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Expression, purification and characterization of a quinoprotein L-sorbose dehydrogenase from *Ketogulonicigenium vulgare* Y25

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It is well known that *Ketogulonicigenium vulgare* Y25 could effectively oxidize L-sorbose to 2-keto-L-gulonic acid (2KGA), an industrial precursor of vitamin C. There in, L-sorbose dehydrogenase is one of the key enzymes responsible for the production of 2KGA. From this organism, the coding region of *sdh* gene was cloned into pET22b plasmid and its transcription product was overexpressed. This procedure allowed purification of L-sorbose dehydrogenase and production of polyclonal antibodies. In Western blot assays, the antibodies gave a positive reaction against bacteria protein extract and purified L-sorbose dehydrogenase. The molecular mass of the enzyme was 60532 Da, and the N-terminal amino acid sequence was determined to be QTAIT. The Native-PAGE and resting-cell reaction assay showed that purified L-sorbose dehydrogenase could convert L-sorbose to 2KGA, and PQQ was found to be indispensable for its activity as prosthetic group. The enzyme showed broad substrates specificity and the K_m value for L-sorbose and 1-propanol was 21.9 mM and 0.13 mM, respectively. The optimum pH of the enzyme activity was 8.0, and the optimum temperature was 35°C. The activity of the L-sorbose dehydrogenase was greatly stimulated by Ca^{2+} and strongly inhibited by Co^{2+} and Cu^{2+}. The results obtained from the present study showed that a PQQ-dependent L-sorbose dehydrogenase could oxidize L-sorbose into 2-keto-L-gulonic acid *in vitro*.

Key words: *Ketogulonicigenium vulgare*, 2-keto-L-gulonic acid, L-sorbose dehydrogenase, quinoprotein.

INTRODUCTION

L-ascorbic acid, commonly known as vitamin C, is generally used in pharmaceutical, food, cosmetic and feedstuff industries as an essential nutrient and a powerful antioxidant. The most popular process for synthesizing vitamin C is Reichstein's method in the last century (Reichstein et al., 1934). Because of economical and ecological advantages, the use of microbial processes to produce vitamin C became attractive in the recent years. In 1990, Yin et al. established a method, converting L-sorbose into 2-keto-L-gulonic acid (2KGA, a precursor of L-ascorbic acid) by an artificial microbial ecosystem consisting of *Gluconobacter oxydans* and *Bacillus* sp. (Yin et al., 1990). So far, the co-culture method has been widely used in China. In the mix culture, *Bacillus* sp. functions as the companion bacteria to accelerate the growth of *G. oxydans*, while *G. oxydans* is responsible

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for the bioconversion of 2KGA from L-sorbose.

It is generally thought that the conversion process in *G. oxydans* comprises two successive reactions concerned with the oxidization of L-sorbose into L-sorbosone by the membrane-bound L-sorbose dehydrogenase and subsequent oxidization of L-sorbosone into 2KGA by the cytoplasmic L-sorbosone dehydrogenase (Miyazaki et al., 2006), namely “sorbosone pathway” (Makover et al., 1975). The rate limiting reaction is the synthesis of L-sorbosone. L-sorbose dehydrogenase is reported to be a 58-kDa protein, containing one molecule of FAD as an electron acceptor. And L-sorbosone dehydrogenase requires NAD(P) as an electron acceptor (molecular mass, approximately 50 kDa).

However, Urbance et al. (2001) proposed the reclassification of *G. oxydans* in vitamin C production to the genus *Ketogulonicigenium*. Moreover, L-sorbose dehydrogenase from *Ketogulonicigenium vulgare* DSM4025 (originally identified as *G. oxydans* DSM4025) was found as a new quinoprotein dehydrogenase catalyzing not only the conversion of L-sorbose to L-sorbosone, but also that of L-sorbosone to 2KGA *in vitro* (Asakura et al., 1999). These results suggested that the characterization of L-sorbose dehydrogenase in *K. vulgare* is obviously different from that described in the sorbosone pathway.

Recently, the whole genome sequencing of *K. vulgare* and *B. megaterium* has been completed (Xiong et al., 2011; Liu et al., 2011a, b). In addition, genome-scale metabolic models (GSMMs) of the two strains have also been reconstructed (Zou et al., 2012, 2013). Much progress has also been made in understanding their relation and the nutrient requirement of *K. vulgare* (Ma et al., 2011, 2012; Zhou et al., 2011.). However, the detailed mechanism of 2KGA biosynthesis in *K. vulgare* remains to be elucidated.

Since L-sorbose dehydrogenase played significant roles in the 2KGA conversion processes, we described the cloning, expression, purification and characterization of L-sorbose dehydrogenase from *K. vulgare* Y25 in the present report.

MATERIALS AND METHODS

Microorganisms and chemicals

The strain of *K. vulgare* Y25 used in this study was obtained from Weisheng Pharmaceutical Co. Ltd., and preserved in our laboratory. *Escherichia coli* strain DH5α and BL21(DE3) were obtained from TransGen Biotech. L-sorbose and 2,6-dichlorophenolindophenol (DCIP) were purchased from Merck Co., Ltd. Phenazine methosulfate (PMS), nitroblue tetrazolium (NBT) and pyrroloquinoline quinone (PQQ) were from Sigma-Aldrich. A goat anti-rabbit horseradish peroxidase conjugate and BCA protein assay kit were supplied from CW Biotech. Protease inhibitor cocktail was bought from Roche. Other chemicals were commercially available and of reagent grade.

Expression and purification of L-sorbose dehydrogenase

The L-sorbose dehydrogenase gene (*sdh*) was amplified by PCR using fresh *K. vulgare* Y25 culture as template. The oligonucleotides were designed based on the reported *sdh* gene coding sequence (EIO_2658). The primers used for *sdh* gene amplification were: forward (5’-GGCATATGAAACCGACTTCG CTGC), reverse (5’-CCGCTCGAGTTATTGCGGCAGGGCGAAG). Restriction sites for *Nde*I and *Xho*I were included in the amplificated fragment. The PCR product was ligated into pET22b and the resultant plasmid was transformed into *E. coli* BL21(DE3) following the manufacturer's instruction. Transformed cells were plated on LB agar, supplemented with ampicillin (100 μg/ml) and cultivated overnight at 37°C. One positive colony was picked from LB plate and inoculated into 5 ml of LB medium containing the same antibiotics. After shake incubation overnight, the culture was transferred to a 1-L conical flask containing 200 ml LB and cultivated for 2 to 5 h (until cell concentration reached an OD of 0.5 to 0.8 at 600 nm). At this time, IPTG was added to the culture medium at a final concentration of 0.5 mM and the cells were incubated for at least 4 h under the same condition. Then, a total of 2 L culture was harvested by centrifugation at 4°C (5,000 ×*g* for 10 min) and the pellet was resuspended in 50 ml of lysis buffer (50 mM Tris-HCl, a tablet of protease inhibitor cocktail, pH 8.0). The above cell suspension was disrupted by sonication (30 min for 5 s pulses of 30 W, leaving 5 s between each pulse) and centrifuged in 4°C at 15,000 ×*g* for 20 min. The supernatant was stored for the following purification.

Step 1: DEAE-sepharose column chromatography

The soluble fraction was loaded on a DEAE-sepharose column, which had been equilibrated with the TE buffer (50 mM Tris-HCl，pH 8.0). After the column was washed with 200 ml of the same buffer, the protein was eluted by a linear gradient of NaCl form 0 to 1.0 M in the same buffer (1 l). The active fractions were collected and used for the next step.

Step 2: Q-sepharose column chromatography

The pooled fraction in the previous step was dialyzed with the citrate buffer (50 mM citric acid-citrate sodium, 50 mM sodium chloride). The sample was put on a Q-sepharose column, which had been equilibrated with the same buffer. After the column was washed with the buffer to the baseline, the elution was done with a linear gradient of 0.2 to 0.4 M NaCl in the same buffer (200 ml). The fractions, which contained a protein peak with specific activity of sorbose dehydrogenase, were desalted and concentrated to 20 ml with PBS by centrifugation-ultrafiltration using a PM-10 membrane.

Measurement of protein concentration

BCA protein assay reagent was used for the determination of protein concentrations with bovine serum albumin as the standard.

Protein electrophoresis

Conventional sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis (PAGE) was done on 12.5% polyacrylamide gels as described by Laemmli et al. (1970). The supernatant samples containing 2 mg protein were subjected to SDS-PAGE analysis and stained with Coomassie Brilliant Blue G-250 (CBB).

Preparation of anti-SDH antibody and immunological analysis

For anti-SDH antibody preparation, 2 ml of an emulsion containing 50 mg purified L-sorbose dehydrogenase in Freund’s complete adjuvant (1:1) was injected intradermally into a healthy male New Zealand white rabbit aged 3 to 4 months (body weight ~2.5 kg) following the protocol described by Hu et al. (2002).

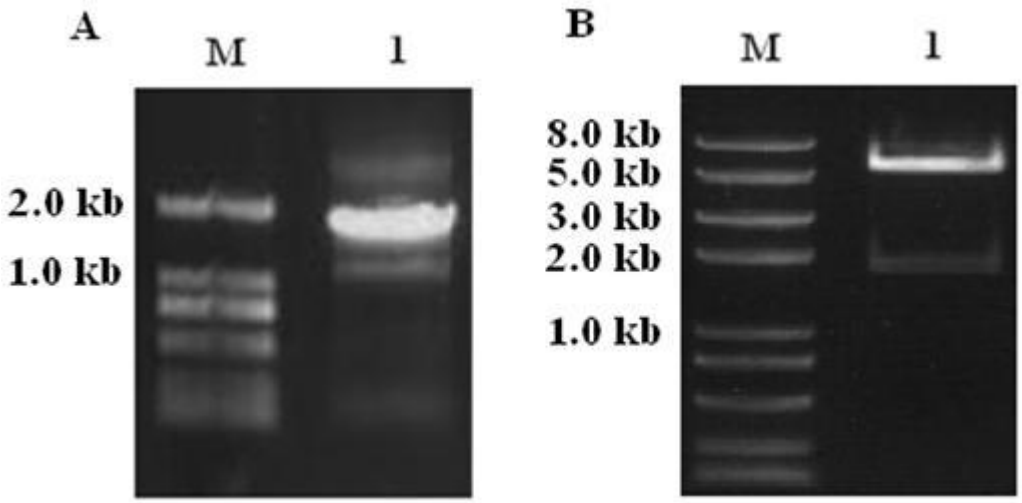

Figure 1. Construction of pET22b-*sdh*. A: Analysis of *sdh* PCR products (lane 1) by 1% agarose gel electrophoresis. B: 1% agarose gel electrophoresis confirming the expression plasmid pET22b-sdh (lane 1) digested by *Nde*I-*Xho*I.

After SDS-PAGE, the proteins were electrotransferred using an electrophoretic transfer cell (Mini Trans-Blot®, BioRad) to a polyvinylidene difluoride (PVDF) membrane previously activated in pure methanol for 20 s. The transference was carried out at 60 V for 90 min in 1X transfer buffer, which contained (l l) 14.4 g glycine, 3.0 g Tris and 100 ml methanol in distilled water. The membranes (with transferred proteins) were incubated at 25°C for 30 min in membrane-washing buffer (0.05% (v/v) Tween 20 and 5% (w/v) skim milk in PBS). Then, the anti-SDH antibodies (1:5,000) were added and incubated for 1 h under the same conditions. The solution was discarded and the membrane were gently washed three times (1 min each) with the same buffer and incubated again under similar conditions in washing buffer with 5% (w/v) skim milk containing goat anti-rabbit HRP conjugate (1:5,000). The membranes were washed three times (1 min each) with membrane-washing buffer and finally developed with 0.05% (w/v) 3,3'-dia-minobenzidine tetrahydrochloride, 0.02% (w/v) nickel chloride hexahydrate and 0.1% (v/v) of 30% hydrogen peroxide. The developing procedure was terminated with distilled water.

Determination of the N-terminal sequences of the protein

Purified L-sorbose dehydrogenase (100 µg) was subjected to SDS-PAGE, and the protein band was electroblotted on a PVDF membrane. The membrane was subjected to N-terminal amino acid sequencing by Edman degradation.

L-sorbose dehydrogenase activity assay

For L-sorbose dehydrogenase activity staining, native PAGE (pH 8.8) was performed with a 10% polyacrylamide gel by using a method described by Davis et al. (1964). The gel was immersed in a staining solution (20 ml) consisting of 100 mM Tris-HCl buffer (pH 8.0), 0.1 mg/ml NBT, 0.1 mg/ml PMS and 2 mg/ml L-sorbose with gently shaking at 25°C for 20 min. The reaction was terminated by immersing the gel in distilled water.

Resting-cell reaction system for *E. coli* transformants

E. coli cells were cultivated in LB medium at 37°C overnight with shaking. Cells were collected from 2 ml of culture broth by centrifugation, and washed with 0.9% NaCl, and suspended in 400 µl of resting cell mixture consisting of 0.3% NaCl, 1% $CaCO_3$, 4% L-sorbose and 1 mM PMS. 1 µg/ml PQQ was added to the reaction mixture because *E. coli* does not produce PQQ. The mixture was incubated at room temperature (25°C) with gently shaking for 20 h. The reaction mixture was detected by Thin-layer chromatography (TLC).

Enzyme assay

The basal reaction mixture for determining the activity of L-sorbose dehydrogenase consisted of 50 mM DCIP and 0.325 mM PMS, which was prepared just before the experiment. A curve had a 1-cm light path and contained 0.4 ml of the basal reaction mixture, 0.1 ml of 0.4 M L-sorbose and the enzyme solution, and water with a total volume of 0.51 ml. The reference cuvette contained all components without substrate. The reaction was started at 25°C with L-sorbose, and the enzyme activity was measured at the initial reduction of 1 µM of DCIP per min.

RESULTS

Construction of pET22b-*sdh*

Based on the *sdh* gene reported in NCBI GenBank (EIO_2658), we designed a pair of PCR primers for amplifying *sdh* fragment; it was clear in 1% agarose stained with 0.5 µg/ml ethidium bromide (Figure 1a). The amplified fragment was digested with *Nde*I-*Xho*I and ligated into vector pET22b digested by the same enzymes. This constructed plasmid was transformed into the competent cells of *E. coli* DH5α to select the positive colonies. The integrity and fidelity of the fragment from the recombinant plasmid was checked by enzymatic digestion (Figure 1b) and sequencing. After sequence alignment between the amplified sequence and original sequence from NCBI GenBank, we found that both the open reading frames

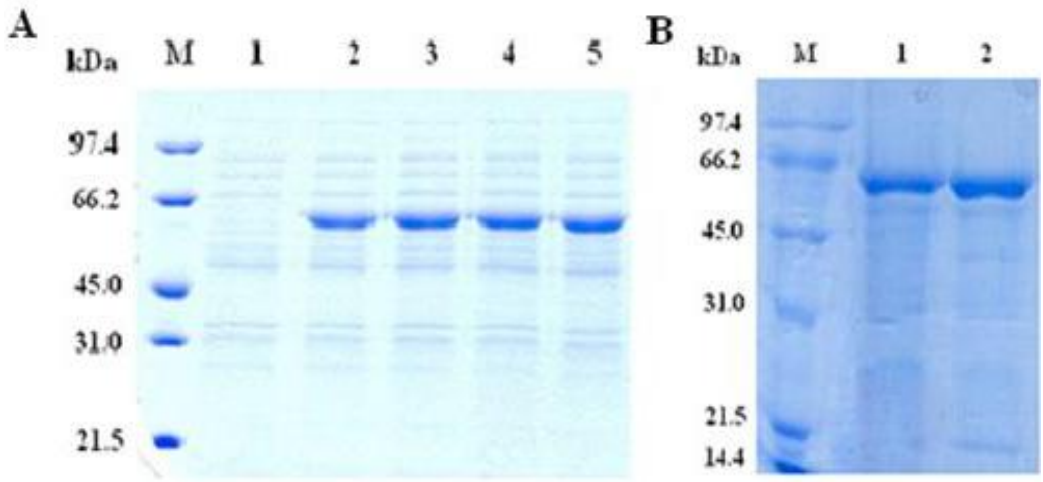

Figure 2. 12.5% SDS-PAGE analysis of the expression of L-sorbose dehydrogenase. A: the crude extracts (lanes 2 to 5) were obtained from pET22b-*sdh* transformed BL21(DE3) cultures grown for 4 h in the presence of 0.5 mM IPTG. Extract (lane 1) was not induced by IPTG. B: 12.5% SDS-PAGE showing the purified L-sorbose dehydrogenase (lanes 1 and 2).

Figure 3. Western blot using rabbit anti-SDH antibodies against extracts from *K. vulgare* Y25 cultures (lane 1), pET22b-sdh/BL21(DE3) cultures after induction for 4 h with 0.5 mM IPTG (lane 2), purified L-sorbose dehydrogenase (lane 3). The extracts were electrophoresed on 12.5% SDS-PAGE

and nucleotides of the two sequeces were identical and were ready for protein expression.

Expression and purification of L-sorbose dehydrogenase

In order to characterize the enzymatic activity of the L-sorbose dehydrogenase, the recombinant protein was expressed and purifed from soluble extract of *E. coli* BL21(DE3), which bare a plasmid containing the putative *sdh* expression cassette and was induced with a final concentration of 0.5 mM IPTG for at least 4 h. Then, 12.5% SDS-PAGE was carried out to detect the protein expression. As seen in Figure 2a, the target protein was successfully over-expressed and migrated as a dominant band with a molecular weight of approximately 60 kD, accounting for about 30% of the total protein. To acquire sufficient amounts of protein, typical purification procedures including DEAE- and Q–sepharose chromategraphy were conducted following the protocol described in materials and methods. After two steps of purification, the enzyme was isolated and the final product purity was calculated to be more than 90% using the software of GelPro Analyzer (Figure 2b).

Polyclonal antibodies production

Polyclonal antibodies against the purified L-sorbose dehydrogenase were produced in rabbits, following the procedure of Hu et al. (2002). As shown in Figure 3, the antibodies gave a positive reaction against both cell extracts from strain Y25, recombinant BL21(DE3) as well as the purified protein.

Structural characterization of L-sorbose dehydrogenase

Different from the deduced amino acid sequence of the protein, the N-terminal residuals of purified protein were Gln-Thr-Ala-Ile-Thr (QTAIT), which indicates that the N-terminal 23 amino acid residuals probably constituting signal peptide were cleaved before the protein maturation (Figure 4). The molecular weight of purified protein was determined to be 60,529 Da by mass spectrum analysis, indicated in Figure 5b that 2KGA production in resting-cell reaction was observed by TLC assay. However, hardly any activitiy of L-sorbose dehydrogenase was shown without adding exogenous PQQ in the reaction mixture. Therefore, we speculated that PQQ act as the prosthetic group of L-sorbose dehydrogenase.

Catalytic properties of L-sorbose dehydrogenase

The substrate specificities of purified enzyme for sugars and sugar alcohols were evaluated using the following

MKPTSLLWASAGALALLAAPALA**QTAIT**DEMLANPPAGEWINYGQNQENYRHSPLTQITADNVGQLQLV
WARGMEAGKIQVTPLVHDGVMYLANPGDVIQAIDAATGDLIWEHRRQLPNIATLNSFGEPTRGMALYGT
NVYFVSWDNHLVALDMGTGQVVFDVDRGQGDERVSNSSGPIVANGTIVAGSTCQYSPFGCFVSGHDSAT
GEELWRNYFIPRAGEEGDETWGNDYESRWMTGAWGQITYDPVTNLVHYGSTAVGPASETQRGTPGGTLY
GTNTRFAVRPDTGEIVWRHQTLPRDNWDQECTFEMMVTNVDVQPSTEMEGLQSINPNAATGERRVLTGV
PCKTGTMWQFDAETGEFLWARDTNYQNMIESIDENGIVTVNEDAILKELDVEYDVCPTFLGGRDWPSAA
LNPDSGIYFIPLNNVCYDMMAVDQEFTSMDVYNTSNVTKLPPGKDMIGRIDAIDISTGRTLWSVERAAAN
YSPVLSTGGGVLFNGGTDRYFRALSQETGETLWQTRLATVASGQAISYEVDGMQYVAIAGGGVSYGSGL
NSALAGERVDSTAIGNAVYVFALPQ.

Figure 4. Deduced amino acid sequence of L-sorbose dehydrogenase. Determined N-terminal sequence of matured protein is **bold** and marked in a box. A possible signal peptide sequence is underlined.

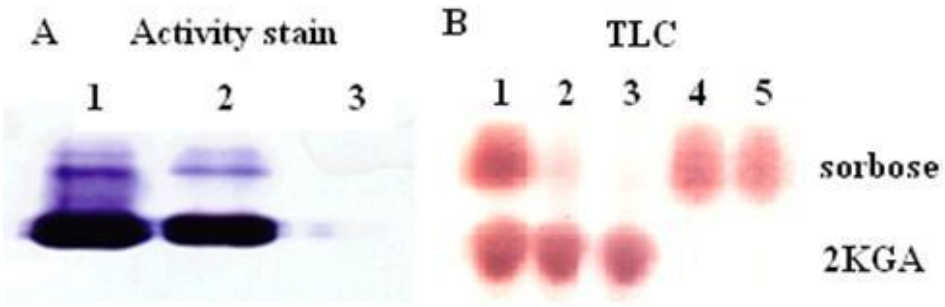

Figure 5. Enzyme activity analysis of L-sorbose dehydrogenase. A: Activity staining of cell extracts of *K. vulgare* (*lane 1*) and purified L-sorbose dehydrogenase with or without PQQ (lane 2 to 3) on a 10% native PAGE. B: TLC analysis of standard of substrate sorbose and product 2KGA (lane 1), *K. vulgare* Y25 resting-cell reaction mixture (lane 2), recombinant *E. coli* resting-cell reaction mixture with PQQ (lane 3), recombinant *E. coli* resting-cell reaction mixture without PQQ (lane 4); reaction mixture without PQQ and recombinant *E. coli* resting-cell (lane 5).

Table 1. Substrate specificities of L-sorbose dehydrogenase. The enzyme was characterized by its substrate specificities using 6 substrates: 1-propanol, D-glucose, D-sorbitol, L-sorbose, D-mannitol and D-fructose. Relative activity is expressed as 100% of the highest reaction rate obtained with L-sorbose.

Substrate	Concentration (mM)	Relative activity (%)
L-sorbose	100	100.0±3.1
1-propanol	100	857.0±30.5
D-glucose	100	6.4±0.2
D-sorbitol	100	32.2±1.1
D-mannitol	100	10.5±0.4
D-fructose	100	43.6±1.9

substrates: L-sorbose, 1-propanol, D-glucose, D-sorbitol, D-mannitol and D-fructose (Table 1). Among the substances tested, 1-propanol was the preferred substrate with a relative activity which is 8-fold higher than that from L-sorbose. The steady-state kinetic which coincided well with the estimated value from PAGE.

Enzyme activity of L-sorbose dehydrogenase

A major activity band was obtained from Native PAGE and staining of the purified protein (Figure 5a). It is analysis was performed by determining the initial velocities of oxidizing reaction using variable concentrations of L-sorbose and 1-propanol from 4 to 160 mM. The K_m values for the two substrates were calculated to be 21.9 and 0.13 mM, respectively, when DCIP was used as the electron acceptor for the reaction. In addition, the optimum pH and temperature for L-sorbose dehydrogenase were about 8.0 and 35°C, respectively, (Figure 6a and b). The enzyme remained stable at 35°C but quickly lost its activity when the temperature reached 45, 55 and 65°C (Figure 6c). The enzyme activity was greatly increased by the addition of Ca^{2+} and strongly inhibited by the trace addition of $Co^{2+}$and Cu^{2+} (Figure 6d).

DISCUSSION

In this study, we cloned a sorbose dehydrogenase gene (EIO_2658) from *K. vulgare* Y25 and presented its enzymatic characters based on protein expression and purification. It is apparent from the results that a PQQ-dependent L-sorbose dehydrogenase could oxidize L-sorbose into 2KGA *in vitro*. Our data provides important information for further study of the physiological function

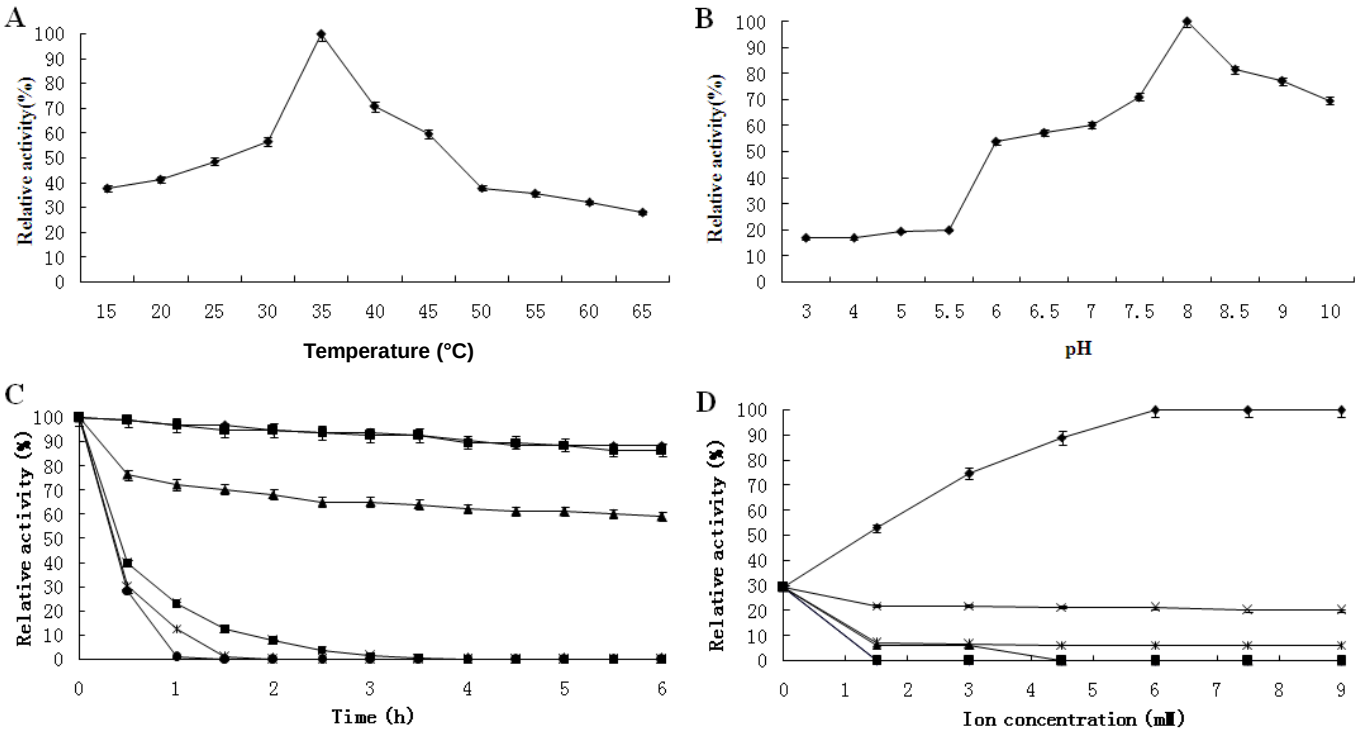

Figure 6. Effects of temperature (A) and pH (B) on the activity of L-sorbose dehydrogenase. C: Thermal stability of L-sorbose dehydrogenase. 10°C (◆) , 25°C (■) , 35°C (▲) , 45°C (×), 55°C (*), 65°C (★). D: Effects of metal ions on the activity of L-sorbose dehydrogenase. The trend curve of $CoCl_2.6H_2O$ is overlapped with that of $CuCl_2.2H_2O$. $CoCl_2.6H_2O$ (■), $CuCl_2.2H_2O$ (★), $ZnCl_2$ (▲), $MgCl_2.6H_2O$(×), $BaCl_2$(*), $CaCl_2$ (◆). Relative activity is expressed as 100% percentage of the highest reaction rate obtained with L-sorbose.

of L-sorbose dehydrogenase *in vivo*.

When compared with L-sorbose/L-sorbosone dehydrogenase in *K. vulgare* DSM4025 reported by Asakura et al. (1999), the L-sorbose dehydrogenase from *K. vulgare* Y25 exhibited similar characters including amino acid sequence, coenzyme, optimum pH and temperature and so on, which was possibly attributed to their close relationship in phylogenesis. However, there were still several different properties. By Mass Spectrum analysis, the molecular weight of mature L-sorbose dehydrogenase from *K. vulgare* Y25 was 60,529 Da, higher than that of L-sorbose/L-sorbosone dehydrogenase from *K. vulgare* DSM4025. Their Km values towards L-sorbose and 1-propanol also showed significant difference. Besides, the activity of L-sorbose dehydrogenase from *K. vulgare* Y25 was greatly increased by the addition of Ca^{2+}, while that of L-sorbose/L-sorbosone dehydrogenase from *K. vulgare* DSM4025 was not affected.

Distinguished from two kinds of dehydrogenase working together in conventional “sorbosone pathway”, sorbose dehydrogenase in *K. vulgare* could sequentially oxidize L-sorbose to 2KGA *in vitro*, which would provide advantage for *K. vulgare* to produce 2KGA (Miyazaki et al., 2006). The unique characterization provides advantage for *K. vulgare* which has been applied to 2KGA production. Secondly, the result of N-terminal sequencing of sorbose dehydrogenase suggested that the 23 N-terminal amino acid were the signal peptide. Hence, sorbose dehydrogenase is considered to be freely soluble in the periplasm or loosely bound to the periplasmic side of the cytoplasmic membrane (Asakura et al., 1999). The active sites of L-sorbose dehydrogenase are exposed to

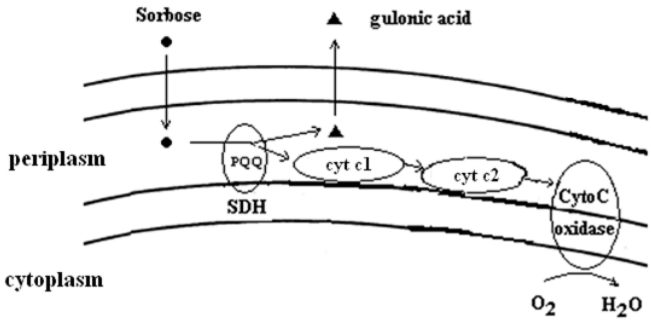

Figure 7. The potential electron transport chain in the sorbose oxidation process. Soluble cytochrome c551 (EIO_2635) is considered to be the first cytochrome c in the electron transport chain, and EIO_0329 or EIO_0898 were estimated to be the second cytochrome c. EIO_2445, EIO_0767 and EIO_0772 were suggested to be the subunit I, II, III of aa3-type cytochrome c oxidase.

the outside of the inner cell membrane, therefore substrate, sorbose, is directly oxidized in the periplasmic space. The product, 2KGA, will be released into the medium via porines presented in the outer membrane of *K. vulgare*. Thus, L-sorbose dehydrogenase seems to lend itself to rapid substrate and harmful molecules oxidation. This property is also favorable to 2KGA production for *K. vulgare*.

L-sorbose dehydrogenase showed very broad substrate specificities, similar to other membrane-bound dehydrogenases in oxidative fermentation (Zhang et al., 2006; Sugisawa et al., 1991). It may be the reason why it is constitutively expressed, but not regulated by the carbon source (data not published). Sorbose oxidation is coupled with the respiratory chain of the organism, and the electrons generated are transferred to the terminal oxidase yielding oxidation energy. According to the property of prosthetic group, L-sorbose dehydrogenase is classified as type I quinoprotein. It is analogous to methanol dehydrogenase in methylotrophic bacteria, in which methanol-oxidizing system consists of a soluble quinoprotein dehydrogenase, soluble cytochrome c, and a membrane-bound terminal oxidase (Anthony et al., 2003; Quilter et al., 1984; Beardmore et al., 1983). The presence of high amounts of soluble cytochrome c551 in the periplasm of *K. vulgare* was observed and it has been proved to physiologically act as an electron acceptor of L-sorbose dehydrogenase by *in vitro* reconstruction of 2KGA production system from L-sorbose (Asakura et al., 1999). Therefore, we speculated that there is a periplasmic electron transport chain responsible for oxidation of L-sorbose to 2KGA in *K. vulgare* as follows: L-sorbose → SDH → cytochrome c1 → cytochrome c2 → cytochrome c oxidase → O_2 (Figure 7). Soluble cytochrome c551 (EIO_2635) is considered to be the first cytochrome c in the electron transport chain, similar to cytochrome c subunit of many dehydrogenases. The second cytochrome c (EIO_0329 or EIO_0898) is estimated to be membrane-bound and low-abundance. According to *K. vulgare* genome annotation and bioinformatics analysis, cytochrome c oxidase (EIO_2445, EIO_0767 and EIO_0772) is suggested to be classified into aa3-type (Drosou et al., 2002; Kishikawa et al., 2010).

Analyzed from the ratio of expressed L-sorbose dehydrogenase in total protein, the yield of sorbose dehydrogenase was estimated to be about 1 g/L. The high expression level and subsequent facile purification process make *E. coli* an efficient and cost-effective host for the large-scale production of L-sorbose dehydrogenase for both research and industrial purpose.

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