Encapsulation of Nitrile Hydratases for Improved Conversion of Nitriles to Amides

A material useful for synthesis of amides, nitrile degradation or remediation

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Field

Industrial synthesis
Nitrile removal

Technology

Purified nitrile hydratase encapsulated in solid support materials

Key Features

- Stereoselective
- Recombinantly purified from E. coli

Key Benefits

- Patent covers the use of a purified enzyme, its encapsulation, and use thereof

Stage of Development

Recombinantly expressed
Purified
Catalytic activity tested
Encapsulated and tested

Status

Seeking licensing partner

Patent Status

Provisional Application

Technology

The ability to degrade nitriles is a common trait of microorganisms, and the use of nitrile-degrading enzymes in organic drug research and discovery, and other chemistry related activities continues to evolve.

Two issues regarding the use of enzymes in organic synthetic processes are: 1) the difficulty in separating the enzyme from the synthetic reaction mixture, and 2) the aprotic solvents so often used in organic synthetic reaction mixtures inactivate most enzymes. One way to overcome these issues is by encapsulating the enzymes in a solid support material that is sufficiently hydrated to protect the protein and sufficiently porous to permit access to small substrates. Some patented technologies use living or encapsulated microbes to perform nitrile hydrations, but there are limitations. Since there are other enzymes in the bacterial nitrile degradation pathways, such as nitrilases, catalysis does not always stop with amide production, but can also progress to carboxylate formation. Thus the use of a single purified enzyme is preferable to the use of whole microbes or crude microbial lysate.

The inventors have studied the thermostable co-type nitrile hydratase from *Psuedonocardia thermophila* JCM 3095 (*Pt*NHase) and the Fe-type NHase from *Comamonas testosteroni* (*Ct*NHase). They have recombinant expression systems for both enzymes in *E. coli* and have purified them to homogeneity. *Ct*NHase was chosen because it preferentially hydrates small aliphatic nitriles and *Pt*NHase because it preferentially hydrates aromatic and halogenated aromatic nitriles.

*Ct*NHase and *Pt*NHase, have been successfully encapsulated in in a solid support material and their specificity and activity towards a variety of nitrile substrates has been (and are continuing to be) characterized. These encapsulated enzymes have been proven to retain their catalytic activity weeks after being removed from the reaction vessel, rinsed, and dried. Furthermore, the catalytic pellets can be treated with trypsin, which proteolytically digests all surface accessible proteins, and shown to retain some activity, indicating that active enzyme is trapped inside the pellets (and is not just attached to the pellet surface).

Potential Market

The invention can convert any nitrile to an amide. The pharmaceutical industry and/or companies supplying products and services to the drug discovery and chemical research community can benefit from the encapsulation of nitrile hydratase technology; multi-step syntheses run under mild conditions can now be planned and performed by organic chemists using these new synthetic reagents. Green chemistry applications could also benefit for the same reasons as stated above. Green chemistry is important as it seeks to 1) design chemical products and use reagents that are less hazardous to human health and the environment, and 2) to design syntheses and other processes that can be less energy and materials intensive.

Opportunity

Loyola University Chicago is looking for commercial licensing partners for this patent.

Inventors

Richard C. Holz

Dr. Holz is Professor and Chair of the Chemistry Department at Loyola University Chicago. Dr. Holz received a B.S. degree in Chemistry from Bemidji State University with minors in biology and mathematics, an M.S. degree in Chemistry from the University of Minnesota-Duluth, and a Ph.D. in Chemistry from The Pennsylvania State University under the direction of Dr. William DeW. Horrocks, Jr. He was an NIH Postdoctoral Research Fellow at the University of Minnesota under the direction of Dr. Larry Que who is the 3M/Alumni Distinguished Professor of Chemistry. He joined the Loyola faculty in 2006 as Chair of the Chemistry Department after spending fourteen years at Utah State University. His current research interests include: structure/function studies on metalloenzymes, biophysical methods such as enzyme kinetics, site-directed mutagenesis, UV-VIS, NMR and EPR spectroscopies, nano and micron-sized biologically powered devices, biomolecule recognition at surfaces, and nano molecular detection devices. Dr. Holz is a Member of the American Chemical Society and the Society of Biological Inorganic Chemistry. He has co-authored more than 75 research articles and has lectured at over 50 universities around the world and has been funded by the National Science Foundation, the National Institutes of Health and the Air Force office of Scientific Research. He resides in the northern suburbs of Chicago with his wife Anna and his two daughters Emilia and Marika.

Timothy E. Elgren

Dr. Elgren is Professor of Chemistry and Chair of the Biochemistry/Molecular Biology Program at Hamilton College in Clinton, New York. He received a B.A. in Chemistry from Hamline University and a Ph.D. in Chemistry from Dartmouth College under the direction of Dr. Dean Wilcox. He did a postdoctoral fellowship at the University of Minnesota with Dr. Larry Que focusing on physical bioinorganic chemistry. Dr. Elgren joined the Hamilton College faculty in 1993. His research efforts have focused on physical characterization of the structure and chemistry associated with metal sites in metalloenzymes. His recent work has centered on the use of silica-derived materials used to encapsulate and stabilize enzymes. These bio-materials catalyze a wide range of reactions including hydrogen production, halogenation and dehalogenation reactions, amine oxidation, and nitryl and amide hydrolysis and serve a variety of functions including biosensers, decontamination catalysts, and feed stock production catalysts. His work has been funded by the National Science Foundation and the National Institutes of Health, been published in a variety of professional journals, and presented a conferences and universities domestically and internationally. He lives in upstate New York with his wife Gail and two children, Ruth and Ben.