ENZYMATIC CONVERSION OF CO_2 TO METHANOL: EFFECTS OF MODIFICATION OF SILICA GEL AND ADH ON ENZYME ACTIVITY

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Introduction

CO_2 can be converted to methanol through an enzymatic approach in the following three steps:

$$CO_2 \xrightarrow[\text{NADH}]{F_{ate}DH} HCOOH \xrightarrow[\text{NADH}]{F_{ald}DH} HCHO \xrightarrow[\text{NADH}]{ADH} CH_3OH$$

$F_{ate}DH$, $F_{ald}DH$ and ADH are formate dehydrogenase, formaldehyde dehydrogenase and alcohol dehydrogenase respectively and are co-encapsulated in a silica gel formed by sol-gel process. Reduced nicotinamide adenine dinucleotide (NADH) is used as a terminal electron donor for each reduction step.[1,2] To understand the whole process more clearly and get the optimized results, the above three reactions are studied separately. In this paper, we concentrate on the third one.

Immobilized enzyme has advantages over free enzyme such as easy separation and reusability. However, most immobilized enzymes show lower activities than that of the free enzymes. Lower productions were also found in our study where the enzymes were immobilized in silica matrix by sol-gel process.[1] This may be due to the diffusion hindrance of the substrate from bulk solution to the gel, the accessibility hindrance of the immobilized enzyme in the three-dimensional silicate network and the microenvironment for the enzymes.

Herein, an improved sol-gel method using polyethylene glycol (PEG) as a multifunctional agent and an enzyme modification method using methoxypolyethylene glycol (mPEG) as a modifier are reported. Larger pore size and more flexible structure of the gel are expected by using the first method while more suitable microenvironment for enzyme is expected by using the second method. Mainly due to their hydrophilicity, linear structure and bio-compatability, PEG and mPEG are selected as modifiers. Modified gel properties were characterized by BET and IR and were compared with unmodified gel. Enzyme (ADH) activities after modification were investigated and compared with unmodified ones by kinetic studies of the conversion reaction of HCHO to CH_3OH.

Experimental

Synthesis of mPEG-modified ADH. 3.5 mg ADH and 10.5 mg activated mPEG were dissolved in 5 mL of 0.1 M phosphate buffer (pH7.0) and the solution was kept gently stirring for 2 h under room temperature. The degree of modification was 90% determined by using trinitrobenzene sulphonic acid (TNBS) to react with the unPEGylated amino groups and measuring the amount of the product that has a characteristic adsorption at 420nm detected by an UV-visible spectrophotometer (U-2800, Hitachi).

Preparation of gel and immobilization of ADH. The improved sol-gel immobilization processes are illustrated in **Figure 1**. It involves two ways: (1) immobilization of enzyme in modified gels and (2) immobilization of chemical modified enzymes in typical gels. For (1), mix tetraethylorthosilicate (TEOS), the precursor, with HCl, the catalyst, and PEG600 (MW=600), the gel-modifier, together first, then regulate the pH of the solution to 7.0 by adding NaOH solution to form the modified sol, and finally add a certain amount of enzyme–containing (3.5 mg/ml) 0.01 M phosphate buffer solution (pH7.0) to the sol. A transparent enzyme-containing gel was formed shortly. For (2), mix TEOS with HCl first, then regulate the pH of the solution to 7.0 by adding NaOH solution to form a typical sol, and add a certain amount of mPEG-modified enzyme–containing phosphate buffer solution to the sol. A transparent modified enzyme-containing gel was formed shortly. The gels prepared by the above two ways were aged at 4℃ for 7 days. The aged enzyme-containing gel was put into a dialysis membrane bag, dipped into 0.1 M phosphate buffer solution (pH7.0), and kept at 4℃ with frequent change of the buffer until there is no alcohol was detected by Gas Chromatographer (HP6890, Agilent). The pore size of the gel and its chemical structure were measured by BET (CHEMBET-3000, QUZNTA CHROME) and IR (Nicolet-560, MAGNA-IR) respectively.

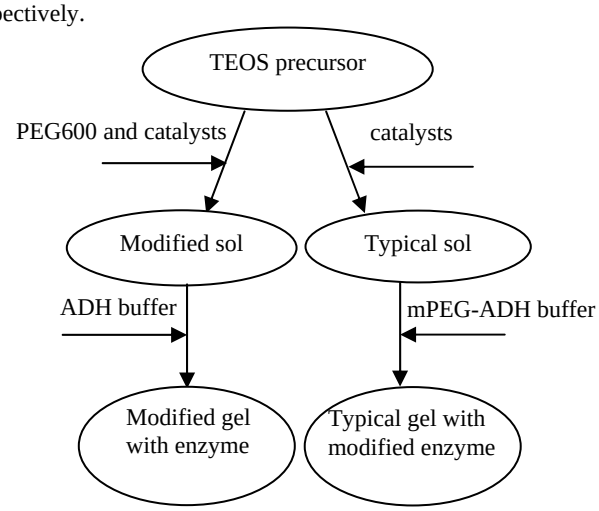

Figure 1. Sol-gel immobilization processes.

Enzymatic reactions. Enzyme activities of ADH were evaluated based on the reaction rates and kinetic Michaelis constants (K_m) of the reduction of HCHO to CH_3OH. Since, during the reaction, the cofactor (NADH), which has a characteristic adsorption at 340 nm, will be converted to NAD^+ that has no adsorption at 340 nm, the reaction progress can be recorded by the detection of the amount of NADH. A UV-visible spectrophotometer (U-2800, Hitachi) was employed to determine the reduction of the concentration of NADH and the reaction rates were calculated accordingly. At 25℃ and pH7.0, by varying the concentration of NADH from 50 to 250 μM and HCHO form 3 to 30 mM, kinetic parameters were obtained based on the ordered mechanism proposed by Dalziel.[3]

Results and Discussion

Structure characterization of gels. The pore size distribution of typical gel and PEG-modified gel are given in **Figure 2**. The average pore size of PEG-modified gel (4.67nm) is larger than that of the typical gel (3.88nm). This is due to the linking function of the PEG between sol particles, resulting in a branched structure with larger mesopores. The infrared spectra of the PEG-modified gels (**Figure 3**) show no new bonds formed, indicating that the linkage between PEG and silica sol particles was by molecular forces or hydrogen bonds.

Stability of free ADH and mPEG-modified ADH in alcohol. The sol-gel process using TEOS as the precursor will produce ethanol

that is harmful for enzyme. The alcohol toleration of both native and mPEG-modified ADH were studied by measuring their reacting activities of free enzymes. After being immersed in 1% ethanol solution for 2 minutes, the mPEG-modified ADH maintained 50% of its original activity, while the native ADH retained 36% of its original one. This denotes that such chemical modification of ADH by mPEG is a valid and significant means of stabilizing ADH in alcohol solutions. This may be due to the less flexibility of the enzyme with a long-chain PEG molecule linked to its surface, leading to steric hindrance of protein unfolding. And also, the hydrophilicity of PEG may provide a more hydrophilic microenvironment that is more preferable for enzyme to stay and function.

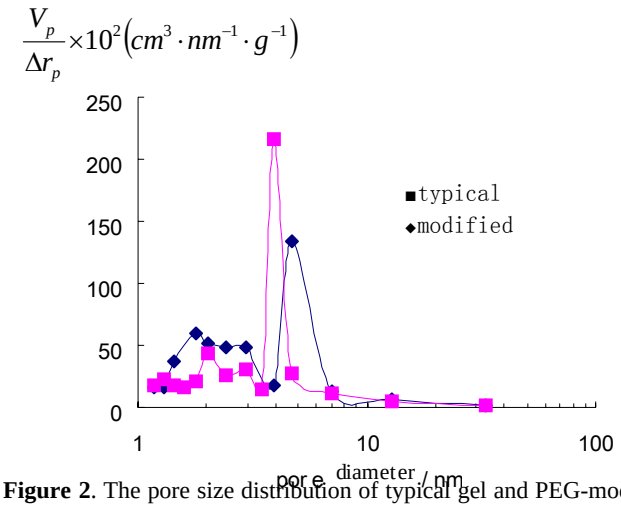

Figure 2. The pore size distribution of typical gel and PEG-modified gel.

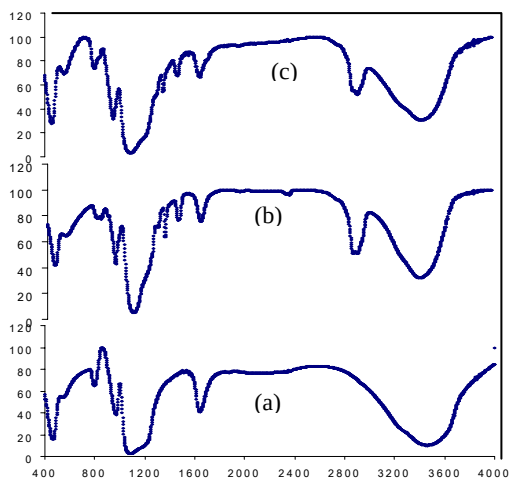

Figure 3. The infrared spectra of (a) typical gel; (b) PEG-modified gel and (c) mPEG-containing gel.

Storage stability of immobilized ADH. As shown in **Figure 4**, the activity of the native ADH encapsulated in typical silica gel was reduced by 17% after 92 days of storage. In contrast, the enzyme activities of ADH in PEG-modified gel and mPEG-modified ADH in typical gel almost showed no decrease after storage for the same period. This may be due to the same reasons mentioned above.

Kinetic parameters. Experimental results showed that all the enzymatic reactions followed Michaelis-Menten kinetics and the kinetic parameters were calculated by Dalziel's double-substrate

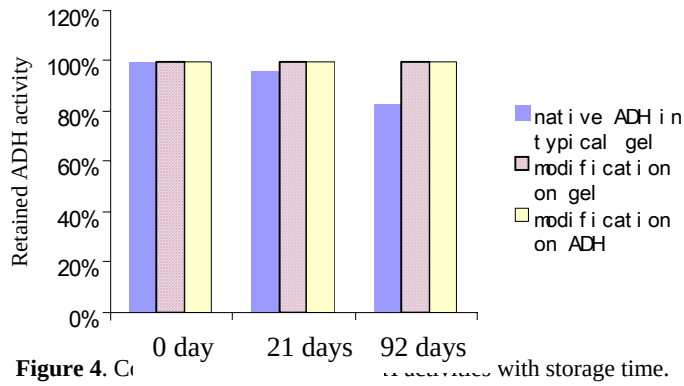

Figure 4. C activities with storage time.

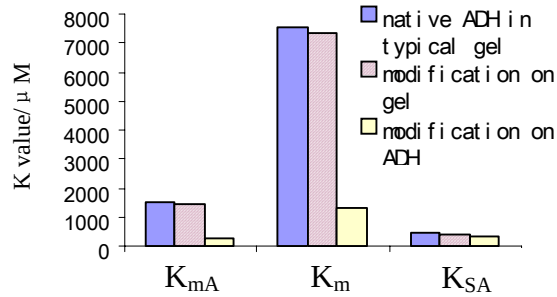

Figure 5. The kinetic Michaelis constants of reactions catalyzed by ADH.

model. Kinetic constants were shown and compared in **Figure 5**. K_{mA} is the Michaelis constant of NADH, while K_{mB} the Michaelis constant of HCHO and K_{SA} the dissociation constant of ADH-NADH pairs. The lower the K_m is, the higher activity the immobilized ADH is. Compared with the unmodified ADH in unmodified gel, both of the two improvement methods work. The mPEG-modified ADH encapsulated in typical gel showed the highest activity among the three cases, indicating that the modification on ADH (improvement method (2)) seems to have a greater effect than the modification on gel (improvement method (1)).

The catalytic activity of ADH immobilized in PEG-modified gel is higher than that in typical gel. This may mainly be attributed to the easier diffusion resulted from the enlarged pore size. These enlarged pores are considered to be more "comfortable" for enzymes to stay and also easier for substrate to diffuse through the pore channel and access to the enzymes. In addition, the improved gel properties such as hydrophilicity and mechanical strength may also provide higher biocompatibility for enzymes.

Acknowledgment

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