A Novel Microbial Aldehyde Oxidase Applicable to Production of Useful Raw Materials, Glycolic Acid and Glyoxylic Acid, from Ethylene Glycol

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Description

Glycolic acid is an attractive raw material which is used as a dyeing and tanning agent in the textile industry, a flavoring agent and preservative in the food processing industry, and a skin care agent in the pharmaceutical industry. It is also utilized for the production of polyglycolic acid and other biocompatible copolymers. Glycolic acid can be isolated from natural sources, such as sugarcane, sugar beets, pineapple, or cantaloupe, but it is also chemically synthesized by hydrogenation of oxalic acid with nascent hydrogen or the hydrolysis of the cyanohydrin derived from formaldehyde. Ethylene glycol is a relatively inexpensive starting material for the production of glycolic acid by an oxidation reaction. However, the chemical oxidation reaction of ethylene glycol has certain drawbacks, such as the formation of formaldehyde and other compounds as by-products. To overcome such drawbacks of chemical synthesis, the production of glycolic acid, one of the preferred methods is to use enzymatic production rather than chemical synthesis. The utilization of microbial enzymes also has the major advantage of promoting simple and eco-friendly industrial-scale production. We therefore designed a new enzymatic method for the production of glycolic acid from ethylene glycol using two microbial oxidases; ethylene glycol is first converted to glycolaldehyde by an ethylene glycol-oxidizing enzyme, and the resulting glycolaldehyde is then oxidized to glycolic acid by an aldehyde oxidase (ALOD) (Figure 1).

In order to establish this new enzymatic method for production of glycolic acid by two oxidases, we previously demonstrated that the alcohol oxidases (EC 1.1.3.13) from methanolytic yeasts such as Candida sp. and Pichia pastoris [1] or glycerol oxidase from Aspergillus japonicus [2] catalyzed the oxidation of ethylene glycol to glyoxal via glycolaldehyde, and the reaction rate of ethylene glycol oxidation was much faster than that of glycolaldehyde oxidation [3]. Thus, these alcohol oxidases and glycerol oxidase are available for accumulation of glycolaldehyde in a high concentration from ethylene glycol [3]. Subsequently, we isolated a new bacterial strain, Burkholderia sp. AIU 129, that produces an ALOD catalyzing the oxidation of glycolaldehyde (Figure 2).

The ALOD from Burkholderia sp. AIU 129 (BurkALOD) exhibited high activity for a wide variety of aldehydes including glycolaldehyde, but no activity for glycolic acid. This substrate specificity is an attractive advantage for the efficient production of glycolic acid from glycolaldehyde, because the enzyme did not catalyze further oxidation of glycolic acid into glyoxylic acid. This ALOD consisted of three different subunits (aβγ structure), in which the a, β, and γ subunits were 76 kDa, 36 kDa, and 14 kDa, respectively. These subunits were similar to a putative molybdenum-binding protein subunit, a putative FAD-binding subunit, and a putative iron-sulfur-binding subunit of xanthine dehydrogenase from Burkholderia phenoliruptrix BR3459a [4], respectively, indicating that the BurkALOD might belong to the ALOD group in the xanthine oxidase family.

Microbial ALODs consisting of three different subunits have also been reported from Pseudomonas sp. KY4690 [5], Pseudomonas stutzeri IFO12695 [6], Methyllobacillus sp. KY4400 [7], and Streptomyces rimosus ATCC10970 [8] (Table 1). However, their N-terminal amino acid sequences were not similar to those of the BurkALOD, although the 39 kDa-subunit of ALOD from Pseudomonas sp. KY4690 was similar to the β subunit of our ALOD (53% identity) (Table 2). In addition, above four ALODs did not exhibit glycolaldehyde-oxidizing activity, although they did exhibit broad substrate specificity for aliphatic and aromatic aldehydes such as formaldehyde, acetaldehyde and benzaldehyde (Table 1).

Moreover, the enzymatic properties of those enzymes, such as optimal pH and temperature, were also different from those of our ALOD. To date, glycolaldehyde oxidase activity has been reported in another two ALODs from Pseudomonas sp. AIU 362 [9] and Pseudomonas sp. MX-058 [10]. However, the structure, molecular mass and enzymatic properties of these ALODs are different from those of the BurkALOD (Table 1).

Thus, the BurkALOD was markedly different from other microbial ALODs, and our ALOD with high glycolaldehyde oxidase activity is the first report of an ALOD consisting of three heterosubunits.

The BurkALOD has potential for the enzymatic production of glycolic acid from ethylene glycol when it is used in combination with the alcohol oxidases or glycerol oxidase. In addition, this enzymatic method could be further expanded to a novel enzymatic method for the production of glyoxylic acid from ethylene glycol by the addition of four ALODs containing a glycolic acid-oxidizing enzyme. We recently found an alcohol oxidase with glycolic acid-oxidizing activity in Ochrobactrum sp. AIU 033 [11] (Figure 1). We are currently performing studies on the optimization
of reaction conditions for efficient production of glycolic acid and glyoxylic acid using a combination of two or three microbial oxidases. Such optimized conditions would make it possible to produce not only glycolic acid but also glyoxylic acid, which is used for the chemical synthesis of vanillin, antibiotics or agrochemicals, from ethylene glycol.

References


10. The authors of the table cited that the purification and characterization of an aldehyde oxidase from Pseudomonas sp. KY 4690, FEMS Microbiol Lett 229: 53-56. The table provides the N-terminal amino acid sequences of ALOD from Burkholderia sp. AIU 129 with other microbial ALODs.