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Biocatalysis in Organic Solvents for the Production of Fine Chemicals and Specialty Polymers

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Biocatalytic approaches to fine chemical and specialty polymer synthesis invariably involves the use of organic solvents as the reaction medium. Both the increased solubility of nonpolar substrates and the elimination of hydrolytic activities of hydrolytic enzymes make the nonaqueous approach particularly appealing for organic and polymer synthesis. This abstract describes approaches used by our group to activate enzymes for use in dehydrated media, to tailor enzyme selectivity for use in organic solvents, and the use of these 'engineered' enzyme preparations for the synthesis of small molecules and higher molecular weight polymeric products.

1. Enzyme Solubilization in Organic Solvents

In our previous work, we demonstrated that enzymes could be dissolved in organic solvents following conversion of the biocatalysts into hydrophobic ion-pairs with the anionic surfactant AOT (= sodium di(2-ethylhexyl) sulfosuccinate) [1]. The enzyme is highly stable in hydrophobic organic solvents, and in hydrophilic solvents upon the addition of added water. The enzyme activity is also high in hydrophobic organic solvents. This is depicted in *Table 1* for subtilisin BPN' catalysis in the soluble state [2]. α-Chymotrypsin is also highly active and stable in such a system [1]. The high stability and activity of these enzymes in organic solutions has led us to examine methods to utilize these enzymes in the synthesis of fine chemicals as well as develop unique enzyme-polymer complexes for use in organic synthesis.

Peptide Synthesis using Soluble Enzymes in Organic Synthesis

Organic solvent-soluble α-chymotrypsin is an effective catalysts for peptide synthesis in homogeneous organic solutions [3]. The soluble enzyme has values of k_{cat}/K_m for the reaction of *N*-Bz-L-Tyr-OEt with L-Leu-NH_2 to yield the dipeptide *N*-Bz-L-Tyr-L-Leu-NH_2 that are over three

Table 1. *Catalytic Constants for Soluble Subtilisin BPN' in Various Solvents*

Solvent	k_{cat}/K_m [$M^{-1}s^{-1}$][a]	Observed half-life [h][c]
Isooctane	370 ± 40	168
tert-Amyl alcohol	1.2 ± 0.2	100
tert-Amyl alcohol + 0.2% (*v/v*) H_2O	2.8 ± 0.4	n.d.
THF	0.04 ± 0.01	0.17
THF + 1.0% (*v/v*) H_2O	0.36 ± 0.06	0.67
Aqueous buffer	3500 ± 200[b]	150
Isooctane (suspended)	0.6 ± 0.1	n.d.

[a]) Catalytic constants determined for transesterification between *N*-Ac-L-Phe-OEt (0.1–250 mM) and 0.5M PrOH using 14–140 μg/ml soluble enzyme.
[b]) Catalytic efficiency determined for hydrolysis of *N*-Ac-L-Phe-OEt (0.1–1.0 mM) in water using 10–50 μg/ml enzyme.
[c]) n.d. = not determined.

Table 2. *Tripeptide Synthesis Catalyzed by Organic Solvent-Soluble α-Chymotrypsin in Isooctane* (containing 30% (*v/v*) THF). The concentrations of enzyme, acyl donor, and acyl acceptor were 85 μg/ml, 5.0 mM, and 7.5 mM, respectively.

Acyl Donor[a])	Acyl Acceptor	Product	Reaction Time [min]	Yield [%][b])
Val-Tyr-OMe	Leu-NH_2	Val-Tyr-Leu-NH_2	120	38
Ala-Phe-OMe	Leu-NH_2	Ala-Phe-Leu-NH_2	120	50
Val-Trp-OMe	Leu-NH_2	Val-Trp-Leu-NH_2	120	51
Val-Tyr-OMe	Leu-NH_2	Val-Tyr-Leu-NH_2	25	97[c])
Ala-Phe-OMe	Leu-NH_2	Ala-Phe-Leu-NH_2	90	81[c])
Val-Trp-OMe	Leu-NH_2	Val-Trp-Leu-NH_2	90	76[c])
Val-Phe-OMe	Leu-NH_2	Val-Phe-Leu-NH_2	90	90[c])
Ile-Phe-OMe	Leu-NH_2	Ile-Phe-Leu-NH_2	90	49[c])
Val-Tyr-OMe	Leu-NH_2	Val-Tyr-Leu-NH_2	60	50[c])
Val-Tyr-OMe	L-Phe-NH_2	Val-Tyr-L-Phe-NH_2	60	21[c])
Val-Tyr-OMe	D-Leu-NH_2	Val-Tyr-D-Leu-NH_2	60	67[c])
Val-Tyr-OMe	D-Phe-NH_2	Val-Tyr-D-Phe-NH_2	60	68[c])

[a]) All acyl donors were the L-isomers and consisted of the *N*-CBZ blocking group.
[b]) Yield based on conversion of the acyl donor.
[c]) The reaction solution contained 0.15% (*v/v*) water.

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orders of magnitude higher than its suspended counterpart in isooctane (containing 30% (*v/v*) tetrahydrofuran (THF) to aid in substrate solubility). Both enzymes are strongly activated in hydrophobic organic solvents. Addition of small concentrations of water (< 0.2 and 1% (*v/v*) in isooctane/THF and AcOEt, respectively) results in up to a 150-fold activation of α-chymotrypsin-catalyzed peptide synthesis. Importantly, added water did not promote hydrolysis in either isooctane/THF or AcOEt; thus, α-chymotrypsin is highly selective toward peptide synthesis in the nearly anhydrous organic solutions (*Fig.*). Using α-chymotrypsin, a variety of tripeptides were produced from dipeptide amino-acid esters (*Table 2*). Reactivity of D-amino-acid amides as acyl acceptors and partially unblocked amino-acid acyl donors further expands the generality of the use of organic solvent-soluble enzymes as peptide-synthesis catalysts.

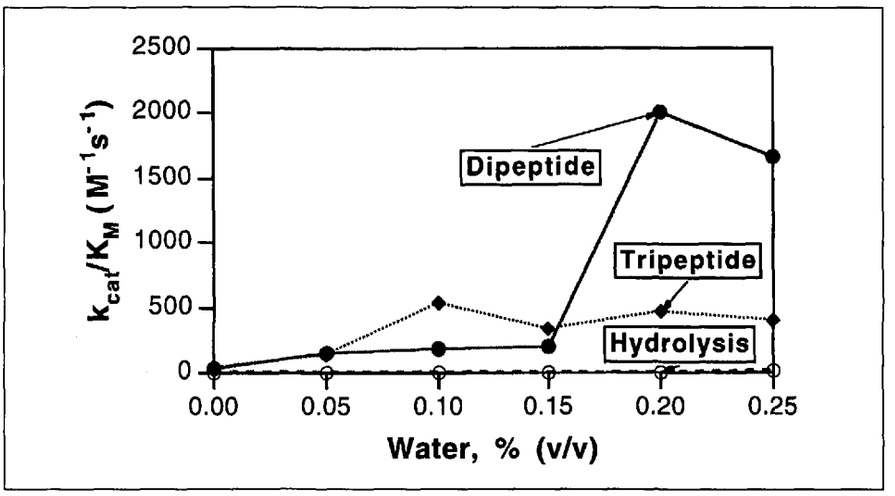

Figure. *Effect of water on α-chymotrypsin-catalyzed peptide synthesis in isooctane/THF and AcOEt*

2. Preparation of 'Plastic enzymes'

The solubility of enzymes in organic solvents led us to develop an approach for the incorporation of enzymes into vinyl-based polymers. These polymers form the basis of a wide range of plastics such as poly(methyl methacrylate) (PMMA) and poly(styrene). Activity of the enzymes embedded the plastic matrix in hexane are up to 30-fold higher than the native chymotrypsin suspended in the solvent. The results for peptide synthesis using the PMMA-entrapped chymotrypsin are particularly striking. The condensation between *N*-Bz-Tyr-OEt and Leu-NH_2 proceeds 500-times faster than with the suspended enzyme in isooctane containing 30% (*v/v*) THF.

[1] V.M. Paradkar, J.S. Dordick, *J. Am. Chem. Soc.* **1994**, *116*, 5009.
[2] P.P. Wangikar, P. C. Michels, D.S. Clark, J. S. Dordick, *J. Am. Chem. Soc.*, submitted.
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Importance of Natural Chemical Resources in Drug Discovery

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'The most important consequence of the structural change in the worldwide pharmaceutical markets is the reduction in R & D returns ... the industry will not realize a satisfactory return on its R & D investment unless research productivity increases substantially'.
(*Lehman Brothers, PharmaPipelines*, 1994)

It is a widely held view in the drug industry today that good margins will only be achieved with innovative drugs that produce significant therapeutic advantage. Also, new markets will be dominated by the first 2–3 drugs approved and, thus, speed in identifying high-quality drug leads provides a competitive edge in pharmaceuticals R & D.

In pursuing new drug leads, the industry has focused on chemical diversity and screening intensity, at times with a focus on vastness of numbers rather than the quality of outputs. Chemical libraries must be biologically relevant and diversity of pharmacophore is much more critical than hundreds of thousands of simple and similar chemicals. Combinatorial chemistry is an exciting and promising new tool but is appropriately gaining a more realistic perspective contrary to its earlier hype.

It has been estimated, that combinatorial chemistry could have provided possibly 10% of the drugs under development today and that methods development might only double this. Therefore, 80% of new drugs will come from other approaches including medicinal chemistry, computational chemistry and natural-products chemistries.

Another important element of success in drug discovery is the use of genomic information to define processes that drive disease and to convert these processes into high throughput screens. The number of screens in large companies are growing from 10 targets per year in the late 1980s to 30 per year in 1995 and over 100 targets per year by the late 1990s. Also, there is a need to extend screening to incorporate *in vivo* factors, including absorption, distribution, biological half-life, metabolism and toxicity.

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