Broadening the scope of biocatalysis to new reactions

In contrast to a few years ago, there are now many different chemistries that can be carried out enzymatically, including reductions, aminations, hydroxylations and oxidations. Spiros Kambourakis and David Rozzell of BioCatalytics review the chemistries available and give examples.

After years of being almost ignored by chemists, biocatalysis is now moving into the mainstream as a result of rapid technological change. Recent research has produced dramatic increases in both the numbers of enzymes available and the different types of chemical reactions that can be catalysed enzymatically. The result has created an unprecedented opportunity for organic chemists: enzymes can now be exploited for chemical synthesis in many more ways than ever before, including many reaction types that were inconceivable as enzyme-catalysed steps just a few years ago.

Lipases have been used for resolution reactions for years, and many people still identify biocatalysis with lipase-catalysed resolutions. In the past only a few different lipases and esterases were available, and if reactions using these few enzymes were not successful, there were no biocatalytic alternatives. Today, screening sets of 20 or more different hydrolytic enzymes are offered, with all enzymes readily available in larger quantities for process development and scale-up. The chances of successfully implementing biocatalytic resolutions at the commercial scale have never been higher.

But the opportunities for chemists in the field of biocatalysis have expanded far beyond the use of lipases for resolving chiral compounds. The combination of high throughput screening methods and bioinformatics has stimulated enzyme discovery research, leading to the development of new enzymatic alternatives for a broad range of chemical reactions. In some cases, such as amino acid synthesis, where only a handful of enzymes were traditionally available, sets of diverse transaminases and reductive aminases capable of catalysing the synthesis of a wide range of different amino acids and amines are now offered commercially. In other cases, including stereoselective ketone reduction or nitrile hydrolysis, large numbers of enzymes are now available where virtually none existed a few years ago. Most recently, enzymes that catalyse chemical reactions that were considered 'off limits' for biocatalysis are being developed. This article highlights some of the new enzyme chemistry platforms that are now available and looks at future opportunities in some new areas as well.

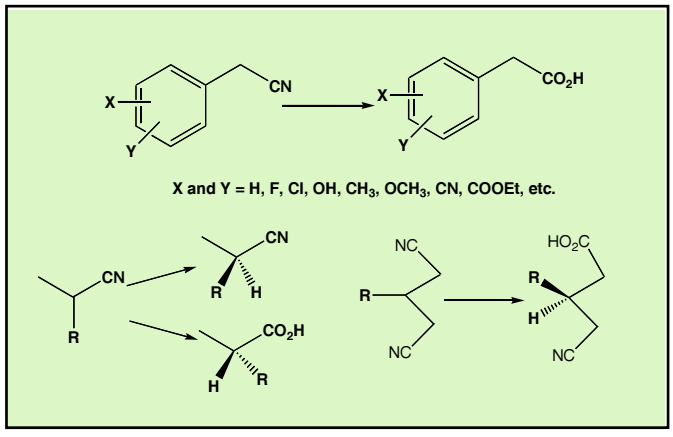

Fig 1. Examples of reactions catalysed by nitrilases.

Other hydrolytic enzymes

The development of broad and diverse sets of amidases and nitrilases has expanded the potential for stereoselective hydrolytic reactions to amides and nitriles. Nitrilases in particular are being used with increasing frequency due to the advantages that enzymes offer for hydrolysing nitriles in high yield under extremely mild conditions. Nitrilases catalyse the efficient hydrolysis of nitriles at neutral pH and room temperature, meaning that nitriles can be converted to carboxylic acids in the presence of other functional groups, such as esters or amides, that are normally more labile. When stereoselectivity is required, single enantiomers of chiral nitriles can be hydrolysed (Fig 1), and pro-chiral dinitriles can be stereoselectively converted to the chiral mono-acids. The latter reaction offers the potential for conversion yields approaching 100 per cent of theoretical.

Redox chemistries

Enzyme-catalysed oxidations and reductions have been known from biochemical research for many years, but it is only recently that these reactions have found commercial applications. Stereoselective ketone reduction has proven to be broadly useful largely due to the explosion of new enzymes in this category over the past few years. Large numbers of new ketoreductases (KREDs) are now available that can catalyse the stereoselective reduction of virtually any ketone to produce either the R or S alcohol (Fig 2). With methods to recycle the nicotinamide cofactor (NADH or NADPH) now well established and inexpensive, the potential exists for the enzyme-catalysed synthesis of a wide range of chiral alcohols cost-effectively. Figure 3 shows a partial list of ketones that can be reduced efficiently by KRED enzymes; the number of examples is expanding dramatically as

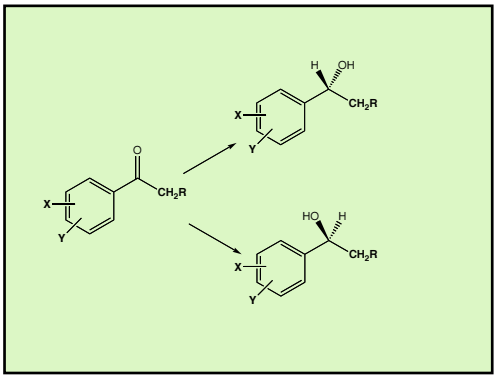

Fig 2. KRED-catalysed stereoselective ketone reduction.

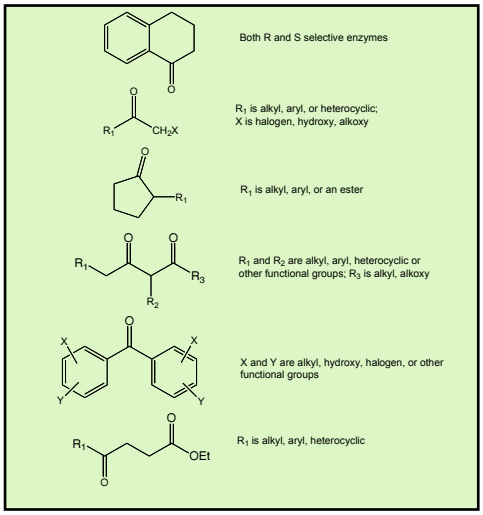

Fig 3. Examples of ketones that can be reduced by KRED enzymes.

this enzyme chemistry platform moves into the mainstream, particularly in the field of medicinal chemistry.

Reductive amination

Reductive amination is a reaction that is especially useful for the production of non-naturally occurring amino acids. Screening sets of enzymes called amino acid dehydrogenases are now available that can be used to rapidly identify the best enzyme for the conversion of almost any 2-ketoacid to the corresponding alpha-amino acid. Enzymes selective for producing L-amino acids are now very well developed, but recently BioCatalytics developed the first D-selective amino acid dehydrogenases for the production of D-amino acids, both naturally-occurring and non-natural (Fig 4).

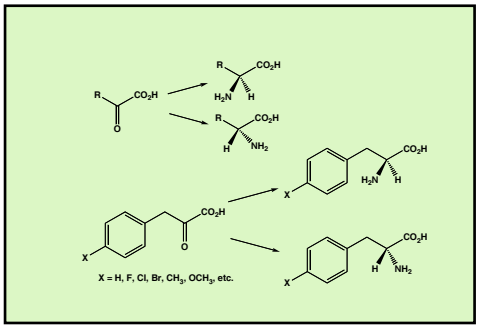

Fig 4. Enzymatic reductive amination to produce unnatural amino acids.

Oxidations

New enzymes that catalyse oxidation reactions are now available. Baeyer-Villiger mono-oxygenases can catalyse the classic conversion of a ketone to an ester, and this reaction is particularly effective for the conversion of cyclic ketones to lactones. Chiral sulfoxidation is also possible, with sulfides being oxidised to chiral sulfoxides. High stereoselectivity is often observed. Other enzyme-catalysed oxidations where stereoselectivity is not required are also useful. Aryl alcohol oxidases catalyse the oxidation of aryl alcohols to the corresponding aldehyde. This is a reaction that is often difficult to carry out in high yield, especially when other oxidation-sensitive functionality is present in the molecule. Aryl alcohol oxidases act on a wide range of structurally diverse aryl alcohols. Compounds containing phenyl, naphthyl, pyridyl, and other aromatic side-chains bearing a wide range of substitution can be oxidised enzymatically to the aldehyde using only air (oxygen) as the oxidant, allowing a green alternative to chromium-based oxidation reagents (Fig 5).

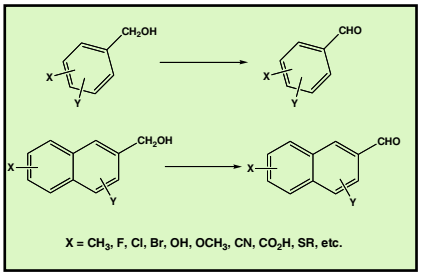

Fig 5. Aryl alcohol oxidase-catalysed reactions.

Transamination reactions

Amine transfer reactions are an alternative to reductive amination for the introduction of an amino group. An advantage of transamination reactions is that by using the appropriate enzyme, either amino acids or amines may be produced. Inexpensive amine donors such as aspartate, glutamate, or simple amines are typically used. Amine transaminases can also be used to resolve a racemic mixture of a chiral amine.

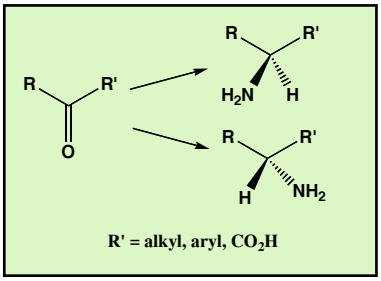

Fig 6. Enzyme-catalysed transamination can produce both optically active amino acids and amines.

Carbon-carbon bond formation

The formation of chiral cyanohydrins is catalysed by a group of enzymes known as oxynitrilases. For years the only oxynitrilase that was commercially available was extracted from almonds, but today there are both R-selective and S-selective oxynitrilases produced microbially. The substrate range is reasonably broad, and these enzymes have enabled the efficient synthesis of compounds such as substituted mandelic acids by the combination of stereoselective cyanohydrin formation with hydrolysis of the resulting hydroxynitrile, which can also be catalysed enzymatically. Aldolases are another developing class of enzymes finding increased interest due to the possibility of catalysing stereoselective aldol condensations to produce chiral beta-hydroxy aldehydes with high stereochemical purity.

Hydroxylation reactions

The hydroxylation of a hydrocarbon or aromatic ring has always been a difficult chemical reaction to carry out. P450 enzymes are known to catalyse these types of reactions, but these types of enzymes have never been available as biocatalysts until recently. Human CYP biocatalysts are now offered for the synthesis of drug metabolites, and these enzymes work well for producing quantities of material for structure identification and preliminary characterisation. The availability of these enzymes will open up new possibilities for the application of enzymes for reactions that were previously not considered for biocatalysis.

C=C double bond reduction

The application of the ability of isolated enzymes to reduce carbon-carbon double bonds is another novel biocatalytic reaction that is now possible. Alkenes can be reduced in the presence of nicotinamide cofactors. The reduction can be stereoselective when the alkene is substituted, thus offering a new, green chiral chemistry platform for organic chemists. sp²

FURTHER INFORMATION

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