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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 for 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 into glycolic acid [4].

The ALOD from *Burkholderia* sp. AIU 129 (*Burk*ALOD) 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 (αβγ structure), in which the α, 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 *Burk*ALOD might belong to the ALOD group in the xanthine oxidase family.

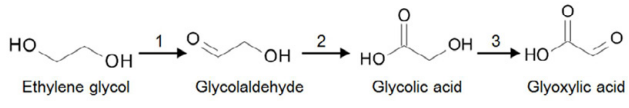

Figure 1: Pathway of glycolic acid and glyoxylic acid production by microbial oxidases. 1, alcohol oxidases from *Candida* sp. and *P. pastoris* or glycerol oxidase from *A. japonicus*; 2, aldehyde oxidase from *Burkholderia* sp. AIU 129; 3, alcohol oxidase from *Ochrobactrum* sp. AIU 033.

Microbial ALODs consisting of three different subunits have also been reported from *Pseudomonas* sp. KY4690 [5], *Pseudomonas stuzeri* IFO12695 [6], *Methylobacillus* sp. KY4400 [7], and *Streptomyces rimosus* ATCC10970 [8] (Table 1). However, their N-terminal amino acid sequences were not similar to those of the *Burk*ALOD, 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 *Burk*ALOD (Table 1).

Thus, the *Burk*ALOD 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 *Burk*ALOD 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 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

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Origin	*Burkholderia* sp. AIU 129	*Pseudomonas* sp. KY4690	*Pseudomonas stutzeri* IFO 12695	*Methylobacillus* sp. KY 4400	*Streptmyces rimosus* ATCC10970	*Pseudomonas* sp. AIU 362	*Pseudomonas* sp. MX-058	
							F10	F13
Substrate specificity (%)								
Acetoaldehyde	100	100	100	100	100	100	100	100
Formaldehyde	68	13	4	286	17	22	-	-
Benzaldehyde	87	90	80	371	57	75	335	131
Glyoxal	70	-	-	-	30	95	160	149
Glycolaldehyde	53	-	-	-	-	7	91	34
Glycolic acid	0	-	-	-	-	0	0	0
Optimum-pH	Below 6.0	-	7.0	3.0-4.0	7.0	6.0	3.5-7.0	3.5-7.0
Optimum-temp (°C)	50	-	37	50	30	40-45	65	60
Molecular mass (kDa)	130	132	160	142	150	95	150	150
Subunit (kDa)	14, 36, 76 (Heterotrimer)	18, 39, 88 (Heterotrimer)	18, 38, 83 (Heterotrimer)	18, 38, 88 (Heterotrimer)	23, 39, 79 (Heterotrimer)	27 (Homotetramer)	9, 14, 39, 80 (Heterotetramer)	9, 14, 22, 39, 58 (Heteropentamer)
Reference	[4]	[5]	[6]	[7]	[8]	[9]	[10]	

Table 1: Comparison of characteristics of ALOD from *Burkholderia* sp. AIU 129 with other microbial ALODs.

Origin	Subunit (kDa)	N-terminal amino acid sequence	Reference
Burkholderia sp. AIU 129	14	LLDVAPSDVQRVPVSFDINGKKEVF	
	36	MNRFSYTRANEVSQAIEQARAKGAA	[4]
	76	IETLPALRASGVPHKDVDGRLK	
Pseudomonas sp. KY4690	18	-	
	39	MNRFGYAKPNAVPDA	[5]
	88	VVHLQKAVPRVDGPL	
Streptmyces rimosus ATCC10970	23	QKPEFVYTXYG	
	39	MKPFRYERATDAAHA	[8]
	79	SESRAVGADFERADA	
Pseudomonas sp. AIU 362	27	MRIAFIGLGNMGAPMARNLIKAGHQLNLFDLNQTVLAELAELGGQVSASPKD	[9]
Pseudomonas sp. MX-058 (F10)	9	TPIPGSSALGOPMDRVDGLKVTGHARYAGEYPEAGLLHSXXV	
	14	AXGHSIALTTNGQTRRVDDQPPTTLLDDLREEQDDVGTKQ	[10]
	39	MNPFSYSKPDDITQAVNLAGPASRFIAGGTNLLDLMKENIARP	
	80	DAAEGSPFRPFYNDRVLYSGQPLALVVA	
Pseudomonas sp. MX-058 (F13)	9	TPIPGSSALGOPMDRVDGLKVTGHARYAGEYPEAGLLHSXXV	
	14	AXGHSIALTTNGQTRRVDDQPPTTLLDDLREEQDDVGTKQ	
	22	ESSHETLEVQIDAKPDDKREGYSTATXH	[10]
	39	MNPFSYSKPDDITQAVNLAGPASRFIAGGTNLLDLMKENIARP	
	58	DAAEGSPFRPFYNDRVLYSGQPLALVVA	

Table 2: N-terminal amino acid sequences of ALOD from *Burkholderia* sp. AIU 129 and other microbial ALODs.

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.

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