Biochemical Engineering Journal 125 (2017) 190–195

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Efficient chemoenzymatic synthesis of gabapentin by control of immobilized biocatalyst activity in a stirred bioreactor

Ya-Ping Xue[a,b], Hu-Jun Zhong[a,b], Shu-Ping Zou[a,b], Yu-Guo Zheng[a,b,*]

[a] Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, China

[b] Engineering Research Center of Bioconversion and Biopurification of Ministry of Education, Zhejiang University of Technology, Hangzhou 310014, China

ARTICLE INFO

Article history:
Received 2 January 2017
Received in revised form 19 April 2017
Accepted 12 June 2017
Available online 13 June 2017

Keywords:
Nitrilase
Gabapentin
Chemoenzymatic synthesis
1-Cyanocyclohexaneacetonitrile
1-Cyanocyclohexaneacetic acid

ABSTRACT

Gabapentin has been widely used for the treatment of epilepsy and neuropathic pain in the world. In this work, an efficient and greener chemoenzymatic manufacturing process for gabapentin has been developed. The whole cells expressing engineered nitrilase were immobilized in diatomite cross-linked with polyethyleneimine and glutaraldehyde to improve their reusability and stability for the synthesis of gabapentin intermediate 1-cyanocyclohexaneacetic acid from 1-cyanocyclohexaneacetonitrile. The initial enzyme activity in the bioreactors at each batch biotransformation was maintained at a constant value by introducing fresh immobilized cells into the system to keep the high conversion (100%) and the same operation time (8 h) in each batch. The resulting 1-cyanocyclohexaneacetic acid was then converted to gabapentin efficiently by simple chemical steps. An E factor (exclude water) of 11.5 was calculated for the chemoenzymatic route relative to 55.7 for the mentioned chemical process. This new route dramatically improved process efficiency compared to the chemical process.

1. Introduction

Gabapentin (GBP)[1-(aminomethyl)cyclohexaneacetic acid] is an amino acid which has a structural analog of the inhibitory neurotransmitter gammaaminobutyric acid. As one of important antiepileptic drugs, GBP was introduced for the treatment of partial seizures in patients with epilepsy [1]. It has also been found to be beneficial for the treatment of neuropathic pain related to reflex sympathetic dystrophy [2], postpoliomyelitis neuropathy [3], and postherpetic neuralgia [4]. The broad applications and increasing demand of GBP have attracted a great deal of attention among synthetic organic chemists.

Over the past decades, many methodologies have been developed for the synthesis of GBP [5–22]. However, most of these methods have certain drawbacks, such as safety issue [5–9], complex sequential operations [7,16–18] or low yield [6–8,10–14]. For example, one of the successful industry-relevant strategies for producing GBP involved as an intermediate a glutaric-anhydride derivative which is prepared in several steps from cyclohexnone. After opening the anhydride, one of the two carboxy group is transformed into an NH_2 group by *Hofmann*, *Curtius*, or *Lossen* rearrangement. These synthesis methods are costly on a technical scale, since they would require complex operations [10,14] or expensive safety precautions for the handling of thermally unstable azides [5]. Alternatively, the reported chemical process starting from cyclohexanone via the synthesis of 1-cyanocyclohexaneacetic acid (1-CA) from 1-cyanocyclohexaneacetonitrile (1-CN) followed by hydrogenation seems to be a potential approach to prepare GBP considering the operational simplicity [11] (Scheme 1). The drawback of this route is that the chemical procedure to convert 1-CN to 1-CA affords product in low yield (<50%) and produce large amounts of unwanted byproducts and inorganic wastes [11]. Fortunately, nitrilases as the catalysts of transforming the nitriles to acids have been discovered and developed, which used efficiently in chemo-, *enantio*- and regioselective synthyesis [23–34]. The nitrilase-mediated hydrolysis of α,ω-dinitriles to cyanocarboxylic acids provides the advantage of a clean and green synthetic process in the absence of organic solvents [27–32]. In addition to operating under milder reaction conditions, this biotransformation of α,ω-dinitriles exhibited high regioselectivity. Several nitrilases from *Acidovorax facilis* [33], *Bradyrhizobium japonicum* [34] have been reported to have the ability to convert α,ω-dinitriles to cyanocarboxylic acids including 1-CN to 1-CA.

* Corresponding author at: Key Laboratory of Bioorganic Synthesis of Zhejiang Province, College of Biotechnology and Bioengineering, Zhejiang University of Technology, Hangzhou 310014, China.
E-mail addresses: zhengyg@zjut.edu.cn, zhengyg88320630@163.com (Y.-G. Zheng).

http://dx.doi.org/10.1016/j.bej.2017.06.008
1369-703X/

Nomenclature

E_0	Active form of enzyme
E_1	Intermediate state of enzyme
E_d	Deactivated form of enzyme
A_0	Initial activity of enzyme in the reactor (U)
A_1	Activity of intermediate specie initially present in the reactor (U)
A_t	Total activity of enzyme in the reactor at time t (U)
$A(n)$	Initial activity at the beginning of each batch (U)
k_d	Deactivation rate of enzyme in the first order deactivation mechanism (h^{-1})
k_0	The first-step deactivation rate of enzyme in the two-step sequential deactivation mechanism (h^{-1})
k_1	The second-step deactivation rate of enzyme in the two-step sequential deactivation mechanism (h^{-1})
E^{fr}	Specific activity of fresh enzyme (U/g)
q_n	Feed quantity of fresh immobilized cells (g)
T_n	Deactivation function of immobilized cells

Greek letters

α_1	Ratio of specific activity of native enzyme to intermediate specie

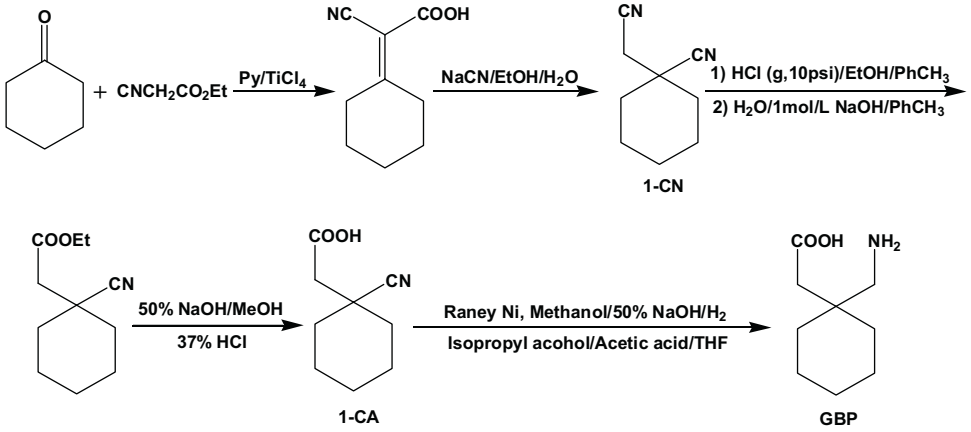
Recently, a nitrilase from *A. facilis* ZJB09122 exhibited high regioselectivity for the hydrolysis of α,ω-dinitrile to cyanocarboxylic acids was discovered [35]. To enable a practical large-scale process, protein engineering were used to increase enzyme specific activity. Consequently, a mutant F168V with extremely high specific activity towards the hydrolysis of 1-CN to 1-CA was obtained based on the homology modeling and "hot spot" mutation [36]. In comparsion to native nitrilase, a great increase in cell specific activity was observed. With this mutant nitrilase, a chemoenzymatic process is designed for the production of high-purity GBP (Scheme 2). The key step is the nitrilase-catalyzed hydrolysis of 1-CN to 1-CA with strict regioselectivity followed by hydrogenation to gabapentin-lactam (LAC) over Raney-nickel [37].

In this work, the whole cells of recombinant *Escherichia coli* expressing the engineered nitrilase were immobilized to recycle the biocatalyst and facilitate the synthesis of 1-CA. The initial enzyme activity in the bioreactors at each batch biotransformation was maintained at a constant value by introducing fresh immobilized biocatalyst into the system so as to compensate for the loss of enzyme activity to keep the high conversion and the same operation time in each batch. The resulting 1-CA was then converted to GBP efficiently by simple chemical steps. The proposed approach may enable the economical and environmentally attractive production of GBP.

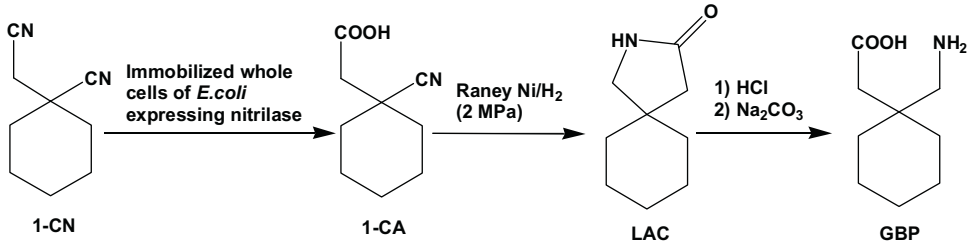
2. Experimental

2.1. Materials

Luria-Bertani (LB) medium: Yeast extract, 5 g/L; tryptone, 10 g/L; NaCl, 10 g/L, Fermentation medium: Yeast extract, 12 g/L; tryptone, 15 g/L; NaCl, 10 g/L; glycerol, 10 g/L; $(NH_4)_2SO_4$, 5 g/L; KH_2PO_4, 1.36 g/L; $K_2HPO_4{\cdot}3H_2O$, 2.28 g/L; $MgSO_4{\cdot}7H_2O$, 0.375 g/L. The recombinant strain *E. coli* BL21(DE3)/pET28b(+)-F168 V was constructed previously [35] and whole cells were obtained by fermentation [37]. 1-CN was provided by Zhejiang Chiral Medicine Chemicals Co., Ltd. (Hangzhou, China). Polyethyleneimine (PEI) and diatomite were purchased from Aladdin Industrial Corporation (Shanghai, China). Glutaraldehyde (GA) was purchased from J&K Chemical Co., Ltd. (Beijing, China). Raney-nickel (RTH-4110) was provided by Dalian Tongyong Chemicals Co. Ltd. (Dalian, China). Hydrochloric acid was purchased from Xilong Scientific Co., Ltd. (Shantou, China).



Scheme 1. Chemical process for the synthesis of GBP starting from cyclohexanone via the formation of 1-CA from 1-CN followed by hydrogenation.



Scheme 2. Chemoenzymatic synthesis of GBP using immobilized whole cells of *E.coli* expressing nitrilase.