

Pseudomonas lipase catalyzed synthesis of ergosterol oleate

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Introduction

Microbial enzymes, particularly those produced by extremophiles, are very often stable and active in organic solvents. Lipases are among the most promising and important biocatalysts for carrying out reactions in both, aqueous and nonaqueous, media. Under natural conditions, lipase catalyzes the hydrolysis of ester bonds at the interface between an insoluble substrate phase and the aqueous phase in which the enzyme is dissolved. In reduced water conditions, lipases catalyze the reverse reaction – esterification. So their potential for the hydrolysis and synthesis made lipases used for production of esters of commercial interest. In the last decade interest for enzyme-catalysed production of esters has increased. One of the advantages of enzyme-catalysed process (besides the energy saving) are mild conditions which allow transformations of sensitive substrates.

Aim

In the present study, the potential of bacterial lipase isolated from *Pseudomonas aeruginosa* NCAIM (P) B 001380 to catalyze the esterification of ergosterol with oleic acid in the simple process, was evaluated. The prepared ergosterol oleate was purified by column chromatography and identification was conducted by NMR.

Results and discussion

Stability and activity of crude enzyme preparation in different organic solvents has been studied previously, and according to this, condition for reaction of esterification were chosen. Reaction of esterification of ergosterol and oleic acid catalyzed by lipase was carried out in n-hexane at 40°C. Preliminary detection of synthesized ergosterol oleate was done by TLC on silica gel. After 24, 48, 72, 96 and 120h product formation was checked by TLC and the total formation of ergosterol oleate was quantified by TLC scanner. Conditions for esterification were: 4 mmol of oleic acid and 100 mg of ergosterol in 6 ml of n-hexane with 100 mg of crude lipase, at 40°C with rotational shaking (250 cycles/min). Compared to two other microbial lipases, our lipase showed similar yield formation after 24h (8%). Lipases from *Candida antarctica* B and *Candida rugosa* had higher degree of esterification after 24h (16.1% and 38.5%). Degree of esterification with our lipase was 64% after 5 days of incubation. These results were obtained without optimization of the conditions.

The product was purified on silica gel 100 (0.063 – 0.200) 60x1cm column, eluted with n-hexane:ethyl-acetate (6:4). Fractions were collected and then detected by TLC. After purification ergosterol oleate was analyzed.

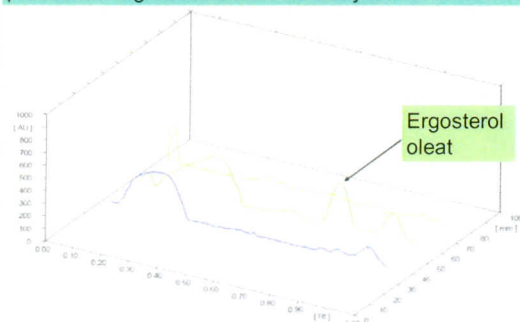


Figure 1. Chromatogram of: **oleic acid**, **ergosterol** and lipase synthesized **ergosterol oleate**.

Material and methods

P. aeruginosa was cultivated at 30°C for 20h in the Luria-Bertani medium (0.5% NaCl, 0.5% yeast extract, and 1% tryptone) with vigorous shaking 250 at cycles/min. An actively growing culture was dispensed into erlenmaer flasks (1%), and fermentation was carried out in the same medium at 30°C for 72h. After 72h, the culture filtrate obtained by centrifugation at 7500 rpm for 15min collected and liophilized. This sample was used as crude lipase. Reaction of esterification was carried out in 20ml hermetically sealed vials. Reaction mixture was consist of 4mmol of acide and 100mg of ergosterol in 6ml of n-hexane with 100mg of crude lipase. Reaction was performed at 40°C with rotational shaking (250 cycles/min). Purified ergosterol oleate was analyzed: ¹H, ¹³C and DEPT¹³C NMR spectra were measured in CDCl₃ as solvent, with Gemini 200 NMR spectrometer. Quantitative analysis of the desired product was done using Camag TLC scanner 3.

Product identification ¹³C NMR spectral data (50 MHz, CDCl₃) of ergosterol oleate was: δ = 11.96 (CH₃-18), 14.06 (CH₃-51), 16.17 (CH₃-19), 17.55 (CH₃-29), 19.59 (CH₃-30), 19.90 (CH₃-31), 21.05 (CH₃-21), 22.63 (CH₂), 22.94 (CH₂), 24.64 (CH₂), 27.15 (CH₂), 28.24 (CH₂), 29.10 (CH₂), 29.48 (CH₂), 29.63 (CH₂), 29.72 (CH₂), 31.74 (CH₂), 31.86 (CH₂), 33.03 (CH-28), 34.03(CH-24) 36.94 (C-10), 38.29 (CH₂-1), 39.04 (CH₂-12), 40.40 (CH₂-4), 40.53(CH-20), 42.77 (C-13), 46.17 (CH-9), 54.50 (CH-17), 55.68 (CH-14), 70.44 (CH-3), 116.31 (CH-7), 119.62 (CH-6), 129.71 (C-43), 129.98 (C-42), 131.95 (CH-23), 135.55 (CH-22), 139.67 (C-8), 141.25 (C-5), 179.72 (C=O-33).

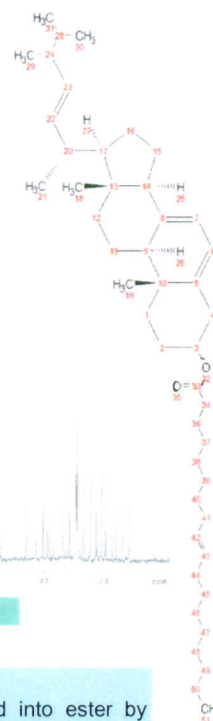


Figure 2. ¹³C NMR spectrum of ergosterol oleat

Conclusion

Our data show that ergosterol can be efficiently converted into ester by lipase. Further investigations such as optimization of synthesis: influence of temperature, effect of substrate molar ratio, effect of molecular sives and synthesis of other steroides, is underway.

Acknowledgment

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Refrence

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Villeneuve P. et al., *Enzyme Microb. Technol.*, 2005; 37:150-155