Preparation of Nanoporous Silica and its Application for Enzyme Immobilization

Jong-Kil Kim, Jin-Koo Park and Ho-Kun Kim
Tel : +82-31-400-5493, Fax : +82-31-407-3863,
e-mail : hkkim@hanyang.ac.kr
Department of Applied Chemistry, Hanyang University Ansan, 425-791, Korea

Introduction

Recently, the bio-conversion processes that can replace chemical synthetic processes in the field of medicine production has been widely studied according to the progress of biotechnology. Studies on the bioconversion processes utilizing an immobilized enzyme especially to produce antibiotics are most active. When an enzyme, which is a kind of protein, is used in the bioconversion reaction, it is necessary to protect the enzyme during the reaction because it has low stability to chemical and physical attacks. Immobilization, the process of fixing an enzme into the specific spaces of inert support, is generally carried out. Thus, the immobilized enzyme can be kept active during the reaction and re-use of the enzyme becomes possible as well. Porous materials of organic, inorganic or organic-inorganic hybrid compounds are usually used as the support for the enzyme immobilization. In this study, nanoporous silicas suitable for the support, were synthesized via a salt route and the pore morphology in silica were characterized. Then,D-amino acid oxidase and Glutaryl-7-ACA acylase,the enzymes for the conversion reaction to synthesize an antibiotic, were immobilized in the above silica and the activity of immobilized enzyme was investigated in relation to the pore morphology.

Experimental section

Silica with 30-80 nm pore diameters that could be used as supports of bio-catalyst were synthesized by acid decomposition reaction using silicate salts as starting materials. 5 N-HCl was added to the starting materials $Na_2O\cdot3.4SiO_2$ in 5 L reactor with a quantitative pump and the overall reactions were controlled by a continuous reaction regulating system.

The hydrothermal process was adopted to control the pore size in the xerogel silica. After the hydrothermal treatment, the surface area and pore volume were measured by the BET method that exploited N2 gas adsorption. The calculation of pore size in silica was done using the following Wheeler's formula.

APD = 40,000 V/S

Here, APD is the average pore diameter in Å unit, V and S are pore volume(ml/g) and surface area(m2/g), respectively.

Aqueous silanization on the pore surfaces was allowed using silane linked to amine group(3-aminopropyltriethoxysilane), then glutaraldehyde was attached to the amine group of the silane and D-amino acid oxidase and Glutaryl-7-ACA acylase for bioconversion process to produce 7-ACA which is an intermediate of antibiotic, was immobilized.

Results and Dicussion

Activity of D-amino acid oxidase as a function of pore diameters are shown in Fig.1 The *maximum value of the activities, 48units/g,* is found in the pore diameters range

of 40–50 nm in Fig 1. In the case of a diameter larger than 50 nm, also in Fig.1, the activities decrease with increasing diameters contrary to the above situation. Particularly, the immobilized enzyme is separated from the support during the bioconversion reaction if the diameter exceeds 60 nm. When the pore diameter is larger than 60 nm, the numbers of active sites in the support become extremely few and it could be one reason of the enzyme separation from support. Entrance of an enzyme with an average length of 2–3 nm into pores with diameter less than 30 nm is difficult, consequently, the activities decrease and fall to nearly 0 in the case of a pore diameter less than 20 nm.
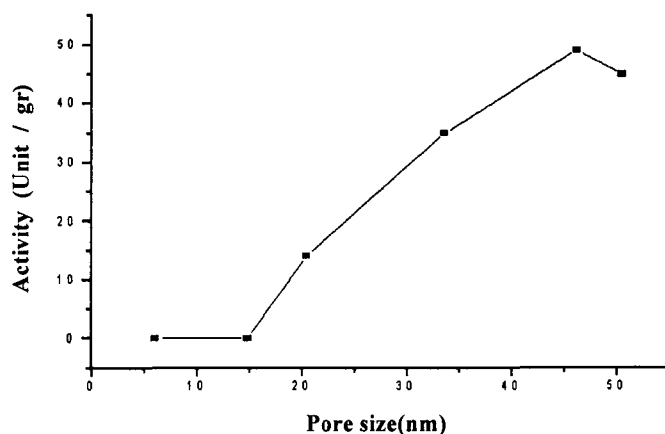

Fig.1.   Activity vs. pore diameter.

REFERENCE

1) W. Tischer, F. Wedekind, Immobilized enzymes : methods and application, Top. Curr. Chem. 200(1999) 95-126

2) Sung, M. H., et. al., *Bioindustry*, **1998**, 11, 23.

3) S. W. Kim, et. Al, *Reactive & Functional polymers*, 2002, 51, 79-92

4) K. H. Chung, et. Al, *Biochemical Eng , J.,* **2002**, 3598, 1-7

5) Park, Y. H., et. al., *Bioindustry*, **1996**, 13, 6.

6) Weeh, H. H., et. al., *Science*, **1996**, 166, 615.

7) A Pierre, et. al., *J. Mol. Catalysis B:Enzymatic*, **2001**, 11, 639.