Alternative biotransformation of retinal to retinoic acid or retinol by aldehyde dehydrogenase from *Bacillus cereus*

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SUPPLEMENTAL DATA

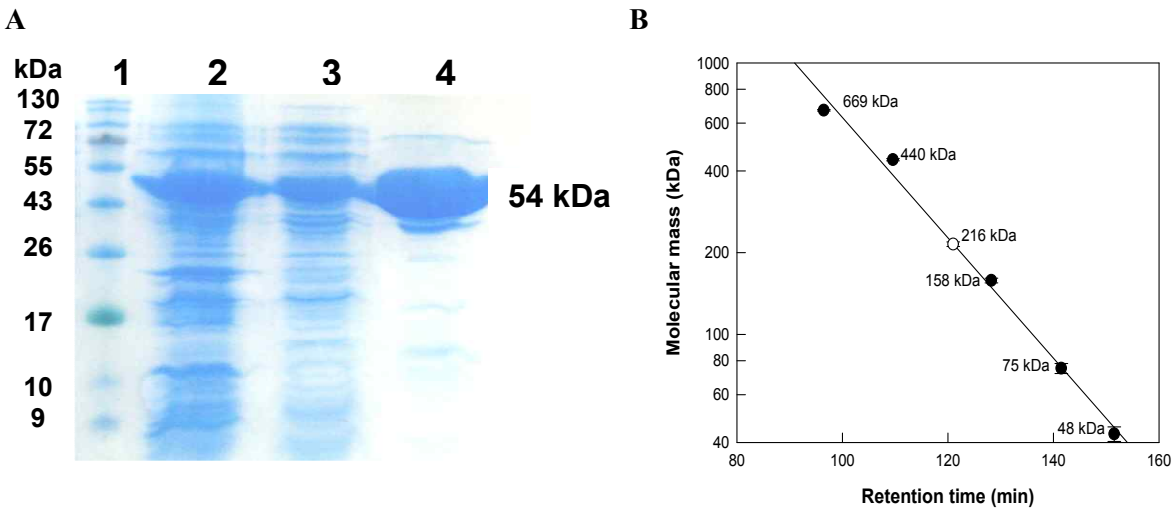

Fig. S1 SDS-PAGE analysis and molecular mass determination of *Bc*ALDH. (A) SDS-PAGE stained with Coomassie blue of *Bc*ALDH. Lane 1, molecular mass marker proteins; lane 2, inclusion body; lane 3, crude extract; and lane 4, purified *Bc*ALDH. (B) Determination of the molecular mass of the purified native enzyme using Sephacryl S-300 HR gel filtration chromatography. The reference proteins (●) were thyroglobulin (689 kDa), ferritin (453 kDa), aldolase (158 kDa), and conalbumin (75 kDa). The enzyme was eluted at a retention time corresponding to 216 kDa. The ALDH eluted at a position corresponding to 216 kDa (○). Data represent the means of three experiments and error bars represent standard deviation.

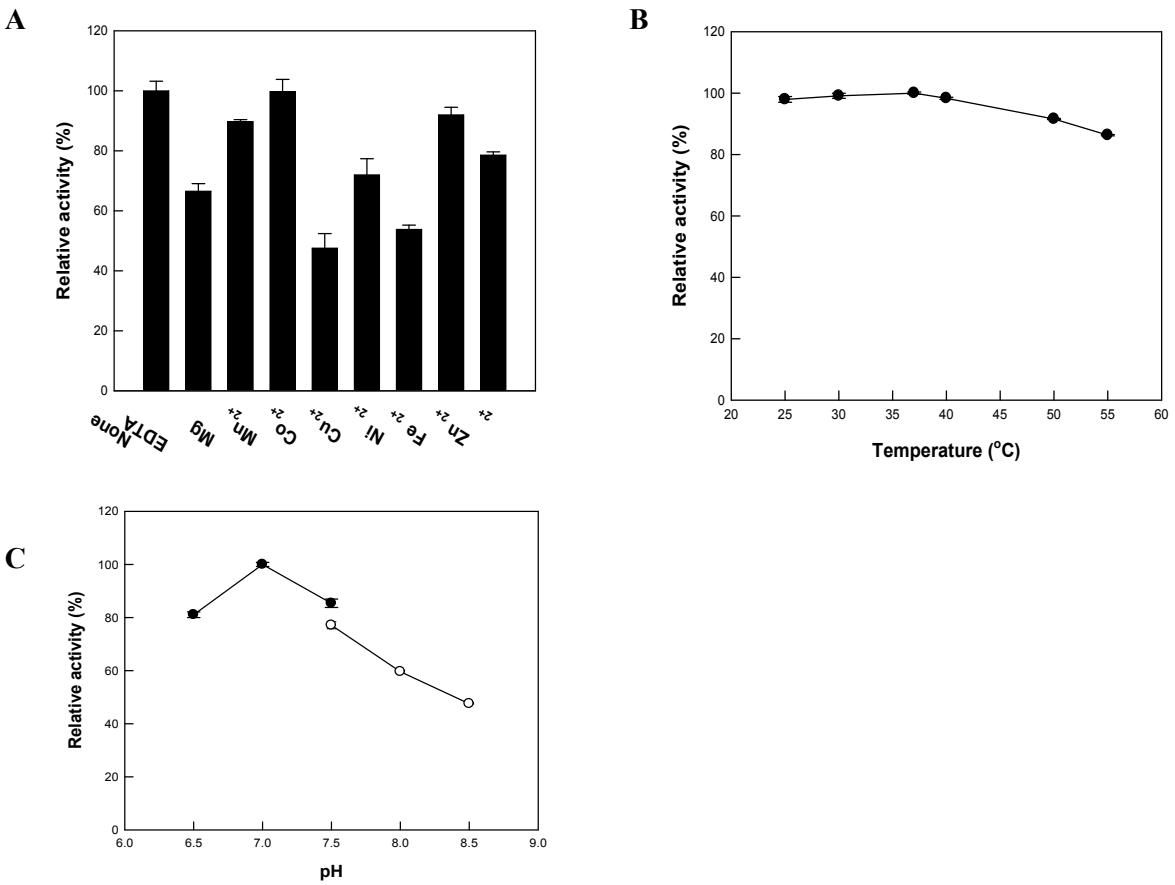

Fig. S2 Effects of metal ions, temperature, and pH on the oxidation activity of *Bc*ALDH for all-*trans*-retinal. (A) Effects of metal ions on the activity of *Bc*ALDH. The reactions were performed in 50 mM PIPES buffer (pH 7.5) containing 0.2 mM all-*trans*-retinal, 4 mM NAD^+, and 1 mM of each metal ion at 37 °C for 30 min. Control is the purified enzyme without EDTA treatment. (B) Effect of temperature on enzyme activity. The reactions were performed in 50 mM PIPES buffer (pH 7.5) containing 0.2 mM all-*trans*-retinal, 0.4 unit/ml enzyme, 1 mM 2-mercaptoethanol (2-ME) and 4 mM NAD^+ for 30 min at temperatures ranging from 25 to 55 °C. (C) Effect of pH on enzyme activity. The reactions were performed in 50 mM PIPES buffer for pH 6.5 to 7.5 (●) and 50 mM HEPPS buffer for pH 7.5 to 8.5 (○) with 0.2 mM all-*trans*-retinal, 0.4 unit/ml enzyme, 1 mM 2-ME, and 4 mM NAD^+ at 37 °C for 30 min. Data represent the means of three experiments and error bars represent standard deviation.

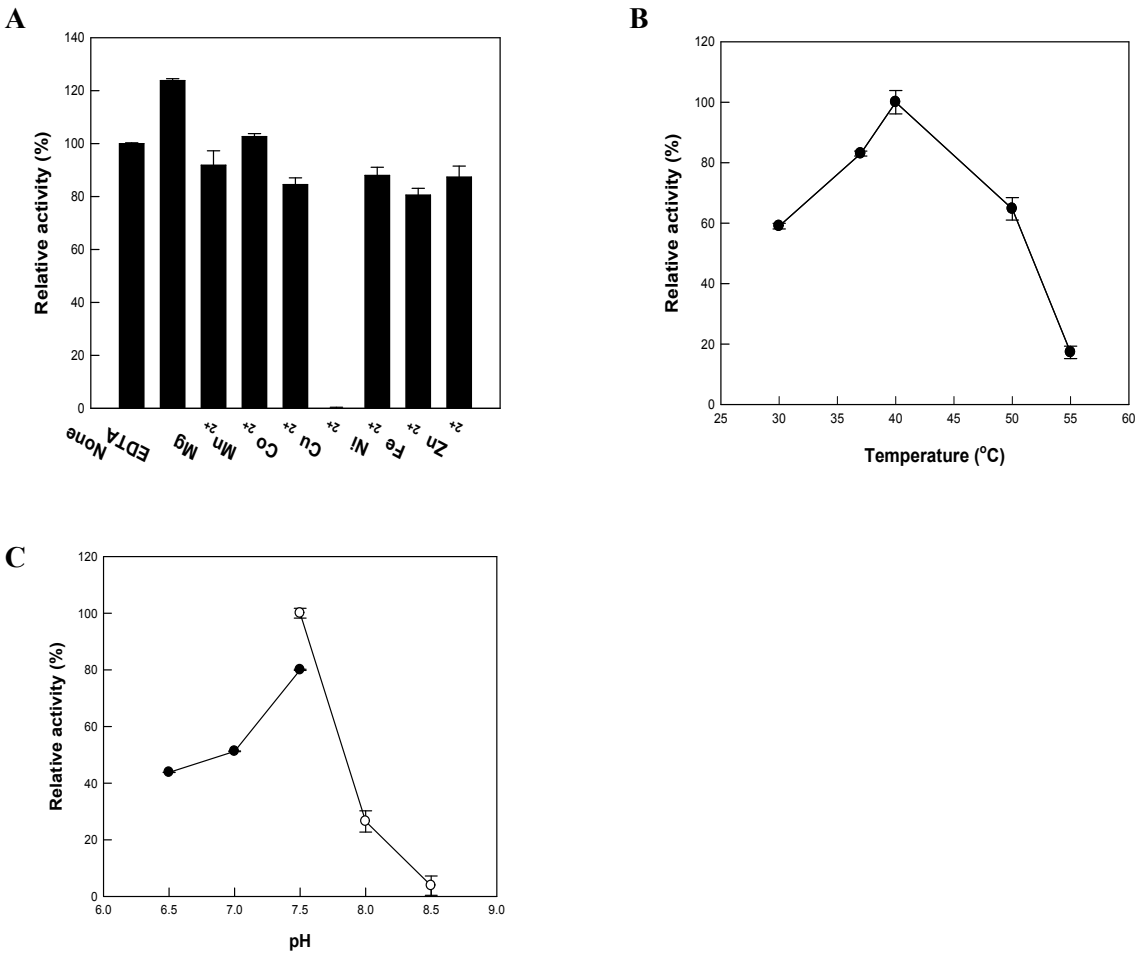

Fig. S3 Effects of metal ions, temperature, and pH on the reduction activity of *B. cereus* ALDH for all-*trans*-retinal. (A) Effects of metal ions on the activity of *Bc*ALDH. The reactions were performed in 50 mM HEPPS buffer (pH 7.5) containing 0.2 mM all-*trans*-retinal, 0.4 unit/ml enzyme, 4 mM NADPH, and 1 mM of each metal ion at 37 °C for 30 min. The control is the purified enzyme without EDTA treatment. (B) Effect of temperature on enzyme activity. The reactions were performed in 50 mM HEPPS buffer (pH 7.5) containing 0.2 mM all-*trans*-retinal, 0.4 unit/ml enzyme, 1 mM 2-ME, and 4 mM NADPH for 30 min at temperatures ranging from 25 to 55 °C. (C) Effect of pH on enzyme activity. The reactions were performed in 50 mM PIPES buffer for pH 6.5 to 7.5 (●) and 50 mM HEPPS buffer for pH 7.5 to 8.5 (○) with 0.2 mM all-*trans*-retinal, 0.4 unit/ml enzyme, 1 mM 2-ME, and 4 mM NADPH at 37 °C for 30 min. Data represent the means of three experiments and error bars represent standard deviation.

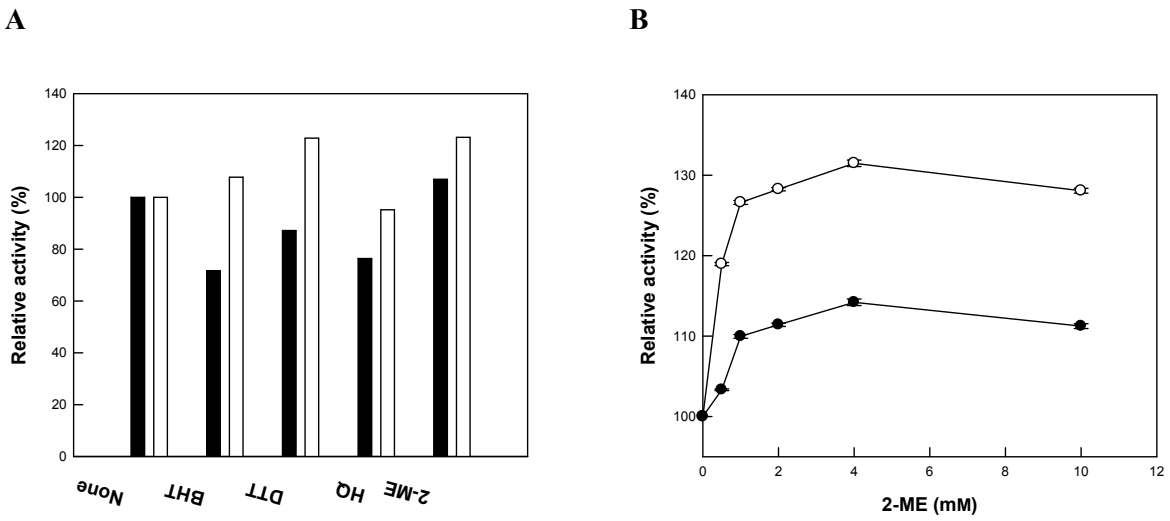

Fig. S4 Effect of reducing agent on the activity of *Bc*ALDH. (A) Effect of reducing agent on the conversion of all-*trans*-retinal to all-*trans*-retinoic acid (□) and all-*trans*-retinol (■). The reactions were performed at 37 °C in 50 mM PIPES buffer (pH 7.5) containing 0.2 mM all-*trans*-retinal, 0.4 unit/ml enzyme, 1 mM reducing agent, and 1 mM NAD^+ or NADPH for 30 min. (B) Effect of reducing agent concentration on the conversion of all-*trans*-retinal to all-*trans*-retinoic acid (○) and all-*trans*-retinol (●). The reactions were performed at 37 °C in 50 mM PIPES buffer (pH 7.5) containing 0.2 mM all-*trans*-retinal, 0.4 unit/ml enzyme, and 1 mM NAD^+ or NADPH, and 2-ME for 30 min. Data represent the means of three experiments and error bars represent standard deviation.

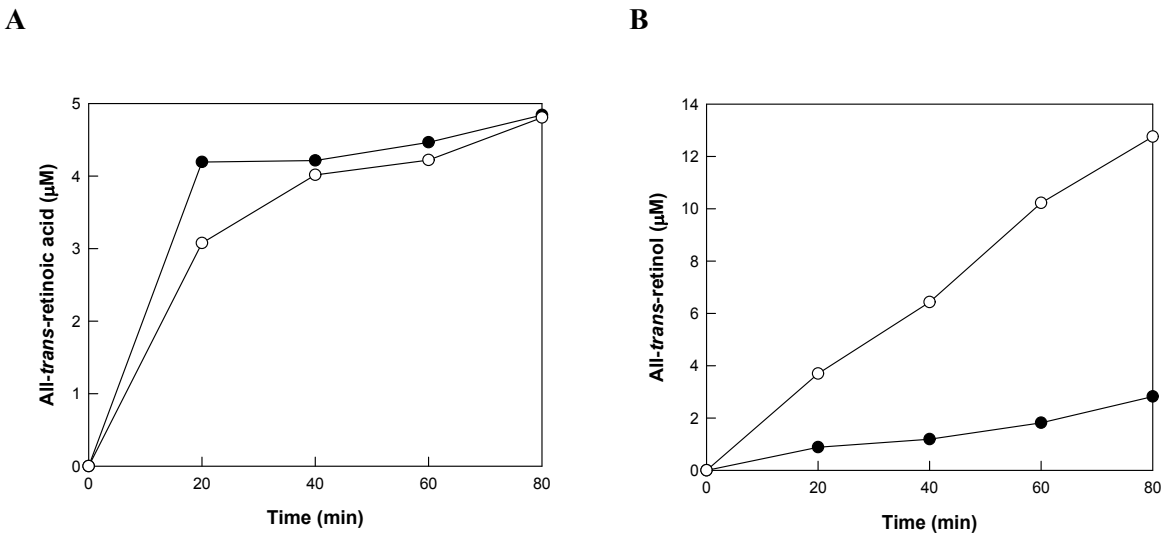

Fig. S5. Biotransformation of all-*trans*-retinal to retinoic acid or retinol by human ALDH with cofactor switch. (A) Time course reactions for the conversion of all-*trans*-retinal to all-*trans*-retinoic acid by the wild-type (●) and E196S (○) variant enzymes of human ALDH with NAD^+. (B) Time course reactions for the conversion of all-*trans*-retinal to all-*trans*-retinol by the wild-type (●) and E196S ALDH (○) variant enzymes from human with NADPH. The reaction was performed in 50 mM EPPS buffer (pH 8.0) containing 0.4 mM all-*trans*-retinal, 0.4 unit/ml enzyme, 1 mM DTT, and 4 mM NAD^+ or NADPH at 25°C for 30 min.

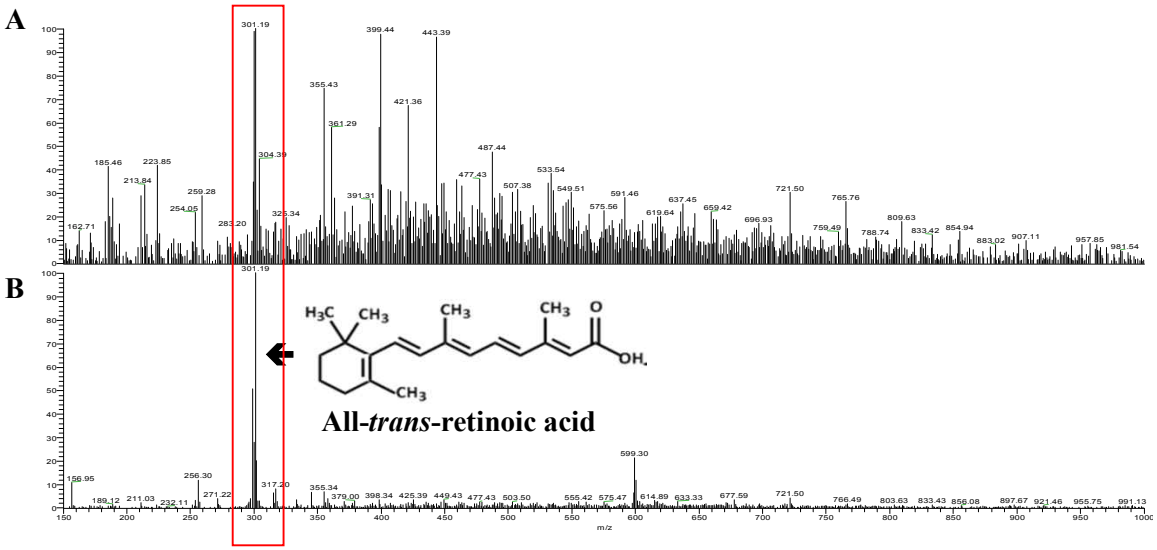

Fig. S6. Identification of the reaction product with the all-*trans*-retinoic acid standard by UPLC-LTQ-XL-IT-MS/MS. (A) Reaction product obtained from all-*trans*-retinal by the reaction of *Bc*ALDH. (B) All-*trans*-retinoic acid standard. UPLC-LTQ-XL-IT-MS/MS comprised of an electrospray interface (Thermo Fischer Scientific, San José, CA, USA) coupled with a DIONEX UltiMate 3000 RS Pump, RS Autosampler, RS Column Compartment, and RS Diode Array Detector (Dionex Corporation, Sunnyvale, CA, USA). Sample was separated on a Thermo Scientific Syncronis C18 UPLC column with 1.7 µm particle size. The mobile phase consisted of A (0.1% [v/v] formic acid in water) and B (0.1% [v/v] formic acid in acetonitrile). The initial condition was 90% solvent A and 10% solvent B, and the ratio of solvent B increased from 10% to 100% of for 18 min, and then re-equilibrated to the initial condition for 4 min. The flow rate was 0.3 mL/min and the injection volume was 10 µL. Temperature of the column during measurement was maintained at 35 °C. The photodiode array was set at 200–600 nm. Ion trap was performed in positive, negative, and full-scan ion modes within a range of 150–1,000 *m/z*. The operating parameters were as follows: source voltage, ±5 kV, capillary voltage, 39 V; capillary temperature, 275 °C. Tandem MS analysis was performed by scan-type turbo data-dependent scanning (DDS) under the same conditions used for MS scanning.

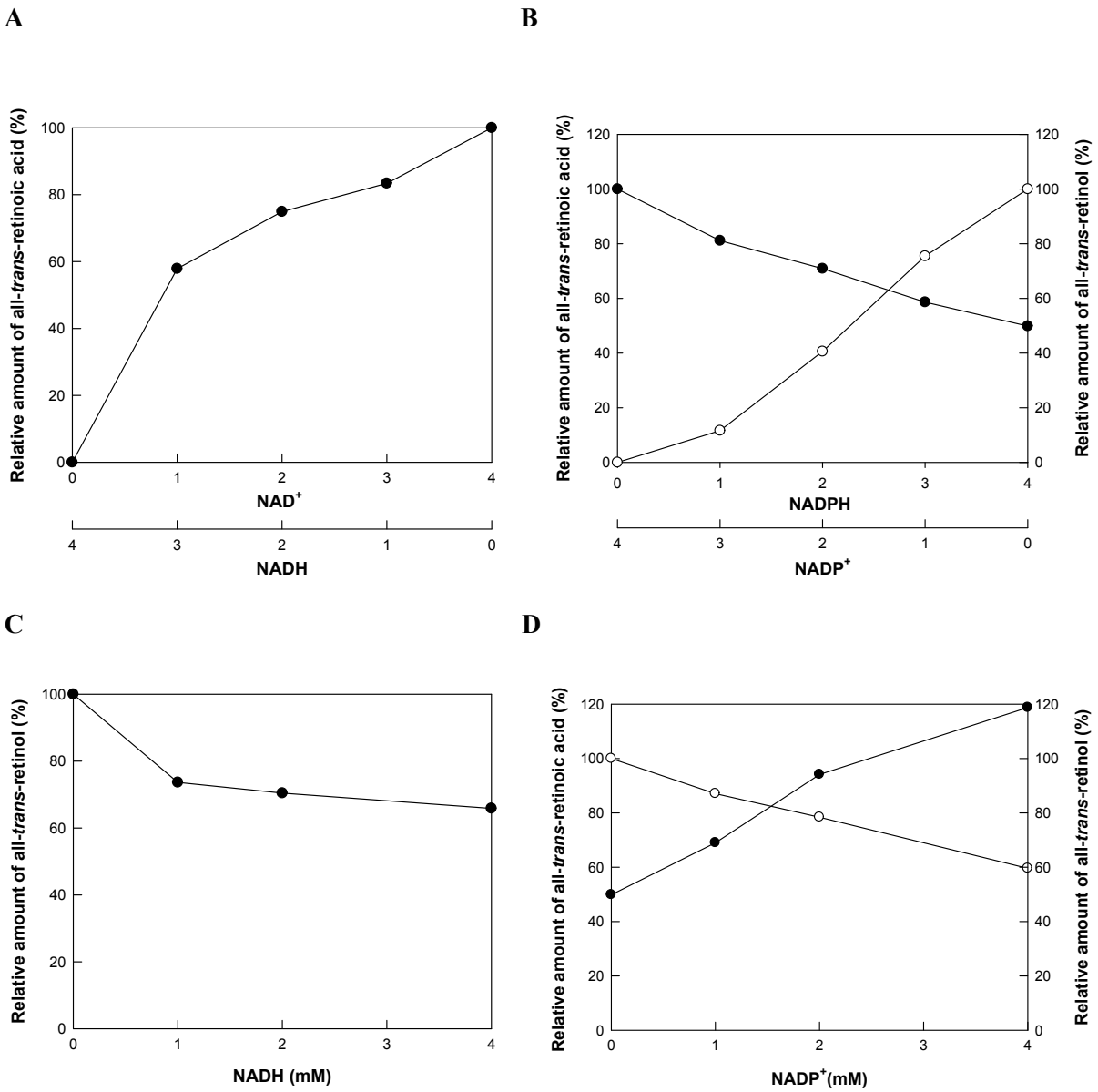

Fig. S7. Effects of supplementation of NAD^+ to NADH, supplementation of NADPH to $NADP^+$, NADH, and $NADP^+$ on the conversion of all-*trans*-retinal to all-*trans*-retinol and all-*trans*-retinoic acid. All-*trans*-retinoic acid (●) and all-*trans*-retinol (○) The enzymatic reaction of *Bc*ALDH for the conversion of all-*trans*-retinal to all-*trans*-retinoic acid or all-*trans*-retinol was performed at 37 °C in 50 mM PIPES buffer (pH 7.0) or at 40 °C in 50 mM HEPPS buffer (pH 7.5), respectively, containing 0.2 mM all-*trans*-retinal, 0.4 unit ml^{-1} enzyme, 5% (v/v) methanol, 4 mM 2-ME, and 0-4 mM NAD^+ or NADPH, respectively, for 30 min. (A) Effect of supplementation of NAD^+ to NADH on the conversion of all-*trans*-retinal to all-*trans*-retinoic acid (B) Effects of supplementation of NADPH to

$NADP^+$ on the conversion of all-*trans*-retinal to all-*trans*-retinol and all-*trans*-retinoic acid. (C) Effect of NADH on the conversion of all-*trans*-retinal to all-*trans*-retinol. (D) Effect of $NADP^+$ on the conversion of all-*trans*-retinal to all-*trans*-retinol and all-*trans*-retinoic acid. The concentration of all-*trans*-retinoic acid and all-*trans*-retinol at 100 % relative activity were 25 and 5.7 µM respectively.