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Expression of Human Mitochondrial Aldehyde Dehydrogenase 2 in Mammalian Cells using Vaccinia Virus-T7 RNA Polymerase

Su-Min Kang, Seung-Ku Yoo*, and Ki-Hwan Lee

Department of Biotechnology, College of Engineering and Bioproducts Research Center, Yonsei University, 134 Shinchon-Dong, Sudaemun-Ku, Seoul 120-749, Korea

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Human mitochondrial aldehyde dehydrogenase 2 (ALDH2) is mainly responsible for oxidation of acetaldehyde generated during alcohol oxidation *in vivo*. A full-length cDNA of human liver ALDH2 was successfully expressed using a vaccinia virus-T7 RNA polymerase system. The expressed ALDH2 had an enzymatic activity as high as the native human liver ALDH2 enzyme.

Key words: Acetaldehyde, human mitochondrial aldehyde dehydrogenase 2 (ALDH2), vaccinia virus T7 RNA polymerase

Aldehyde dehydrogenases are a group of enzymes which catalyze a broad range of aldehydes to corresponding acids by NAD^+-dependent irreversible reactions. ALDH isozymes from liver tissues of several mammals, including human, horse, sheep, and pig have been studied (10, 16). Several ALDH isozymes have been characterized and classified by their kinetics, isoelectric focusing characteristics, and tissue localizations (5). Complementary DNAs of class 1, 2, and 3 ALDHs (cytosolic, mitochondrial, and human stomach) have been cloned and expressed in *Escherichia coli* (*E. coli*)(17). Other ALDH isozymes, such as ALDH 1-7, have also been cloned and expressed in *E. coli* (6).

Among the isozymes of human mitochondrial aldehyde dehydrogenase, ALDH2 has a low Km value for acetaldehyde and a high catalytic efficiency and is known to be mainly responsible for oxidation of acetaldehyde generated during alcohol oxidation *in vivo* (2). ALDH2 is a homotetrameric enzyme composed of 4 subunits of 55 kDa each. Genetic polymorphism of ALDH2 was also investigated (9). A glutamate to a lysine substitution at residue 487 of the ALDH2 gene renders the enzyme inactive (15). Uncatalyzed acetaldehydes in the body cause flushing and hangover after alcohol absorption (11, 13).

ALDH inhibitors such as disulfiram (12, 18) and activators such as cyanamide, benzaldehyde, methylene blue, nitroxyl analogs, and thyroxine analogs (7) have been developed. ALDH2 produced in an *E. coli* system has been used primarily for screening ALDH inhibitors and activators. Native ALDH2 or an ALDH2 similar to the native variety need to be tested for effects of ALDH inhibitors and activators screened by *E. coli*-derived ALDH2 system since the ALDH2 expressed in *E. coli* may be different from native ALDH2 in several aspects such as folding, glycosylation, phosphorylation, subunit assembly, secretion, and proteolytic processing which would affect the enzyme activity (1, 8).

There have been previous reports regarding expression of human ALDH2 in HeLa cells by retroviral vector (15). Vaccinia virus-T7 RNA polymerase expression system which was used in this study has some merits compared with the previous reports (15). In vaccinia virus-T7 RNA polymerase expression system, induction time can be easily controlled and high level of expression can be achieved by the T7 RNA polymerase of recombinant vaccinia virus in the cytoplasm. However, in our system, ALDH2 is expressed transiently and continuous production of ALDH2 is unobtainable (4).

As part of a basic study for expression of eukaryotic proteins and for developing a complementary screening system for ALDH2 activators and inhibitors, this report describes successful expression of the ALDH2 protein using a vaccinia virus-T7 RNA polymerase expression system in mammalian cells (3).

Materials and methods

Plasmids

6×His tag sequence and ALDH2 cDNA were ob-

* To whom correspondence should be addressed.
(Tel) 82-2-361-2887; (Fax) 82-2-362-7265
(E-mail) skyoo@bubble.yonsei.ac.kr

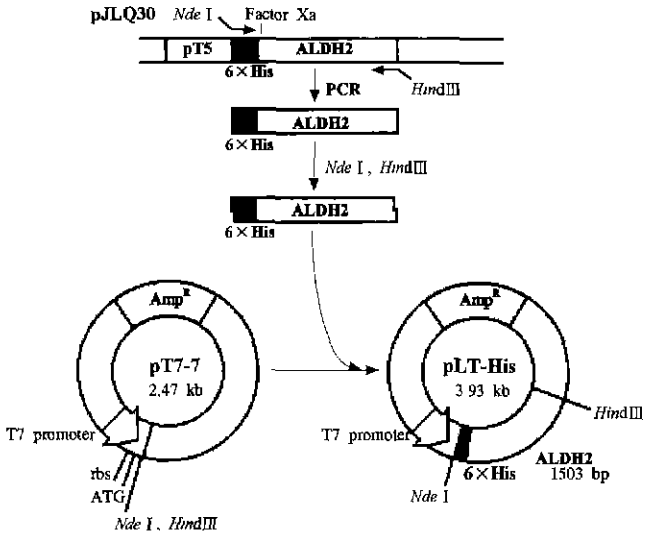

Fig. 1. Construction of recombinant plasmid used for ALDH 2 expression.

tained from pJLQ30 vector by polymerase chain reaction (PCR)(8). Primer sequences used in PCR are shown below:

primer1 : CATATGAGAGGATCGCATCACCATC-AC

primer2 : ATCAAGCTTATGAGTTCTTCTGAGG

The recombinant plasmid pLT-His was constructed by inserting 6×His tag and ALDH2 sequence into pT7-7 vector using *Nde*I/*Hind*III restriction sites (Fig. 1). The recombinant plasmids (pLT-His) used for transfection were obtained from bacterial strain JM109 by alkali lysis and purified by polyethyleneglycol (PEG) precipitation.

Cells and viruses

Recombinant vaccinia virus (vTF7-3) which expresses T7 RNA polymerase was obtained from the American Type Culture Collection (ATCC). Recombinant vTF7-3 viruses were grown in HeLa S3 cells. They were titered and expressed in BS-C-1 cells. HeLa S3 cells and BS-C-1 cells were also obtained from ATCC. Cell lines were grown in minimal essential medium(MEM) containing 10% fetal bovine serum (FBS).

Expression of human liver ALDH2 by lipofection

HeLa S3 and BS-C-1 cells were grown in 25 cm^2 flasks with MEM plus 10% FBS. When the cells had grown to 90% confluency, they were infected with vTF 7-3 at a multiplicity of infection (m.o.i.) of 5~30 for 2 h. The inoculum was removed, and 2.5% FBS (5 ml) containing 1 mg ml-1 lipofectin (DOTAP from Boehringer Mannheim) and 5 μg of pLT-His were added to the flask. The culture was incubated at 37°C for 24~36 h.

Expression of human liver ALDH2 by electroporation

HeLa S3 cells were cultured in a 175 cm^2 flask with 10% FBS-MEM to 90% confluency. Then 2.7×10^6 cells were harvested and washed with phosphate-buffered saline (PBS: 137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4). After resuspension in 900 μl PBS, the cells were transfected with 20 μg of pLT-His and 50 μg of salmon sperm carrier DNAs under electroporation conditions of 200V, 18.2 ms, 1050 μF, and 125 Ohm. The transfected cells were added gently to 10% FBS containing MEM, infected with vTF7-3 with 10 m.o.i, and incubated at 37°C for 24~36 h.

Western blot analysis

Whole cell protein extract (900 μg) was denatured using SDS, separated by polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, incubated with 6×His antibodies for 30 min, then washed with TBST (20 mM Tris, 150 mM NaCl, 0.05% Tween 20, pH7.5) twice. Membranes were then incubated for 30 min with anti-mouse IgG whole molecule conjugated to alkaline phosphatase, washed twice with TBST, and developed using a NBT/BCIP solution.

ALDH2 enzyme activity

ALDH2 activity toward acetaldehydes was measured spectrophotometrically as an increase in absorbance at 340 nm due to reduction of NAD^+. The assay was performed 3 times at 25°C in a 0.1 M pyrophosphate buffer containing 10 mM propylaldehyde as a substrate and 10 mM NAD as a cofactor (14). The blank test was performed without cofacter NAD^+. Electroporated HeLa S3 cells were sonicated by microsonicator for 1 min, and soluble proteins were used for enzyme assay. One unit of ALDH activity was defined as the amount of enzyme required to catalyze the reduction of 1 mol of NAD' per minute at 25°C. A molar absorbancy of 6220 $M^{-1}cm^{-1}$ for NADH at 340 nm was used for calculations.

Results

Both the lipofection and electroporation transfection methods were used for expression of ALDH2. First, vTF7-3 infected HeLa S3 cells at different multiplicity of infection values (5, 10, and 30 m.o.i.) were transfected with pLT-His. Expressed ALDH2 was compared with purified ALDH2, which was expressed in *E. coli*. As shown in Fig. 2(A), Western blot analysis after SDS-PAGE confirmed that pLT-His transfected by lipofection and vTF7-3 infected

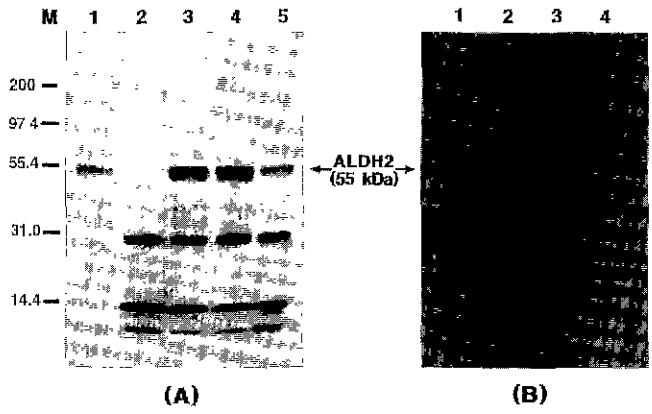

Fig. 2. Western blot analysis of ALDH2 expressed in HeLa S3 cells. (A) ALDH2 expressed in HeLa S3 cells by lipofection. Lane 1, ALDH2 expressed in *E. coli*; Lane 2, HeLa S 3 cells infected with a m.o.i. value of 10 for vTF7-3 alone; Lanes 3, 4, and 5, HeLa S3 cells transfected with 5 μg of pLT-His by lipofection and infected with m.o.i. values of 5, 10, and 30 for vTF7-3, respectively. (B) ALDH2 expressed in HeLa S3 cells by electroporation. Lane 1, ALDH2 expressed in *E. coli*; Lane 2, HeLa S3 cells infected with a m.o.i. value of 10 for vTF7-3 alone; Lanes 3 and 4, HeLa S3 cells infected with a m.o.i. value of 10 for vTF7-3 and transfected with 20 μg of pLT-His by electroporation. 2.7×10^6 HeLa S3 cells were used for both lipofection and electroporation.

cells expressed 55 kDa ALDH2. A value of 10 m.o.i. for vTF7-3 was appropriate for ALDH2 expression. Transfection by electroporation also resulted in expression of 55 kDa ALDH2 (Fig. 2(B)). The expression level was lower than that for lipofection, probably due to fetal cell damage during the electric shock.

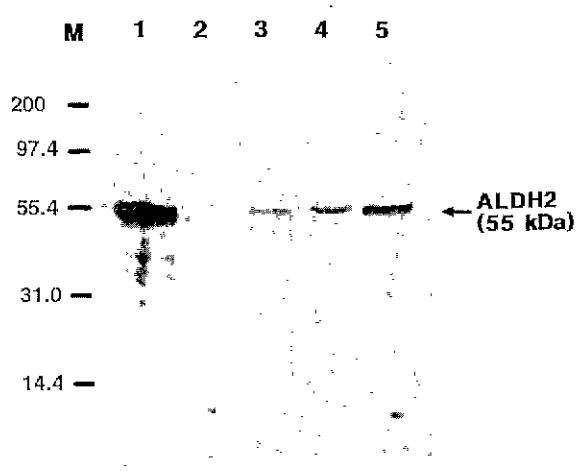

Fig. 3. Western blot analysis of ALDH2 expressed in BS-C-1 cells. Lane 1, ALDH2 expressed in *E. coli*; Lane 2, BS-C-1 cells infected with a m.o.i. value of 10 for vTF7-3 alone; Lanes 3, 4, and 5, BS-C-1 cells transfected with 5 μg of pLT-His by lipofection and infected with m.o.i. values of 5, 10, and 30 for vTF7-3, respectively. 1.8×10^6 BS-C-1 cells were used for expression.

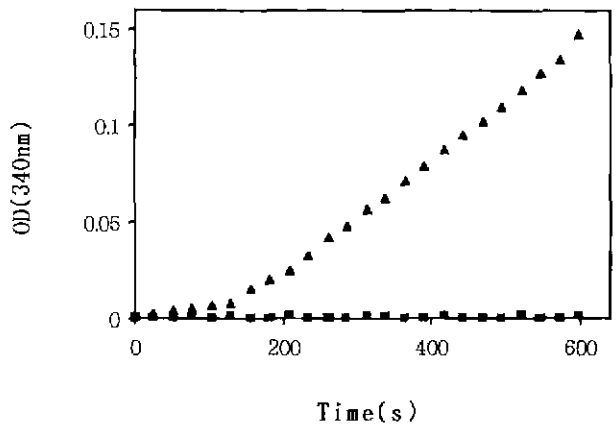

Fig. 4. Enzyme activity of ALDH2 expressed in HeLa S3 cells. ▲: ALDH2 catalytic activity measured by an increase in absorbance at 340 nm with 50 μl of the soluble proteins, 50 μl of 10 mM pyrophosphate buffer, 50 μl of propylaldehyde, and 10 μl of NAD. ■: ALDH2 catalytic activity measured without cofacter NAD. One mg of crude protein from HeLa S3 cells exhibited 23.5 mU of enzyme activity.

We tried to express the ALDH2 protein in the BS-C-1 mammalian cells which were used for vaccinia virus titering, using the same method for the HeLa S3 cell line. As shown in Fig. 3, expression of ALDH2 in BS-C-1 cells were also confirmed by Western blot analysis. In the case of BS-C-1 cells, a m.o.i. value of 30 for vTF7-3 was more efficient for ALDH2 expression than a m.o.i. of either 5 or 10. Additional background band patterns were different from the patterns for HeLa S3 cells. This difference may be due to the fact that each cell produces its own specific proteins.

To determine whether the expressed ALDH2 exhibited enzymatic activity, a spectrometric assay was performed at 25°C for 3 times. Fig. 4 illustrates the average enzyme activity of expressed ALDH2. Based on the fact that the amount of NADH produced by ALDH2 catalytic activity is propotional to the absorbance at 340 nm and that the molar absorbancy of NADH at 340 nm is 6220 $M^{-1}cm^{-1}$, the catalytic activity of ALDH2 was calculated. One mg of crude protein from HeLa S3 cells exhibited 23.5 mU of enzyme activity. As the expressed ALDH2 was about 2. 5% of the total protein according to SDS-PAGE, we can estimate the pure enzyme activity to be 9.5 U/mg which is similar to the expression level of the native human liver ALDH2 and ALDH2 expressed in *E. coli* (17)(Fig. 4).

Discussion

Successful expression of human liver mitochond-

rial aldehyde dehydrogenase (ALDH2) as soluble active protein was achieved using a recombinant vaccinia virus-T7 RNA polymerase/plasmid vector system. Both the lipofection and electroporation methods of transfection were used for introduction of plasmid vectors into cells. Both methods were efficient for transfection of plasmid DNAs. Lipofection was more efficient for ALDH2 expression but electroporation was more economical for repetitive transient expression of ALDH2 genes in the tested cell lines. The expressed ALDH2 had an enzymatic activity as high as the native human liver ALDH2 enzyme (17). Our results showed that the vTF7-3/pLT-His system was effective for expression of human liver ALDH2. ALDH2 plays an important role in acetaldehyde detoxification during alcohol absorption. ALDH2 has been intensively studied because of its importance in human metabolism. However, most of ALDH2 inhibitors or activators have been developed through *E. coli*-derived ALDH2 screening system. We plan to establish an improved complementary screening system for novel ALDH2 inhibitors and activators using the human ALDH2 obtained in this study. It is believed that ALDH2 proteins expressed in human cell lines are more similar to native human liver ALDH2 than proteins which are expressed in *E. coli* in some respects such as posttranslational modification.

Acknowledgment

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