16,17-TrXB₁(15)</td>
<td>15,16,17-Trihydroxydocosa-4,7,10,13,19-pentaenoic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>13,14,15-TrXB₁(γ)</td>
<td>13,14,15-Trihydroxyeicosa-8,11-dienoic acid</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15,16,17-TrXB₁(γ)</td>
<td>15,16,17-Trihydroxydocosa-7,10,13,19-pentaenoic acid</td>
<td>This study</td>
</tr>
<tr>
<td>C</td>
<td></td>
<td>TrXC₃</td>
<td>8,9,12-Trihydroxyeicosa-5,10,14-tetraenoic acid</td>
<td>8</td>
</tr>
<tr>
<td>D</td>
<td></td>
<td>TrXD₃</td>
<td>11,12,13-Trihydroxyeicosa-5,8,14-tetraenoic acid</td>
<td>5</td>
</tr>
<tr>
<td>E</td>
<td></td>
<td>TrXE₃</td>
<td>11,12,15-Trihydroxyeicosa-5,8,13-tetraenoic acid</td>
<td>5</td>
</tr>
</tbody>
</table>
Table S2. Isolated yields, purities, and regression equations for calibration curves of PUFAs and HFAs, HXs, and TrXs obtained from PUFAs by ARA 15-LOX from *B. thailandensis* without and with EH from *M. xanthus*.

<table>
<thead>
<tr>
<th>Type</th>
<th>Product</th>
<th>Isolated yield (w/w)</th>
<th>Purity (w/w)</th>
<th>Regression equation (^{[a]})</th>
<th>(r^2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PUFA</td>
<td>ARA (1)</td>
<td>–</td>
<td>≥ 98.5%</td>
<td>(y = 0.00003583x - 0.0562)</td>
<td>0.9936</td>
</tr>
<tr>
<td></td>
<td>EPA (6)</td>
<td>–</td>
<td>≥ 99%</td>
<td>(y = 0.00003014x - 0.0941)</td>
<td>0.9994</td>
</tr>
<tr>
<td></td>
<td>DHA (11)</td>
<td>–</td>
<td>≥ 98%</td>
<td>(y = 0.00002772x - 0.0171)</td>
<td>0.9948</td>
</tr>
<tr>
<td></td>
<td>ADA (16)</td>
<td>–</td>
<td>≥ 98%</td>
<td>(y = 0.0005711x + 0.0119)</td>
<td>0.9978</td>
</tr>
<tr>
<td></td>
<td>DGLA (21)</td>
<td>–</td>
<td>≥ 99%</td>
<td>(y = 0.0002046x - 0.0543)</td>
<td>0.9992</td>
</tr>
<tr>
<td>HFA</td>
<td>15-HETE (3)</td>
<td>–</td>
<td>≥ 95%</td>
<td>(y = 0.00009662x + 0.0056)</td>
<td>0.9922</td>
</tr>
<tr>
<td></td>
<td>15-HEPE (8)</td>
<td>–</td>
<td>≥ 98%</td>
<td>(y = 0.0002302x + 0.0735)</td>
<td>0.9765</td>
</tr>
<tr>
<td></td>
<td>17-HDoHE (13)</td>
<td>–</td>
<td>≥ 98%</td>
<td>(y = 0.0006845x + 0.0156)</td>
<td>0.9922</td>
</tr>
<tr>
<td></td>
<td>17-HDoTE (18)</td>
<td>86%</td>
<td>96%</td>
<td>(y = 0.001988x - 0.0148)</td>
<td>0.9952</td>
</tr>
<tr>
<td></td>
<td>15-HETrE(γ) (23)</td>
<td>88%</td>
<td>94%</td>
<td>(y = 0.001069x + 0.0641)</td>
<td>0.9844</td>
</tr>
<tr>
<td>HX</td>
<td>14,15-HXB(_3) (4)</td>
<td>80%</td>
<td>95%</td>
<td>(y = 0.0002206x - 0.0346)</td>
<td>0.9928</td>
</tr>
<tr>
<td></td>
<td>14,15-HXB(_4) (9)</td>
<td>70%</td>
<td>91%</td>
<td>(y = 0.0002392x - 0.0426)</td>
<td>0.9919</td>
</tr>
<tr>
<td></td>
<td>16,17-HXB(_3) (14)</td>
<td>82%</td>
<td>93%</td>
<td>(y = 0.0004638x - 0.0195)</td>
<td>0.9950</td>
</tr>
<tr>
<td></td>
<td>16,17-HXB(_3) (19)</td>
<td>81%</td>
<td>94%</td>
<td>(y = 0.0002720x - 0.0280)</td>
<td>0.9903</td>
</tr>
<tr>
<td></td>
<td>16,17-HXB(_3) (24)</td>
<td>75%</td>
<td>92%</td>
<td>(y = 0.001083x - 0.00371)</td>
<td>0.9947</td>
</tr>
<tr>
<td>TrX</td>
<td>13,14,15-TrXB(_3) (5)</td>
<td>78%</td>
<td>93%</td>
<td>(y = 0.0005837x - 0.00822)</td>
<td>0.9971</td>
</tr>
<tr>
<td></td>
<td>13,14,15-TrXB(_4) (10)</td>
<td>73%</td>
<td>91%</td>
<td>(y = 0.0005802x + 0.00537)</td>
<td>0.9987</td>
</tr>
<tr>
<td></td>
<td>15,16,17-TrXB(_3) (15)</td>
<td>86%</td>
<td>98%</td>
<td>(y = 0.0003952x + 0.04184)</td>
<td>0.9903</td>
</tr>
<tr>
<td></td>
<td>15,16,17-TrXB(_3) (20)</td>
<td>83%</td>
<td>96%</td>
<td>(y = 0.0006904x + 0.0904)</td>
<td>0.9904</td>
</tr>
</tbody>
</table>

\(x\), peak area in HPLC profile; \(y\), concentration of standard in mg mL\(^{-1}\).
Table S3. Specific activity of ARA 15-LOX form *B. thailandensis* (BT) and ARA 11-LOX, ARA 12-LOX, and EH from *M. xanthus* (MX) towards C20 and C22 PUFAs.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Product</th>
<th>Specific activity (U mg⁻¹[^a])</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>BT 15-LOX</td>
<td>ARA (1)</td>
<td>15-HpETE (2)</td>
<td>23.3 ± 0.3</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>EPA (6)</td>
<td>15-HpEPE (7)</td>
<td>21.4 ± 0.1</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>DHA (11)</td>
<td>17-HpDoHE (12)</td>
<td>15.4 ± 0.5</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>DGLA (21)</td>
<td>15-HpETrE(γ) (22)</td>
<td>4.9 ± 0.2</td>
<td>This study</td>
</tr>
<tr>
<td></td>
<td>ADA (16)</td>
<td>17-HpDoTE (17)</td>
<td>10.3 ± 0.2</td>
<td>This study</td>
</tr>
<tr>
<td>MX EH</td>
<td>14,15-HXB₃ (4)</td>
<td>14,15-TrXB₃ (5)</td>
<td>54.0 ± 0.6</td>
<td>This study</td>
</tr>
<tr>
<td>MX 11-LOX</td>
<td>ARA (1)</td>
<td>11-HpETE</td>
<td>5.2 ± 0.1</td>
<td>[^9]</td>
</tr>
<tr>
<td>MX 12-LOX</td>
<td>ARA (1)</td>
<td>12-HpEPE</td>
<td>15.4 ± 0.1</td>
<td>[^10]</td>
</tr>
</tbody>
</table>

[^a]: The specific activity calculated by the reaction of purified ARA 15-LOX from *B. thailandensis* or EH from *M. xanthus* with 1 mM substrate for 1 min.

Supporting Figures

Fig. S1 Nomenclature rules of HXs and TrXs. Compounds of HXA and HXB series contain a hydroxyl group at C8 for C20 PUFAs and at C10 for C22 PUFAs; and at C10 for C20 PUFAs and at C12 for C22 PUFAs, respectively. HXD$_3$ and HXE$_3$ have a hydroxyl group at C13 and C15, respectively. Compounds of TrXA and TrXB series contain three hydroxyl groups at C8, C11, and C12 for C20 PUFAs and at C10, C13, and C14 for C22 PUFAs; and at C10, C11, and C12 for C20 PUFAs and at C12, C13, and C14 for C22 PUFAs, respectively. TrXC$_3$, TrXD$_3$, TrXE$_3$ have three hydroxyl groups at C8, C9, and C12; C11, C12, and C13; and C11, C12, and C15, respectively. Compounds of HX series contain an epoxide group at C11 and C12 for C20 PUFAs and at C13 and C14 for C22 PUFAs, while compounds of 14,15-HX and 16,17-HX series have an epoxide group at C14 and C15 for C20 PUFAs and at C16 and C17 for C22 PUFAs, respectively. Compounds of TrXA and TrXA series have three hydroxyl groups at C8, C11, and C12 for C20 PUFAs and at C10, C13, and C14 for C22 PUFAs, while compounds of 13,14,15-TrX and 15,16,17-TrX series have three hydroxyl groups at C13, C14, and C15 for C20 PUFAs and at C15, C16, and C17 for C22 PUFAs, respectively.
Fig. S2 Biosynthetic pathways of PUFAs into TrXs via HXs previously reported and identified in this study.
Fig. S3 Biosynthetic pathway of DGLA (21) into 13,14,15-TrXB$_2(\gamma)$ (25) via 14,15-HXB$_2(\gamma)$ (24).

The compound 25 (dotted bracket) was identified by only LC-MSMS due to the low activity of *E. coli* expressing ARA 15-LOX from *B. thailandensis* and EH from *M. xanthus*. 
Fig. S4 HPLC chromatograms for the conversion of 15-HpETE to 15-HETE with 50 mM HEPES buffer (red line), *E. coli* cells without plasmid (blue line), and 15-LOX from *B. thailandensis* (green line). The reaction were performed in 50 mM HEPES buffer (pH 7.5) containing 0.5 mM 15-HpETE at 25 °C for 60 min. The concentrations of *E. coli* cells without plasmid and 15-LOX were 6 g L$^{-1}$ and 0.4 g L$^{-1}$, respectively.
**Fig. S5** HPLC chromatograms for the standard and purified compounds of 15-HETE. Green, red, and blue lines represent 15-HETE standard, purified 15-HETE using Prep-LC and SP825 adsorbent resin, and purified 15-HETE using Prep-LC, respectively.
**Fig. S6** Peak areas of 15-HpETE and 15-HETE at the same concentrations. The peak area of 15-HpETE is the same as that of 15-HETE.
**Fig. S7** HPLC profiles of metabolites from ARA by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis*. A) Chromatogram of reaction solution containing 1 mM ARA. B) Chromatogram of reaction solution after the reaction with 1 mM ARA and 15 g L\(^{-1}\) *E. coli* without plasmid for 60 min. C) Chromatogram of reaction solution after the reaction with 1 mM ARA and 15 g L\(^{-1}\) *E. coli* expressing ARA 15-LOX from *B. thailandensis* for 60 min.
**Fig. S8** SDS-PAGE analysis of ARA 15-LOX expressed in recombinant *E. coli* ER2566. Lane M indicates the marker proteins. *M*, molecular mass marker proteins (180, 135, 100, 75, 63, 48, 35, 25, 17, and 11 kDa); *lane 1*, crude extract; *lane 2*, pellet, and *lane 3*, purified ARA 15-LOX.
Fig. S9 Biotransformation of DGLA (21) into 14,15-HXB$_2$(γ) (24) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis*. The symbols indicate the concentrations of DGLA (●; 21), 15-hydroxyperoxyeicosa-8,11,13-trienoic acid (▲; 22), 15-hydroxyeicosa-8,11,13-trienoic acid (■; 23), and 14,15-HXB$_2$(γ) (○; 24). Data represent the means of three experiments and error bars represent the standard deviation.
Fig. S10 Increase of the ratio of HFAs to HXs by adding cysteine as a reducing agent. Blue line represents the chromatogram of reaction without cysteine, green line represents the chromatogram of reaction by adding 50 mM cysteine, and red line represent the chromatogram of reaction by adding 100 mM cysteine. All the reaction performed in 50 mM HEPES buffer (pH 7.5) containing 1 mM ARA and 15 g L$^{-1}$ E. coli cells at 25 °C for 60 min.
Fig. S11 LC-MS/MS analysis of the products obtained after biotransformation of ARA (1) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 15-HETE (3). B) 14,15-HXB₃ (4). C) 13,14,15-TrXB₃ (5).
Fig. S12 LC-MS/MS analysis of the products obtained after biotransformation of EPA (6) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 15-HEPE (8). B) 14,15-HXB₄ (9). C) 13,14,15-TrXB₄ (10).
Fig. S13 LC-MS/MS analysis of the products obtained after biotransformation of DHA (11) by recombinant E. coli expressing ARA 15-LOX from B. thailandensis without and with EH form M. xanthus. A) 17-HDOHE (13). B) 16,17-HXB (14). C) 15,16,17-TrXB (15).
Fig. S14 LC-MS/MS analysis of the products obtained after biotransformation of ADA (16) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 17-HDOTE (18). B) 16,17-HXB₃ (19). C) 15,16,17-TrXB₃ (20).
Fig. S15 LC-MS/MS analysis of the products obtained after biotransformation of DGLA (21) by recombinant *E. coli* expressing ARA 15-LOX from *B. thailandensis* without and with EH form *M. xanthus*. A) 15-HETrE(γ) (23). B) 14,15-HXB₂(γ) (24). C) 13,14,15-TrXB₂ (γ) (25).
Fig. S16 NMR data of 14,15-HXB₃ (4). A) Chemical structure of compound 4. B) $^1$H NMR peak of compound 4. C) $^{13}$C NMR peak of compound 4.
Fig. S18 NMR data of 14,15-HXB₄ (9). A) Chemical structure of compound 9. B) $^1$H NMR peak of compound 9. C) $^{13}$C NMR peak of compound 9.
Fig. S20 NMR data of 16,17-HXB₅ (14). A) Chemical structure of compound 14. B) ¹H NMR peak of compound 14. C) ¹³C NMR peak of compound 14.
Fig. S22 NMR data of 16,17-HXB₃ (19). A) Chemical structure of compound 19. B) $^1$H NMR peak of compound 19. C) $^{13}$C NMR peak of compound 19.
Fig. S24 NMR data of 14,15-HXB₂(γ) (24). A) Chemical structure of compound 24. B) $^1$H NMR peak of compound 24. C) $^{13}$C NMR peak of compound 24.
**Fig. S26** SDS-PAGE analysis of ARA 15-LOX and EH expressed in recombinant *E. coli* ER2566. Lane M indicates the marker proteins. The crude extract (lane 1) and pellet (lane 2) of the cell lysate of *E. coli* expressing *B. thailandensis* ARA 15-LOX and *M. xanthus* EH. Red box and green box indicate the proteins, *B. thailandensis* ARA 15-LOX, and *M. xanthus* EH, respectively.
Fig. S27 NMR data of 13,14,15-TrXB₃ (5). A) Chemical structure of compound 5. B) $^1$H NMR peak of compound 5. C) $^{13}$C NMR peak of compound 5.
**Fig. S28** 2D NMR data of 13,14,15-TrXB₃ (5). A) COSY spectrum of compound 5. B) TOCSY spectrum of compound 5. C) ROESY spectrum of compound 5. D) HSQC spectrum of compound 5. E) HMBC spectrum of compound 5.
**Fig. S29** NMR data of 13,14,15-TrXB₄ (10). A) Chemical structure of compound 10. B) $^1$H NMR peak of compound 10. C) $^{13}$C NMR peak of compound 10.
Fig. S31 NMR data of 15,16,17-TrXB₃ (15). A) Chemical structure of compound 15. B) ¹H NMR peak of compound 15. C) ¹³C NMR peak of compound 15.
Fig. S32 2D NMR data of 15,16,17-TrXB$_3$ (15). A) COSY spectrum of compound 15. B) TOCSY spectrum of compound 15. C) ROESY spectrum of compound 15. D) HSQC spectrum of compound 15. E) HMBC spectrum of compound 15.
Fig. S33 NMR data of 15,16,17-TrXB₃ (20). A) Chemical structure of compound 20. B) ¹H NMR peak of compound 20. C) ¹³C NMR peak of compound 20.
**Fig. S35** Effects of pH and temperature on the activity of ARA 15-LOX from *B. thailandensis*. A) Effect of pH. The buffers used were 50 mM HEPES buffer (●, pH 6.5–7.5) and 50 mM Tris-HCl buffer (○, pH 7.5–9) and the reactions were performed at 25 °C with 1 mM ARA and 6 g L⁻¹ cells for 5 min. B) Effect of temperature. The reactions were performed in 50 mM HEPES buffer (pH 7.5) containing 1 mM ARA and 6 g L⁻¹ cells for 5 min. Data represent the means of three experiments and error bars represent the standard deviation.
References


