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ENZYME SYSTEM COMPRISING AN ENZYME BONDED IN A POROUS MATRIX

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FIELD OF THE INVENTION

The present invention relates to proteins in porous supports, methods of supporting proteins, and methods of using supported proteins. The invention also provides an improved method for making organophosphorous hydrolase ("OPH").

BACKGROUND OF THE INVENTION

The usefulness of proteins for facilitating chemical reactions outside biological organisms has long been known and used to great advantage. There is the potential for much greater use of proteins in facilitating a much larger variety of reactions and facilitating these reactions on a larger scale. However, there are many challenges to be overcome before this potential can be fully realized. These challenges include: the need for highly active protein systems; the need for protein systems that maintain high activity under a range of conditions; and the ability to densely pack active protein onto a porous support.

One example of a protein that is useful for catalyzing a variety of useful reactions is organophosphorous hydrolase, ("OPH"). OPH is an enzyme that might be used to inactivate chemical weapons or organophosphorous pesticides. Chemical weapons (i.e. nerve gases, especially sarin and VX) and organophosphorous pesticides (e.g. parathion, paraoxon and acephate) are highly toxic to higher organisms. Therefore, there is a need for methods of cleaning up undesirable discharges of the chemical weapons and organophosphorous pesticides in accidental spills or production plant contamination. The OPH enzyme offers the potential to inactivate chemical weapons or organophosphorous pesticide without the need for complex and expensive incineration facilities. Despite its potential, the lack of suitable methods for the large scale production of systems with active and stable OPH have limited the application of this enzyme.

The present invention provides improved protein systems that can better meet the challenges described above. Although the invention generally applies to immobilized enzyme systems, etc., in some specific examples, the invention also provides an improved method for making OPH and systems containing active OPH.

SUMMARY OF THE INVENTION

One concept of the invention is the engineering of support structures that match protein sizes to support structure pore sizes. It has been surprisingly found that well-matched sizes can produce protein systems having desirable qualities such as high activity, enhanced stability, and a relatively high density of active protein. Coupling of proteins in pores that are either too small or too large results in inferior properties. Other factors, such as surface area, pore density, pore uniformity and distribution, protein population within a support, and type and density of cross-linking sites may also be utilized to control the characteristics of the protein system.

In one aspect, the invention provides a protein system for use in facilitating chemical reactions. The system includes a porous matrix material that has pores within a solid matrix. In

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another aspect, the protein system comprises: a porous matrix material having a pore volume wherein at least 90% of the pore volume is composed of pores having sizes in the range of 50 to 400 Å, and a chemically-active protein bonded to the matrix material. "Bonded" refers to covalent, ionic and/or electrostatic attachment to the matrix material. In preferred embodiments, the protein is covalently bonded to the matrix through coupling groups.

In another aspect, the protein system comprises: a porous matrix material being sized such that the protein system comprises 8 to 125 mg of protein per gram of matrix material and wherein the protein in the protein system exhibits an activity of at least 65% that of the activity of the protein in the active state.

The invention also provides a method of forming a protein system comprising the steps of: providing a porous matrix material having a pore volume wherein at least 90% of the pore volume is composed of pores having sizes in the range of 50 to 400 Å, and reacting the porous matrix material with a protein so that the protein chemically bonds to the porous matrix material.

The invention also provides a method of making OPH. In this method, a host cell is transfected with a vector comprising a sequence encoding OPH, the sequence being operably linked to a T7 expression control sequence. The transfected host cell is cultured under conditions permitting expression under the control of the expression control sequence. The OPH is purified from the cell or the medium of the cell.

The protein system is engineered to match the size of the individual protein with the size of the individual pores, in preferred embodiments, the volume of the individual protein occupies between 5 and 40% of the average volume of each pore.

The invention also includes methods of using these systems in facilitating chemical processes (i.e., processes of making chemicals) such as hydrolysis, oxidation, hydrogenation, and proteolysis. The invention also encompasses the use of active enzymes in porous supports in filtration equipment for individual soldiers, pesticide workers, vehicles, aircrafts, ships and buildings such as civilian and military defense shelters, to perform detoxifications.

Various embodiments of the present invention can provide numerous advantages including: high protein activities on a porous support; stability under a variety of conditions; high densities of active protein; capability in industrial-scale applications; and providing environmentally safe methods of destroying chemical weapons and organophosphorous pesticides, and avoid the dangers inherent in burning these materials. Other advantages can be envisioned in view of the following descriptions and examples.

BRIEF DESCRIPTIONS OF THE DRAWINGS

FIG. 1 is a conceptualized, cross-sectional representation of an enzyme disposed in a porous substrate.

FIG. 2 is a ribbon diagram for OPH plasmid.

FIG. 3 is the relevant DNA sequence from the construct's BamH I to Bgl II site that encompasses the region immediately preceding the T7 promoter to just beyond the OPH stop codon, SEQ ID: 2.

FIG. 4 is the OPH amino acid sequence, SEQ ID: 1.

DETAILED DESCRIPTION OF THE INVENTION

A conceptual illustration of one embodiment of the protein system 2 of the present invention is shown in FIG. 1. A matrix material 4 has pores 6 containing protein 8. The protein 8 is