ELSEVIER

Process Biochemistry 40 (2005) 131–137

PROCESS BIOCHEMISTRY

www.elsevier.com/locate/procbio

Biosynthesis of 1-octen-3-ol and 10-oxo-*trans*-8-decenoic acid using a crude homogenate of *Agaricus bisporus* Optimization of the reaction: kinetic factors

Ruben O. Morawicki, Robert B. Beelman*, Devin Peterson, Gregory Ziegler

Department of Food Science, The Pennsylvania State University, 116 Borland Laboratory, University Park, PA 16802, USA

Received 3 April 2003; received in revised form 12 November 2003; accepted 25 November 2003

Abstract

1-Octen-3-ol and 10-oxo-*trans*-8-decenoic acid (ODA) are the products of the enzymic breakdown of linoleic acid (LA) in mushrooms by a lipoxygenase and a hydroperoxide lyase. Based on this reaction, natural 1-octen-3-ol and ODA can be produced with a crude enzyme homogenate from mushrooms in a bioreactor. The influence on this reaction of temperature (5–24 °C), concentration of LA (1.3–13.3 mg/g mushrooms), stirring speed (500–1500 rpm), concentration of oxygen (23.2–100% saturation), and pH (6.5–7.5) was studied in a 1 l standard bioreactor by monitoring the production of 1-octen-3-ol. A central composite rotatable and an algorithmic experimental design were used to study the five factors. Only LA concentration and temperature were found to affect significantly the yield of the reaction. The optimal parameters that maximized the production of 1-octen-3-ol were 12 mg of linoleic acid (99% purity) per gram of mushroom, 7.8 °C, and a pH of 7.5; with a predicted yield of 2.7 ± 0.4 mg of 1-octen-3-ol per gram of mushroom.

Keywords: 1-Octen-3-ol; 10-Oxo-*trans*-8-decenoic acid; ODA; Lipoxygenase; Mushroom flavor; Mushroom hormone

1. Introduction

The biotransformation of linoleic acid (LA) by mushroom enzymes produces 1-octen-3-ol, a key component of mushroom aroma, and 10-oxo-*trans*-8-decenoic acid (ODA) [1]. The first step in the reaction is the incorporation of molecular oxygen into the pentadienyl moiety of the linoleic acid by a lipoxygenase (EC 1.13.1.13), producing the intermediate 10-hydroperoxy-*trans*-8-*cis*-12-octadecadienoic acid (10-HPOD). Subsequently, a hydroperoxide lyase cleaves the 10-HPOD generating 1-octen-3-ol and ODA [2].

In addition to biological interest, this biosynthetic pathway can be used industrially to produce 1-octen-3-ol, an important flavour constituent that can be claimed "natural" [3], and ODA, a compound that shows potential as a fungal growth regulator [4,5]. While the yield of this reaction has been increased consistently in the last decade [4,6–9] the optimization of the reaction could produce further improvements in the yield that would make the commercial use of these two compounds more economical.

In most enzymic reactions temperature and concentration of substrate significantly affect the yield of the reaction. Additionally, in this reaction, the limited solubility of the two substrates—linoleic acid and oxygen—in water will likely require the need of an efficient mixing of the media, thus making stirring speed another factor to be studied. Stability of the enzymic system is another concern in this study. Wurzenberger and Grosch [2] reported that a partially purified protein fraction of *Psalliota bispora* lost its capacity to produce 1-octen-3-ol after being stored for 5 h at 0 °C. Champavier et al. [8] reported that the activity of an enzyme preparation of *Agaricus bisporus* decayed quickly when stored at −4 or −20 °C. This suggests that deactivation of the enzymes can be expected during the course of the reaction.

Previous researchers have studied the biosynthetic pathway leading to the formation of 1-octen-3-ol and ODA by conducting the reactions at room temperature, 22–25 °C

* Corresponding author. Tel.: +1-814-863-2964; fax: +1-814-863-6132.
E-mail address: rbb6@psu.edu (R.B. Beelman).

0032-9592/$ – see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.procbio.2003.11.037

[4,6–9]. Champavier et al. [8] also reported that incubations at 40 °C produced higher yields than at 25 °C. However, results have not been reported about the effect of temperatures lower than the ambient on the stability and consequently the yield of the reaction.

When substrates are water insoluble, stirring may become very important to increase the contact between enzymes and substrate. Mixing also affects the oxygen transfer rate when oxygen is one of the substrates of the reaction. However, excessive shear forces created during stirring may speed up enzyme denaturation. Similarly to denaturation by heat, denaturation by shear forces depends on the intensity of the energy added and the time of exposure [10]. An interaction between stirring rate and temperature may also be expected [11].

The first objective of this research was to evaluate stability as a function of the temperature of the enzymes that generate 1-octen-3-ol and ODA using a crude homogenate of *A. bisporus* mushroom. The second objective was to study simultaneously the effect of temperature, concentration of linoleic acid, concentration of dissolved oxygen, and stirring speed on the production of 1-octen-3-ol and ODA in order to maximize the yield. Additionally, the pH of the reaction mixture was studied to determine if pH interacted with other factors.

2. Materials and methods

2.1. Materials

Fresh mushrooms (*A. bisporus*) were obtained from the Mushroom Test and Demonstration Facility (MTDF) at the Pennsylvania State University, University Park, PA. Mushrooms were stored at 4 °C within 30 min after collection. Phosphate buffer (0.1 M), at different levels of pH, was prepared fresh on a weekly basis and stored at 4 °C. The temperature of mushroom and buffer was conditioned before each experiment. 1-Octen-3-ol (98%), 2-heptanone (98%), *cis*-pinonic acid (98%), and linoleic acid (99%) were purchased from Aldrich Chemical Company (Milwaukee, WI). 10-Oxo-*trans*-8-decenoic acid (98%), prepared from mushrooms, was obtained from Center Ingredient Technology, Inc. (State College, PA).

2.2. Enzyme stability studies

Enzyme stability was assayed at 4 and 24 °C.

2.2.1. Enzyme preparation

Mushroom homogenates were prepared as follows: 50 g of mushrooms were blended in 100 ml of 0.1 M phosphate buffer, pH 7.0 during 30 s at 4 and 24 °C, respectively. The homogenates were stored at the homogenization temperatures and used as enzyme preparation.

2.2.2. Substrate

An emulsion was prepared with 500 mg of 99% pure linoleic acid and 6 μl of Tween 20 dispersed in 5 ml of 0.1 M phosphate buffer. The dispersion was homogenized by passing 10 times through a micro-emulsifying needle 21G 1/2 connected between two syringes.

2.2.3. Assay

Enzyme activity was followed over a period of time of 3 and 0.5 h for the assay at 4 and 24 °C, respectively. Time zero assays were performed immediately after homogenization. The reaction was started by mixing 14.5 ml of buffer, with 500 μl of the substrate emulsion, and 15 ml of the enzyme preparation in a test tube with a working volume of 30 ml. The tube was inverted once and mixed with a magnetic stirrer while oxygen was bubbled during the whole reaction. Samples were taken at 0, 2, 6, and 10 min and analyzed for 1-octen-3-ol content. The concentration of 1-octen-3-ol versus time was plotted and the slope of the regression line was taken as the activity of the enzyme in mg/min. A normalized chart of activity in logarithmic units versus time was then plotted and the slope of the regression line was taken as the constant for the first-order inactivation rate.

Buffers used for the assay were conditioned at 4 °C for the homogenate stored at 24 °C and vice versa for the homogenate stored at 24 °C in order to have the same temperature for the assay (approximately 14 °C).

2.3. Optimization of the reaction

2.3.1. Reactor

Optimization of the reaction was conducted in a 1 l standard bioreactor (LH Fermentation, Emeryville CA) equipped with four baffles, a stirrer, and a sparger. Temperature was maintained by circulating water through a jacket surrounding the reactor. A heated/refrigerated circulator bath (VWR 1140) was used to control the temperature of the water with a precision of 0.5 °C. Dissolved oxygen was monitored with an ORION Sensor Link™ PCM800 Dissolved Oxygen Measurement System (ORION Research Inc., Beverly, MA) connected to an IBM computer used for data acquisition. Calibration of the probe was carried out with deionized water saturated with air at 25 °C following the method suggested by the manufacturer. No correction was made for the presence of solids in the reaction broth. pH was monitored with a Model 25 Accumet pH meter (Denver Instruments Company, Arvada, CO). The stirrer was powered by a variable speed motor (Bodine Electric Company, Chicago, IL) and the rotation speed monitored with a non-contact hand digital tachometer (SHIMPO, Lincolnwood, IL).

2.3.2. Reaction

Assays were initiated by blending 150 g of mushrooms, 300 ml of buffer, and adequate amount of linoleic acid for 30 s. Without delay, the homogenate was transferred to the