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TOPIC(s) : Enzyme production, immobilization

Application of linoleate 13-hydratase in stereoselective hydration of 18-carbon unsaturated fatty acids

AUTHORS

Joanna GACH / WROCLAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES, C.K. NORWIDA 25, WROCLAW

Stefano SERRA / C.N.R. SCITEC ISTITUTO DI SCIENZE E TECNOLOGIE CHIMICHE, VIA MANCINELLI 7, MILANO

Stefano MARZORATI / C.N.R. SCITEC ISTITUTO DI SCIENZE E TECNOLOGIE CHIMICHE, VIA MANCINELLI 7, MILANO

Teresa OLEJNICZAK / WROCLAW UNIVERSITY OF ENVIRONMENTAL AND LIFE SCIENCES, C.K. NORWIDA 25, WROCLAW

PURPOSE OF THE ABSTRACT

Fatty acid hydratases are one of the most common enzymes able to catalyze the water addition to the non-activated double bonds. Their physiological function in the microorganisms presumably may be linked with the process of detoxification of fatty acids or alteration in host-microbe interactions [1]. From the industrial point of view, the resulting products, namely the hydroxy fatty acids, find use as starting materials for production of signaling molecules, biopolymers, emulsifiers, stabilizers and aroma compounds [2-5].

In this work, we investigated the biocatalytic potential of the linoleate 13-hydratase from *Lactobacillus acidophilus*, which is able to catalyze the addition of water to the C-12 double bond of linoleic acid. For this purpose, we overexpressed the latter enzyme using *Escherichia coli* BL21(DE3) as a heterologous host. Then, the obtained biocatalyst was purified in order to study its catalytic properties. More specifically, we evaluated both the activity and the regio- and stereoselectivity of the hydration reaction using different C18 unsaturated fatty acids as substrates.

Beside linoleic acid, which is the natural substrate of 13-hydratase from *L. acidophilus*, we have tested the substrate specificity using other C18 Δ -12 unsaturated fatty acids. In particular, we employed the synthetically derived C18:1 Δ -12 fatty acid and two isomeric C18:3 polyunsaturated fatty acids, namely Δ -9,12,15 (linolenic acid) and C18:3 Δ -6,9,12 (γ -linolenic acid). The hydration of C18:1 Δ -9 (oleic acid) was also checked as negative control. The obtained hydroxyl derivatives were extracted from the biotransformation mixture, purified using column chromatography and characterized by nuclear magnetic resonance (^1H NMR and ^{13}C NMR). The gas chromatography analysis coupled with mass spectrometry (GC-MS) allow determining the regioselectivity of the hydration reaction. The enantiomeric excess of the products was measured by NMR analysis after derivatization of the hydroxy-fatty acids to their corresponding (S)-O-acetylmandelate esters.

Overall, we established that the aforementioned enzyme catalyzes the hydration of the unsaturated fatty acids with complete regioselectivity with exclusive formation of the 13-hydroxy derivatives in very high enantioselectivity (ee >95%), regardless of the kind of substrate used. This substrate flexibility points to the potential use of 13-hydratase from *Lactobacillus acidophilus* for the preparative synthesis of different 13-hydroxy-acid derivatives.

FIGURES

FIGURE 1

FIGURE 2

KEYWORDS

fatty acid hydratase | substrate specificity | stereoselectivity | heterologous expression

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