

Electrochemical oxidation of alcohols with alcohol dehydrogenases from thermophilic archaea

Haruka Mishiba¹, Yumi Kariya¹, Hirotohi Matsumura², Nobuhumi Nakamura¹, Masafumi Yohda¹, and Hiroyuki Ohno¹

¹Department of Biotechnology and Life Science, Tokyo University of Agriculture and Technology, Koganei, Tokyo 184-8588, Japan
E-mail: nobu1@cc.tuat.ac.jp

²Department of Biomaterials Sciences, Graduate School of Agriculture and Life Science, The University of Tokyo, Bunkyo-ku, Tokyo 113-8657, Japan

Alcohol dehydrogenases are an important enzyme of biocatalysts because they can be used efficiently in the synthesis of optically active alcohols, which are key building blocks for the chemical industry. Also, many amperometric studies show that the NAD(P)⁺/NAD(P)H depending enzyme have potential to a broad range of dehydrogenase-based devices such as biosensors and biofuel cells.

NAD(P)-dependent alcohol dehydrogenases (ADHs; EC 1.1.1.1) belong to the oxidoreductase family, which catalyze the interconversion reaction of alcohols with the corresponding aldehydes or ketones using NAD(P) as the cofactor. The enzymes can be divided into three groups: Group I, zinc-dependent long chain ADHs; Group II, short chain zinc-independent ADHs; Group III, iron-activated ADHs. Of these three NAD(P)-dependent groups, Group I ADHs are the most studied enzymes, while Group III ADHs have been little studied due to their structural unstabilities.

In this study, for developing our understanding of the properties for Group III ADHs, we have expressed the ADH derived from hyperthermophilic archaeon, *Pyrococcus horikoshii* OT3 (PhADH) with *Escherichia coli*. For comparing to PhADH, we have also constructed a thermoacidophilic archaeal Group I ADH from *Sulfolobus tokodaii* strain 7 (StADH). Electrochemical oxidation of ethanol by using these thermophilic ADHs has been investigated.

The electrochemical responses of NAD⁺-dependent ADHs were measured by cyclic voltammetry. The potential was scanned between 0.3 and -0.3 V vs. Ag/AgCl with a platinum wire as the counter electrode. All electrochemical measurements were performed under a N₂ atmosphere. A oxidized carbon black (CB) coated on a grassy carbon electrode (GCE) was used as a working electrode. For preparation of the CB-cast electrode [1], CB was refluxed in 30% HNO₃ for 24 h at 140 °C. After refluxed, the resulting solution was centrifuged and the precipitate was washed with water. After CB solution was then neutralized to pH 7.0 with appropriate NaOH, the resulting solution was employed for preparation of electrode. The pretreated grassy carbon electrode (GCE) dropped by 1 wt% poly (diallyldimethylammonium chloride) (PDDA) was vacuumed to evaporate water, and the potential sweeps was performed using the PDDA coated GCE for obtaining the CB adsorbed PDDA electrode. The ADH solutions were mixed with an equal amount of 1 wt% PDDA in water. This mixed solution was cast onto the CB/PDDA electrode followed by evaporating water in air.

The purified recombinant PhADH and StADH previously migrated as a single band with apparent molecular mass 42.0 kDa and 37.6 kDa on SDS-PAGE respectively, in good agreement with that deduced from the gene sequences (Figure 1).

When an electrochemical measurement using the PDDA coated electrode in the CB solution, the redox responses estimated from the midpoint of peak-to-peak

potential was -76 mV vs. Ag/AgCl, assigned to the treating CB appeared. The fact that the responses were observed after rinsed with water shows the treating CB particles entrapped into PDDA. Also, the redox potential depends linearly on pH with a slope of -62 mV/pH between pH 4 and 10. The redox potential and pH dependent studies using the CB/PDDA-modified electrode exhibit the redox species could be quinone which is well known to mediate electron transfer from NADH. Upon addition of 10 mM NADH to electrochemical solution, the cyclic voltammogram showed the increase in oxidation peak at ca. 75 mV vs. Ag/AgCl.

The StADH solution mixed with PDDA was cast onto the CB/PDDA-modified electrode. When 1 M ethanol was added to the sample solution, an increase in anodic current in the voltammogram with the CB/PDDA/StADH electrode in phosphate buffer at pH 7 was observed. This result indicates the catalytic reduction of ethanol. Using just the CB/PDDA-modified electrode, the increase in current was not observed. The result indicates that StADH was entrapped on the electrode surface and catalyzed the alcohol oxidation reaction with NAD⁺ in PDDA. Furthermore, the effect of temperature on the electrochemical alcohol oxidation was investigated. StADH-catalyzed oxidation of ethanol was achieved at even 70 °C in the electrode system, as seen in Figure 2. The catalytic current of ethanol oxidation at 70 °C was increased by about 10 times as that at 25 °C.

The investigation of alcohol oxidation with PhADH is now under consideration. We will discuss that the difference of electrochemical properties between Group I StADH and Group III PhADH.

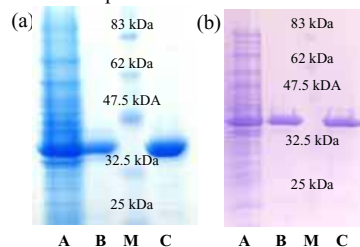


Figure 1 SDS-PAGE of (a) PhADH, and (b) StADH.

A : Crude extract of *E. coli* BL21 harboring the plasmid of ADHs

B : Soluble fraction after heat treatment

M : Protein Marker

C : After purification

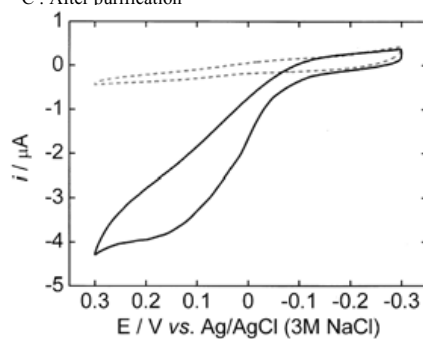


Figure 2 Cyclic voltammograms using CB/PDDA-StADH electrode in 100 mM potassium phosphate buffer (pH 7.0) containing 10 mM NAD⁺ and 1 M ethanol at 25 °C (dotted line) and 70 °C (solid line).

Reference

- [1] Lina Wu, Xueji Zhang, Huangxian Ju, *Anal. Chem.* **2007**, *79*, 453-458