Topics

Super Enzyme - Enzyme Stabilization in Mesoporous Materials

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1. Introduction

Recently, much attention has been given to environmental engineering involving enzymes that are friendly to the global environment. Enzymes have superb and specific reactivity at ordinary temperatures so they seem to be ideal catalysts. Since they are designed to work inside organisms, for use in environmental technology they are required to have high functionality such as reactivity in organic solvents and improved heat stability. On the other hand, many enzyme molecules are approximately 2 to 20 nm in size, which roughly coincides with the pore diameters of mesoporous materials. We attempted to provide enzymes with high functionality by immobilizing them with FSM-16 (Folded Sheet Mesoporous Material) developed by this laboratory

2. Method

We synthesized FSM-16 that has various pore diameters by using a swelling agent. Enzymes are immobilized by admixing them with FSM-16 at 4°C in an aqueous solution of horseradish peroxidase (HRP). To determine reactivity in an organic solvent, the oxidative activity of 1,2-diaminobenzene was measured in toluene. Thermal stability was monitored with phenol polymerization reactivity after heat-treating the enzyme solution at 70°C.

3. Result

Fig. 1 shows the catalytic activity of HRP, which was immobilized in FSM-16 having various pore diameters and other porous materials in an organic solvent. While the native enzyme is inactivated immediately, immobilized HRP shows high enzyme activity. Particularly if immobilized with FSM-16 having a slightly larger pore diameter of about 5 nm (**Fig. 2**-c) compared with that of the enzyme (Fig. 2-a), HRP shows maximum activity. It showed much higher reactivity than when immobilized with silica gel or by other conventional immobilization methods. Similarly, with the thermal stability test, the best stability was obtained when the enzyme was immobilized with FSM-16 having a slightly larger pore diameter than that of the enzyme molecule. Enzymes are adsorbed well by FSM-16 or MCM-41 synthesized using a cationic surfactant for the template. It was discovered that only 1/10 or less of the enzyme is adsorbed by SBA-15 that uses a nonionic surfactant for the template.

When the enzymes are immobilized by FSM-16 having a suitable pore diameter, the silanol group and the amino acid residue of the enzyme would from non-covalent binding. It is also suggested that the immobilization is insufficient and susceptible to circumstantial changes if the size of the pores of FSM-16 is too small compared with the size of the enzyme molecules (about 3nm,Fig. 2-b) or too large

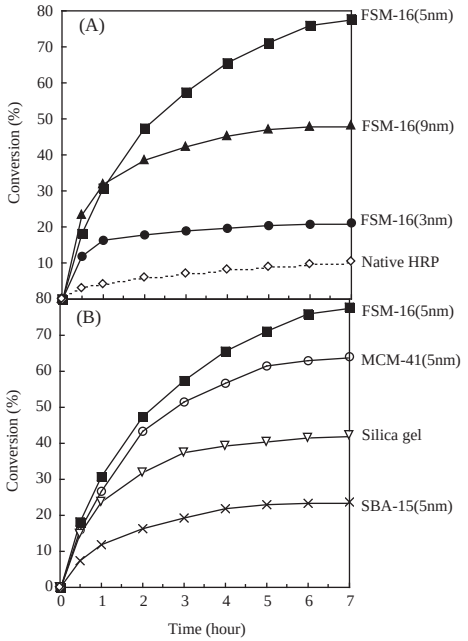

Fig. 1 Catalytic activity of HRP immobilized in FSM-16 with various pore sizes(A), and that of HRP immobilized in various porous silica materials with the same pore size(B).

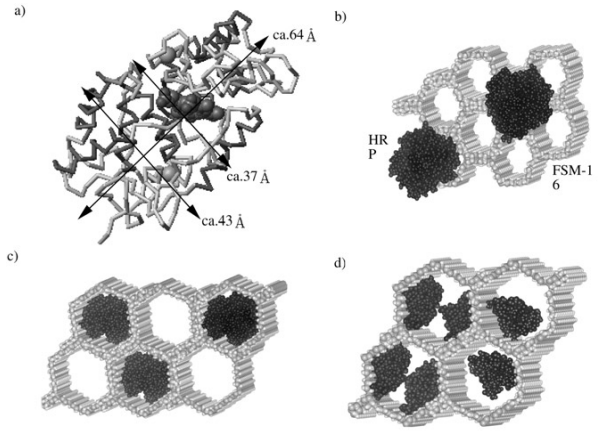

Fig. 2 Structural model of HRP molecule (a) and image models of immobilized HRP in FSM-16 with various pore sizes (b-d) using a computer schematic model.

(about 9nm, Fig. 2-d).

4. Conclusion

Reactivity in an organic solvent and thermal stability of enzymes have been sharply improved by immobilizing them with FSM-16 having a pore diameter that matches the molecular size of the enzyme. Consequently, we can propose a new green chemistry method for the 21st century including the bio-bleaching of pulp and decomposition of pollutants by combining the present technology with the direct evolution method.

Reference

1) Miyazaki, C. et al. : Protein Eng., 12-5(1999), 407

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