LWT - Food Science and Technology 82 (2017) 268–273

Contents lists available at ScienceDirect

LWT - Food Science and Technology

journal homepage: www.elsevier.com/locate/lwt

Kinetic characterization of arginase from *Saccharomyces cerevisiae* during alcoholic fermentation at different temperatures

Ilaria Benucci, Vittorio Fiorelli, Claudio Lombardelli, Katia Liburdi*, Marco Esti

Dept. for Innovation in Biological, Agro-food and Forest Systems, University of Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy

ARTICLE INFO

Article history:
Received 18 November 2016
Received in revised form
6 March 2017
Accepted 16 April 2017
Available online 19 April 2017

Keywords:
Yeast arginase activity
Alcoholic fermentation
Saccharomyces cerevisiae
Kinetic parameters
arginine

ABSTRACT

The kinetic characterization of arginase activity of a commercial *S. cerevisiae* strain was carried out for the first time, estimating the kinetic parameters (V_{max}, $K_{0.5}$ and $V_{max}/K_{0.5}$) throughout alcoholic fermentation in order to investigate the catalytic efficiency of the enzyme and its ability in metabolizing arginine to sustain biosynthetic processes. Alcoholic fermentation was carried out at three different temperatures (15, 20, 25 °C) in semi-synthetic grape juice added with arginine at usual maximal concentration (1 g L^{-1}) in grape must.

Arginine uptake was quite constant throughout fermentation process and it was more effectively assimilated during high temperature fermentation (20 and 25 °C) than at 15 °C. The sigmoidal behavior of yeast arginase kinetic curves, well fitted to the Hill equation, indicated a mechanism of positive cooperativity for the trimeric enzyme. The highest V_{max} (4740.0 U mg^{-1}_{BSAeq}) and the maximal catalytic efficiency (78.87 min^{-1}) were observed when fermentation was at 20 °C approximately 3 days after the inoculum. Moreover, the $K_{0.5}$ value was similar (53–60 mg mL^{-1}) when maximal catalytic efficiency was achieved, thus indicating that the affinity of enzyme for the substrate is not altered by fermentation temperature which only affected product release velocity and therefore $V_{max}/K_{0.5}$ ratio.

1. Introduction

During alcoholic fermentation the metabolic pathway of *Saccharomyces cerevisiae* is characterized by a complex interaction between amino acid assimilation, production and breakdown (Ough, Crowell, & Mooney, 1988). In wine yeast cells, arginase hydrolyzes arginine into L-ornithine and urea (Henschke & Ough, 1991; Monteiro, Trousdale, & Bisson, 1989; Pretorius, 2000; Romagnoli et al., 2014), which can either be excreted from the yeast cell or can be further catabolized to ammonia and CO_2 through an energy-dependent two-step process (Monteiro & Bisson, 1991) catalyzed by urea amidolyase (Coulon et al., 2006). The excretion and re-absorbtion rates of urea from/into yeast cells affect the content of urea in wine at the end of alcoholic fermentation.

As described by Bisson (1996), during the alcoholic fermentation of grape must, the cleavage of urea via urea amidolyase does not always immediately follow the arginine metabolism. This phenomenon may cause an excessive accumulation of urea in the cytoplasm and its subsequent release into the must/wine. Despite commercial yeast strains differ for their ability to catabolize urea as a nitrogen source (An & Ough, 1993), as well as for their capability in urea excretion during the fermentation process (An & Ough, 1993), these information are not usually supplied in the technical sheet. Moreover, fermentation conditions affect both the excretion and reabsorption rates of urea and *S. cerevisiae* secretes more urea at higher fermentation temperatures, while high ammonia concentrations suppress the reabsorption of urea by the yeast as described by Pretorius (2000).

Arginine metabolism in *S. cerevisiae* is regulated through a mechanism defined as 'epiarginase control' (Messenguy & Dubois, 2000), which is modulated by a multienzyme complex found in the cytoplasm composed of two biocatalysts: ornithine transcarbamoylase (EC 2.1.3.3) and arginase (EC 3.5.3.1), which are associated face-to-face with a one-to-one stoichiometry (Green, Eisenstein, McPhie, & Hensley, 1990). As reported by Eisenstein, Duong, Ornberg, Osborne, and Hensley (1986), the multienzyme complex is located at the interface between the anabolic and catabolic arginine pathways and it regulates the flux of metabolites in response to the changes of their levels. Thus, the two

* Corresponding author.
E-mail address: k.liburdi@unitus.it (K. Liburdi).

http://dx.doi.org/10.1016/j.lwt.2017.04.044
0023-6438/

cytoplasmatic enzymes form the complex when both active-site ligands (ornithine and arginine) are present, whereas both enzymes are dissociated and active in their absence. Numerous authors (Jauniaux, Urrestarazu, & Wiame, 1978; Messenguy & Dubois, 2000) have proved that ornithine transcarbamoylase activity, which catalyzes the first committed step in arginine biosynthesis, is reversibly inhibited by the binding of active-site ligands (Middelhoven, 1969; Penninckx & Wiame, 1976) while arginase activity remains unmodified (Eisenstein et al., 1986).

Arginase from *S. cerevisiae* was extracted, purified and characterized (Chan & Cossins, 1973; Green et al., 1990) as a trimeric enzyme, which is composed of three identical subunits with a molecular weight of 39 kDa (Penninckx & Wiame, 1976). Numerous studies have proved that metals, especially Mn^{2+}, play an important role in stabilizing the tertiary and quaternary structures of arginase from various sources, as well as in the maintenance of catalytic activity (Green, Ginsburg, Lewis, & Hensley, 1991; Green et al., 1990; Middelhoven, 1964), which is heat-activated in the presence of Mn^{2+} (Green et al., 1990). It is widely recognized that nitrogen uptake significantly affects the arginine metabolism of *S. cerevisiae* (Ough, Huang, An, & Stevens, 1991) and arginase activity is known to be an effective marker of nitrogen limitation during alcoholic fermentation (Carrasco, Pérez-Ortın, & del Olmo, 2003; Jiménez-Martí, Agustín, Mendes-Ferreira, Mendes-Faia, & Lí del Olmo, 2007). Previous studies (Beltran, Novo, Rozès, Mas, & Guillamón, 2004; Crépin, Nidelet, Sanchez, Dequin, & Camarasa, 2012; Jiranek, Langridge, & Henschke, 1995; Martin, Brandriss, Schneider, & Bakalinsky, 2003) investigated nitrogen assimilation by *S. cerevisiae* during alcoholic fermentation, by comparing the consumption profile of yeast assimilable nitrogen (YAN) and individual amino acids (i.e. arginine). As yet there is little information on the kinetic behavior of yeast arginase during alcoholic fermentation. Therefore, the main objectives of this study were the arginase kinetic characterization and the estimation of the kinetic parameters of the enzyme from yeast (*S. cerevisiae* strain) crude extract throughout the fermentation process, carried out at three different temperatures. Concurrently, the assimilation of arginine added to the semi-synthetic grape juice at its usual maximal concentration (1 g L^{-1}) found in grape must was evaluated.

2. Materials and methods

2.1. Semi-synthetic grape juice preparation and fermentation conditions

The semi-synthetic grape juice was prepared by diluting the juice obtained from Cabernet Franc grape with sugar and nitrogen added back to appropriate levels, as described below. As reported by Coleman, Fish, and Block (2007), the purpose of diluting the grape juice was to create a medium so that it was possible to manipulate the sugar and nitrogen levels while all other nutrients were equal throughout the experiments. The grape juice was from the 2015 vintage and was supplied by the Vigne del Patrimonio winery (Viterbo, Italy). The juice was composed as follows: Brix 23.5; titratable acidity, 6.2 g L^{-1}; pH, 3.35. Sodium metabisulfite was added to the juice at 50 mg L^{-1}.

The grape juice was diluted 3:1, thus 3 parts of 0.03 M tartaric acid/sodium tartrate solution (pH 3.2) were added to 1 part of juice. Sugar was replaced by adding a stock solution composed of 50% glucose and 50% fructose (Sigma Aldrich, Milan, Italy) dissolved in sterile, filtered, deionized water, reaching a final concentration of 200 g L^{-1}. The yeast assimilable nitrogen (YAN) content (200 mg N/L) was obtained by adding ammonium chloride and amino acids from stock solutions, in order to complement the amount of arginine (1 g L^{-1}).

The commercial *Saccharomyces cerevisiae* r.f. *cerevisiae* yeast strain used in this study was Fervens BERRY (DAL CIN GILDO SpA, Concorezzo - Italy). Active dry yeast (0.5 g) was rehydrated in 5 mL water at 37 °C for 15 min and then acclimatized in 400 mL semi-synthetic grape juice according to the manufacturer's instructions. The yeast cells were then added to fresh semi-synthetic grape juice at an inoculation level of approximately 5×10^6 cells mL^{-1}, reaching a total volume of 2 L. Alcoholic fermentation, carried out in triplicate, was performed in 3 L flasks with stoppers containing a Müller valve that only releases CO_2 from the system. Incubation was carried out at three different temperatures (15, 20 and 25 °C) in a temperature-controlled incubator (Zhicheng ZHWY-200B, Shanghai, China) with shaker tables set at a constant speed of 20 rpm. Temperature and agitation levels were maintained throughout the fermentation process.

According to other authors (Romano, Fiore, Paraggio, Caruso, & Capece, 2003) weight loss was used as a parameter for monitoring the evolution of fermentation process. The total weight of samples was determined daily and the weight loss, resulting from CO_2 release, was measured using a Europe 4000 AR balance (Gibertini, Elettronica Srl, Novate, Milan, Italy; accuracy: ± 0.01 g; maximum capacity: 4 kg).

2.2. Yeast crude extract preparation

Crude protein extracts were obtained at various time points (16, 24, 48, 66, 112 and 204 h) throughout alcoholic fermentation at three temperatures (15, 20 and 25 °C) and then arginase activity was measured. The protocol described by Carrasco et al. (2003) was applied for preparing the crude protein extracts with some modifications. Yeast biomass, from aliquots of semi-synthetic grape juice (100 mL) collected during fermentation, was separated by centrifugation (15000 × g 15 min at 4 °C) and weighed. Cell lysis was achieved by grinding the collected biomass into liquid nitrogen and then resuspending it in a volume of breaking buffer (Tris-HCl 5 mM pH 7.5, $MnCl_2$ 1 mM) 10-times greater than the weight of the biomass. The mixture was kept under magnetic stirring at 4 °C for 10 min in order to achieve complete cell lysis. The lysate was centrifuged (5000 × g 5 min at 4 °C) and the supernatant was used as crude protein extract. Protein concentration was determined with Bradford Protein Assay (Bradford, 1976) using Coomassie brilliant blue reagent and measuring absorbance at 595 nm. BSA was used as standard protein.

2.3. Arginase activity assay

The arginase activity of the crude protein extracts obtained during alcoholic fermentation was determined in accordance with the protocol described by Konarska and Tomaszewski (1975) and reported by other authors (Carrasco et al., 2003; Jiménez-Martí et al., 2007) with some modifications. 200 μl of extract was mixed with McIlvaine buffer (0–790 μl) 0.1 M, pH 9 prewarmed at 37 °C. With the aim of starting arginase reaction, various amounts of arginine solution were added in order to reach a concentration ranging between 0 and 170 mg mL^{-1} in a final assay volume of 1 mL. The samples were mixed and incubated in a termoblock at 37 °C. Aliquots of 100 μl were removed every 3 min and the reactions were stopped by adding 10 μl of trichloroacetic acid (2 M). Arginine hydrolysis in the sample was detected spectrophotometrically by means of a K-LARGE enzymatic kit (Megazyme International Ireland Ltd., Wicklow, Ireland) and its concentration was calculated according to the manufacturer's instructions. Blanks containing no protein extract or no arginine were also included in the assay.