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JOURNAL OF BIOCHEMISTRY, MICROBIOLOGY AND BIOTECHNOLOGY

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Expression, purification and characterization of *meta*-cleavage enzyme CarBaBb from *Novosphiongobium* sp. K

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History

Received: 30 September 2013

Received in revised form: 25 November 2013

Accepted: 2 December 2013

Available online: 25 December 2013

KEYWORDS

meta-cleavage enzyme,
extradiol dioxygenase
carbazole degradation

ABSTRACT

The *meta*-cleavage enzyme carbabb of carbazole-degrader *novosphiongobium* sp. Ka1 were cloned, expressed and purified to homogeneity in *escherichia coli* strain. The enzyme was cloned with 6x histidine residues attached at the c-terminal of large subunit carbb for purification using affinity chromatography method prior to gel filtration chromatography. The carbabb, a two-subunit *meta*-cleavage enzyme, approximately 30 kda for carbb dan 10 kda for carba, was found to be $\alpha_2\beta_2$-heterotetrameric (*m*r 80,000), showed highest activity at ph 8.5 and temperature 30°c. Carbabb showed highest catalytic activity towards 2,3-dihydroxybiphenyl with k_{cat}/k_m 4.1 $m^{-1}s^{-1}$, and overall higher catalytic activities towards biphenyl-type substrates in comparison to catechol-type substrates. Based on the similarities, this *meta*-cleavage enzyme from *novosphiongobium* sp. Ka1 would also be a good candidate for protein crystallization and structural studies apart from carbabb from strain *p. Resinovorans* strain ca10.

INTRODUCTION

Carbazole is a compound produced from impurities of fossil fuel which share the similar plane structure of the dioxin family and is also characterized to be a recalcitrant chemical and was reported to have carcinogen properties [1]. Isolation of bacteria capable of degrading carbazole as sole carbon and nitrogen source have been reported and genes responsible for degradation of aromatic compounds have been discovered and characterized extensively [2, 3, 4, 5].

Generally in the carbazole degradation pathway, 2 molecules of oxygen are added to carbazole through the process of angular dioxygenation, which produces a diol derivative which is later converted to 2'-aminobiphenyl-2,3-diol through spontaneous ring cleavage [4, 5, 6]. Extradiol dioxygenase also known as the *meta*-cleavege enzyme then catalyzes the ring cleavage opening at the C-C bond next to the added hydroxy groups. In contrast, *ortho*-cleavage is the process of cleaving C-C bond between the two hydroxy groups. Product of *meta*-cleavage will be hydrolyzed by a *meta*-cleavage product hydrolase which will further transform substrates for downstream pathway before assimilation through TCA cycle [5].

The *car* genes of *Pseudomonas resinovorans* CA10 have been studied most extensively among other carbazole degrading bacteria. Extradiol dioxygenase of strain CA10 is a class III extradiol enzyme designated CarBaBb coded by carBa and carBb genes [7, 8]. Although most extradiol dioxygenase are homo-multimeric, CarBaBb is made of two proteins like LigAB enzyme [10]. CarBaBb is made of CarBb, a larger subunit which carries the catalytic site and CarBa, a smaller subunit which function remains unknown. CarBaBb from strain CA10 showed strong *meta*-cleavage activity towards biphenyl-type substrates but showed weak activity towards catechol-type substrates [11]. Both *carBa* and *carBb* genes were also found in car genes of *Novosphiongobium* sp. KA1 [9]. Significant differences between car_{CA10} and car_{KA1} gene cluster have been shown but the similarity of both CarBaBb is approximately 40% [12]. However, to date, the crystal structure of CarBaBb enzyme has not been reported [13]. Here, we report the expression, purification and characterization of *meta*-cleavage enzyme from *Novosphiongobium* sp. KA1.

MATERIALS AND METHODOLOGY

Bacterial strains, plasmids, media and culture conditions.

Escherichia coli strains DH5α (Takara Bio Co., Ltd., Kyoto, Japan), and BL21(DE3) (Novagen, Inc., Madison, USA) were used for expression plasmid construction and protein expression. Expression plasmids were constructed based on plasmids pUC119 and pET26b [14]. Relevant information regarding the strains and plasmids used for this study is as listed in table 1. *E. coli* strains were grown on LB medium or 2xYT medium containing appropriate antibiotics (final concentration of 100 μg/ml ampicillin) at 37°C with shaking (300 strokes/min for glass tubes or 120 rpm for flasks). For plate cultures, media solidified with 2.0% agar (wt/vol) were used. Appropriate amount of isopropyl-B-D-thiogalactopyranoside (IPTG) was added to the medium to induce expression [15].

DNA manipulation

Plasmid DNA was prepared from the *E. coli* host strain by alkaline lysis method [15, 16]. Restriction endonucleases and DNA