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Biocatalysis for Sustainable Organic Synthesis

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Biocatalysis offers mild reaction conditions, an environmentally attractive catalyst–solvent system, high activities, and chemo-, regio-, and stereoselectivities, while the use of enzymes generally circumvents the need for functional group activation and avoids protection/deprotection steps required in traditional organic syntheses. This review, using β-lactam antibiotics as an example, discusses recent advances in biocatalysis research towards the goal of 'green' methodologies for the manufacture of (fine) chemicals and the emulation of a cell's enzymatic cascade processes.

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Introduction

There is currently much attention being focused on the application of catalytic methods—homogeneous, heterogeneous, and enzymatic—as atom-efficient, cleaner alternatives to traditional organic syntheses.[1] The goal is the development of green, sustainable methodologies for the manufacture of (fine) chemicals.

In this context, biocatalysis has many potential benefits: mild reaction conditions (physiological pH and temperature), an environmentally attractive catalyst (an enzyme) and solvent (often water), high activities, and chemo-, regio-, and stereoselectivities. Furthermore, the use of enzymes generally circumvents the need for functional group activation and avoids protection and deprotection steps required in traditional organic syntheses. This affords synthetic routes which are shorter, generate less waste, and, hence, are both environmentally and economically more attractive. The ultimate role model is the living cell in which numerous enzymatic steps are exquisitely orchestrated in multi-step organic syntheses. A major goal is, therefore, to emulate nature's cell factories by developing enzymatic cascade processes, either de novo or by improving existing metabolic pathways (metabolic pathway engineering).

The time is ripe for the widespread application of biocatalysis in industrial organic synthesis.[2–13] Advances in recombinant DNA techniques[14] have made it, in principle, possible to produce virtually any enzyme for a commercially acceptable price. Moreover, advances in protein engineering have made it possible, using techniques such as site-directed mutagenesis and in vitro evolution by means of gene shuffling,[15–18] to manipulate enzymes such that they exhibit the desired properties: substrate specificity, activity, selectivity, stability, pH profile, and so forth. Furthermore, the development of an ever-increasing arsenal of immobilization techniques has provided effective methods for optimizing the operational performance and the recovery and re-use of enzymes.

Green Synthesis of β-Lactam Antibiotics

An excellent example of the impact that biocatalysis can have on the replacement of traditional organic syntheses by cleaner, greener alternatives is provided by the industrial synthesis of the β-lactam antibiotics, comprising the semi-synthetic penicillins and cephalosporins (Scheme 1).[19,20]

Up to the mid-1980s, these semi-synthetic penicillins and cephalosporins were produced via chemical procedures, with the exception of the raw material, penicillin G, which was produced by fermentation of *Penicillium chrysogenum*. The first step involved cleavage of the phenylacetyl side-chain, by a chemical procedure, affording 6-aminopenicillanic acid (6-APA), the key intermediate in the synthesis of semi-synthetic penicillins (Scheme 2). The key intermediate for semi-synthetic cephalosporins, 7-aminodeacetylcephalosporanic acid (7-ADCA), was obtained from penicillin G via a chemical ring expansion, followed by analogous chemical cleavage of the phenylacetyl side-chain.

These 'stoichiometric' chemical transformations, involving protection and deprotection steps, generate copious amounts of waste, that is, they have high *E*-factors,[1] and employ environmentally unattractive reagents and solvents. For example, the production of 1 kg of 6-APA involves the use of 0.6 kg Me_3SiCl, 1.2 kg of PCl_5, 1.6 kg of $PhNMe_2$, 0.2 kg of NH_3, 8.4 L of Bu^nOH, and 8.4 L of CH_2Cl_2.[21] Moreover, the reaction is performed at −40°C.

Although enzymatic cleavage of penicillin G was already known in the 1960s, the procedure was inconvenient, inefficient, and expensive, owing to low productivities, large

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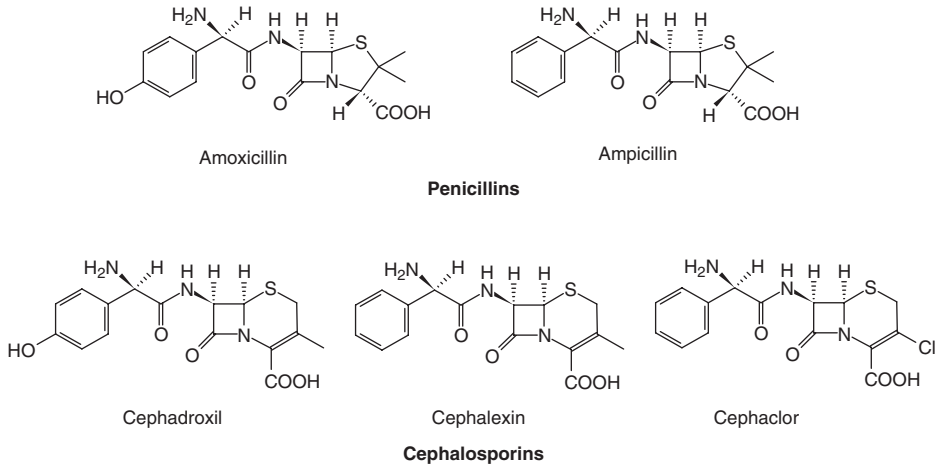
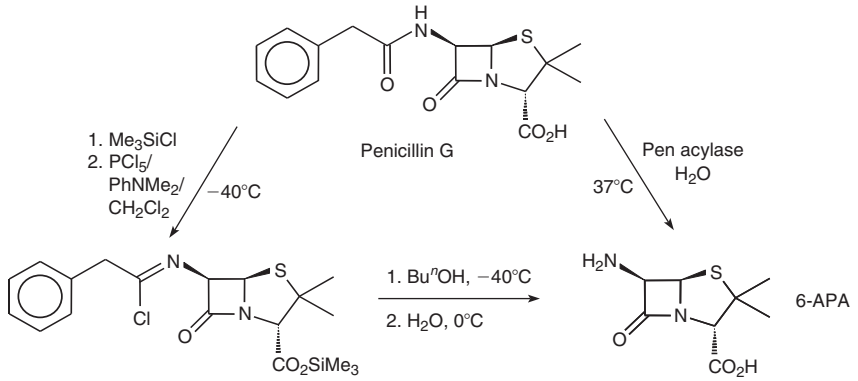


Scheme 1. Structures of penicillins and cephalosporins.



Scheme 2. Enzymatic versus chemical process for 6-APA.

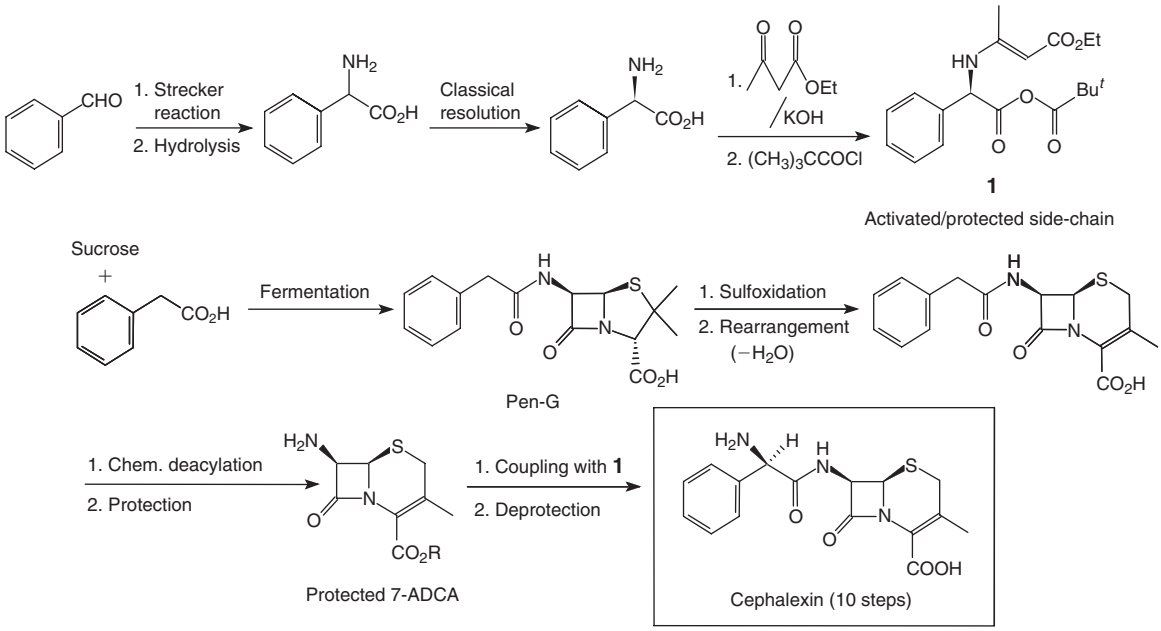
reaction volumes, and discarding the enzyme, pencillin acylase, after one use. However, in the 1980s pencillin acylases with improved stability were obtained and, by employing recombinant DNA technology, efficient production of the enzyme became possible. Combined with the development of effective procedures for immobilization of the enzyme, which made recycling possible, dramatic reductions in enzyme costs and overall process costs were forthcoming.

The enzymatic cleavage of penicillin G (Scheme 2) is performed in water at 37°C and the only reagent used is ammonia (2 L H_2O and 0.09 kg NH_3 for 1 kg of 6-APA) to control pH. The economic and environmental benefits of enzymatic versus chemical deacylation are obvious, and this has led to the widespread replacement of the latter by the former in the last 15 years. Similarly, chemical deacylation has been universally replaced by enzymatic deacylation in the manufacture of the cephalosporin nucleus, 7-ADCA.

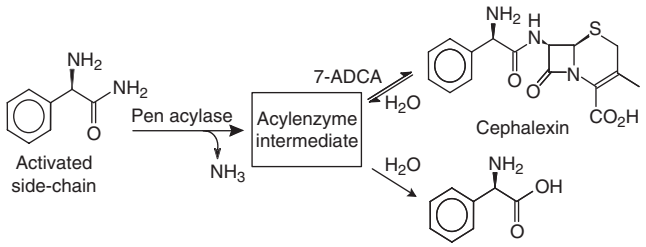
Nonetheless, a cursory perusal of the overall process for the manufacture of cephalexin, the largest cephalosporin with an annual production of about 3000 tons worldwide, reveals a total of ten, largely classical, chemical steps (Scheme 3). This results in the generation of approximately 40 kg of waste per kilogramme of cephalexin. The above-mentioned replacement of the chemical deacylation step by an enzymatic one constitutes a substantial improvement but clearly there are still many opportunities for the substitution of classical chemical procedures with cleaner biocatalytic alternatives. For example, many of these steps involve protection, deprotection, and activation of functional groups, which presumably could be avoided in an enzymatic procedure.

The next step to be addressed, in this context, was the enzymatic coupling of the side-chain, (*R*)-phenylglycine, to the 7-ADCA nucleus. This is essentially the reverse of enzymatic cleavage but with a different acid, (*R*)-phenylglycine instead of phenylacetic acid. In principle, this can be achieved either through thermodynamic control (reversal of the hydrolytic process) or kinetic control (transacylation using a reactive side-chain donor such as an ester or an amide). Thermodynamically controlled condensation is not feasible as the equilibrium, in water, is unfavourable.

The main obstacle confronting the kinetically controlled synthesis is competing hydrolysis of the side-chain donor, either directly or indirectly via hydrolysis of the cephalexin product (Scheme 4), which necessitates the use of an excess of side-chain donor. Hence, the synthesis/hydrolysis ratio (S/H; mole of product per mole of hydrolyzed side-chain donor) is a good indicator of the economic viability of the process. For an economically viable process, the S/H ratio should be as high as possible at high (>90%) 7-ADCA conversion, since the latter is the expensive component in the coupling reaction. The Dutch chemical company DSM is currently producing cephalexin using such an enzymatic coupling process.



Scheme 3. Cephalexin synthesis—classical route.



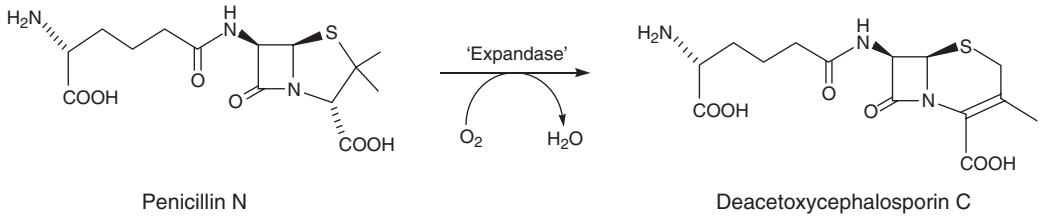
Scheme 4. Enzymatic synthesis of cephalexin.

Notwithstanding the above-mentioned improvements, the manufacture of cephalexin (Scheme 3) still involves many chemical steps. The key 7-ADCA nucleus is obtained from penicillin G by sulfoxidation and dehydration/ring expansion followed by (enzymatic) deacylation. Replacement of this two-step chemical procedure for ring expansion by a biotransformation would have obvious economic and environmental benefits. In the biosynthetic pathway the crucial step is the deacetoxycephalosporinase (expandase)-catalyzed expansion of the 5-membered ring of penicillin N to afford deacetoxycephalosporin C (Scheme 5). However, penicillin N is not commercially available and, unfortunately, expandase does not accept penicillin G (or penicillin V) as a substrate. A solution to this problem was found by introducing the genes for isopenicillin N epimerase and penicillin N expandase from *Streptomyces clavuligerus* into *Penicillium chrysogenum*, which naturally does not produce either penicillin N or any cephalosporin. Substitution of phenylacetic acid with adipic acid in the fermentation of this transgenic *Penicillium sp.* leads to the formation of adipyl-7-ADCA. Enzymatic deacylation of the latter, using a specially developed acylase, and enzymatic coupling of the resulting 7-ADCA with the side-chain donor affords a green, six-step process for the manufacture of cephalexin (Scheme 6).

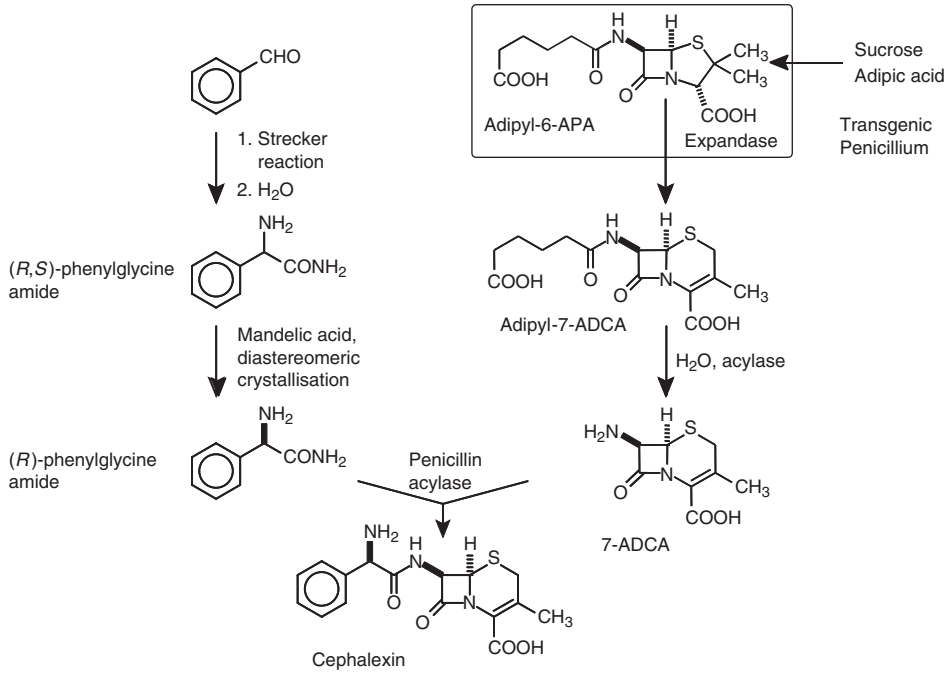
Examination of Scheme 6 reveals that the nucleus part of the synthesis involves a fermentation followed by two enzymatic steps. However, the synthesis of the side-chain donor still involves a chemical procedure. Hence, a current goal is to replace these steps by, preferably, one enzymatic step from benzaldehyde. Currently, the most economical process for the synthesis of the side-chain donor, (*R*)-phenylglycine amide, involves a Strecker reaction of benzaldehyde with HCN/NH_3 followed by selective hydrolysis to the racemic amino amide and a dynamic resolution of the latter involving an asymmetric transformation of its salt with (*R*)-mandelic acid (see Scheme 6). DSM has also developed an enzymatic procedure for conversion of the racemic amino amide to the (*R*)-isomer using an amidase from a *Pseudomonas putida* sp.[19] However, a distinct disadvantage of this process is that it produces the desired product in a maximum yield of 50%, together with 50% of the (*S*)-acid (Scheme 7). Recycling of the latter to the racemic amino amide would require three extra steps, esterification, racemization, and ammoniolysis, with concomitant generation of copious amounts of salts. This is clearly not a viable proposition.

Nitrile-Converting Enzymes

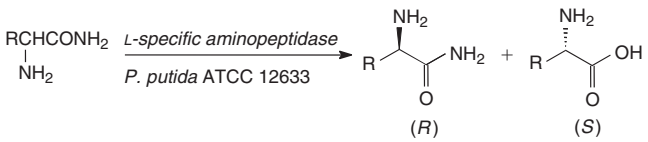
It is clear that, in order to obtain a short enzymatic synthesis of the (*R*)-amino amide, it is necessary to use a nitrile-converting enzyme. Catabolic pathways for the in vivo degradation of nitriles involve two types of nitrile-converting enzymes, as shown in Scheme 8.[22] The first type, nitrilases, catalyzes the hydrolysis of a nitrile directly to the corresponding carboxylic acid. These enzymes are often highly enantioselective but are clearly not interesting in the context of amino amide synthesis. The second type comprises the nitrile hydratases, which catalyze the conversion of a nitrile to the corresponding amide. Microorganisms which



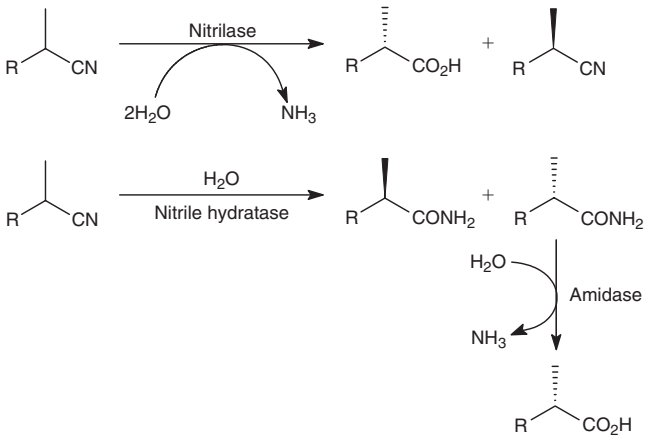
Scheme 5. Biosynthesis of deacetoxycephalosporin C.



Scheme 6. A green process for cephalexin in six steps.



Scheme 7. Enzymatic production of (*R*)-phenylglycine amide. R = C_6H_5.

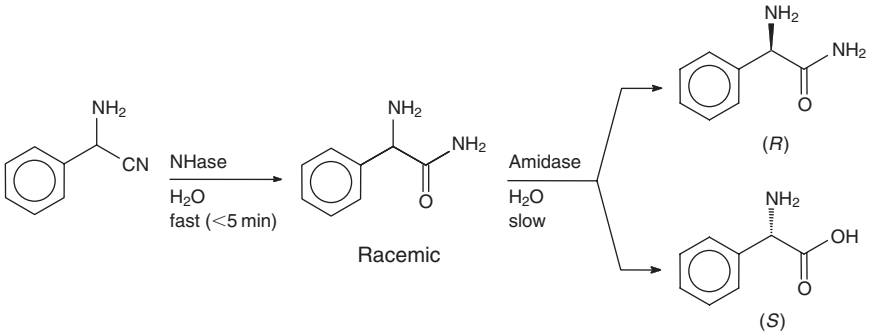


Scheme 8. Nitrile-converting enzymes. Nitrile hydratase is aselective; amidase is highly enantioselective.

utilize this pathway also contain an [usually (*S*)-selective] amidase for further conversion of the amino amide. Clearly the nitrile hydratase/amidase route is also not a viable option (see discussion of the DSM amidase process above).

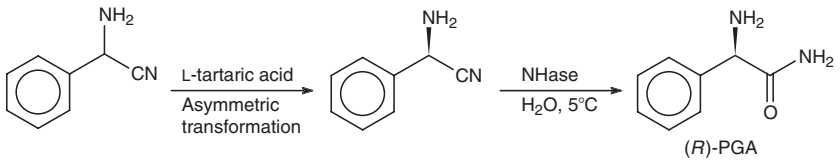
The direct conversion of racemic phenylglycine nitrile into (*R*)-phenylglycine amide, using an (*R*)-selective nitrile hydratase in a dynamic kinetic resolution (the nitrile is known to racemize easily), would clearly be superior to all existing methodologies. In this context, it is worth pointing out that biocatalytic conversions of nitriles are usually performed with whole cells rather than free enzymes. This usually means that the whole-cell biocatalyst contains, in addition to a nitrile hydratase, an amidase which is generally (*S*)-selective. We screened about 60 nitrile hydratase-harbouring strains and found five that converted racemic phenylglycine nitrile.[23] However, none of these contained an enantioselective nitrile hydratase; the observed enantioselectivity (Scheme 9) was entirely due to the (*S*)-selective amidase present. The nitrile hydratase is very fast and aselective and the amidase is slow and very enantioselective.

Why is the nitrile hydratase completely non-stereoselective? This is unusual for an enzyme. One possible reason is that racemization of the product or an intermediate, occurs in situ. In order to test this we studied the nitrile



Strain	(*R*)-PGA		(*S*)-PG		*E*
	Yield [%]	*e.e.* [%]	Yield [%]	*e.e.* [%]	
R. globerulus MAWA	48	>99	52	>97	>100
R. rhodococcus MAWA	43	85	38	>99	>100

Scheme 9. Enzymatic hydrolysis of racemic phenylglycine nitrile.



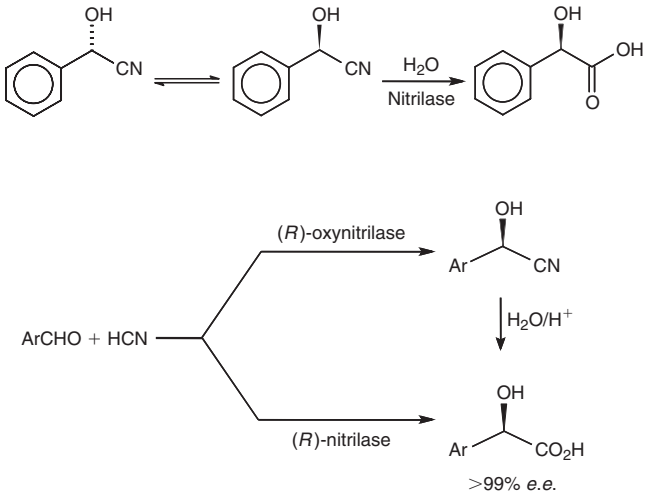
Strain	Time [h]	Yield [%]	*e.e.* [%]
SP 361	7	96.0	95.0
R. globerulus MAWA	4	98.7	94.6
R. erythropolis MAWF	7	95.6	97.4
R. rhodococcus MAWE	7	98.6	96.8
R. rhodococcus MAWB	6	95.4	98.3
	22	95.1	99.5

Scheme 10. Stereoretentive hydrolysis of (*R*)-phenylglycine nitrile.

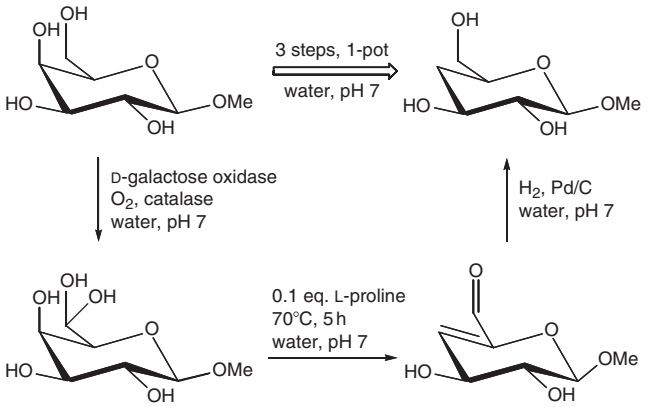
hydratase catalyzed hydration of (*R*)-phenylglycine nitrile, prepared by asymmetric transformation of the L-tartaric acid salt of the racemate.[24] We found that the troublesome retro-Strecker reaction could largely be circumvented by conducting the reaction in a fed-batch mode (to maintain a low concentration of nitrile) at pH 7 and 5°C. (*R*)-Phenylglycine amide was obtained in >95% yield and >95% *e.e.* (Scheme 10). Interestingly, the enantiomeric purity of the product increased at high conversions. This was a result of the slow conversion of the small amount of the (*S*)-isomer in the amino amide product (the amino nitrile substrate was not enantiomerically pure). Hence, the amidase serves to upgrade the (*R*)-amino amide product. These results clearly show that in situ racemization is not the reason for the observed lack of stereoselectivity. Furthermore, based on what is known regarding the mechanism of nitrile hydratases, there does not seem to be any mechanistic rationale for this lack of stereoselectivity. This suggests that an enantioselective nitrile hydratase for amino nitriles will be found. One could say that this is one of the holy grails of biocatalysis. The search continues.

If the product required is the carboxylic acid, rather than the amide, then a nitrilase-based process becomes interesting. This is of particular interest in the synthesis of enantiomerically pure α-hydroxy acids by hydrocyanation of an aldehyde followed by hydrolysis of the resulting cyanohydrin. There are two possibilities for employing a biocatalyst in this transformation (Scheme 11): enantioselective hydrocyanation catalyzed by an oxynitrilase followed by chemical hydrolysis of the cyanohydrin, or enantioselective nitrilase-catalyzed hydrolysis of racemic cyanohydrin (formed by non-enzymatic hydrocyanation). In the latter case, the cyanohydrin enantiomers rapidly equilibrate under the reaction conditions, resulting in a dynamic kinetic resolution, that is, an attractive one-step process from a benzaldehyde to the corresponding (*R*)-mandelic acid. This concept has been applied by Mitsubishi in a commercial process for the manufacture of (*R*)-mandelic acid.[22]

Based on these interesting results we expect that nitrilases, which have become commercially available only recently, will find wide application in the future. Nitriles are, after all, intermediates in many organic syntheses.



Scheme 11. Enzymatic synthesis of α-hydroxy acids.



Scheme 12. A catalytic cascade process for the synthesis of a deoxy sugar.

Fine Chemical Processes of the Future: Catalytic Cascades

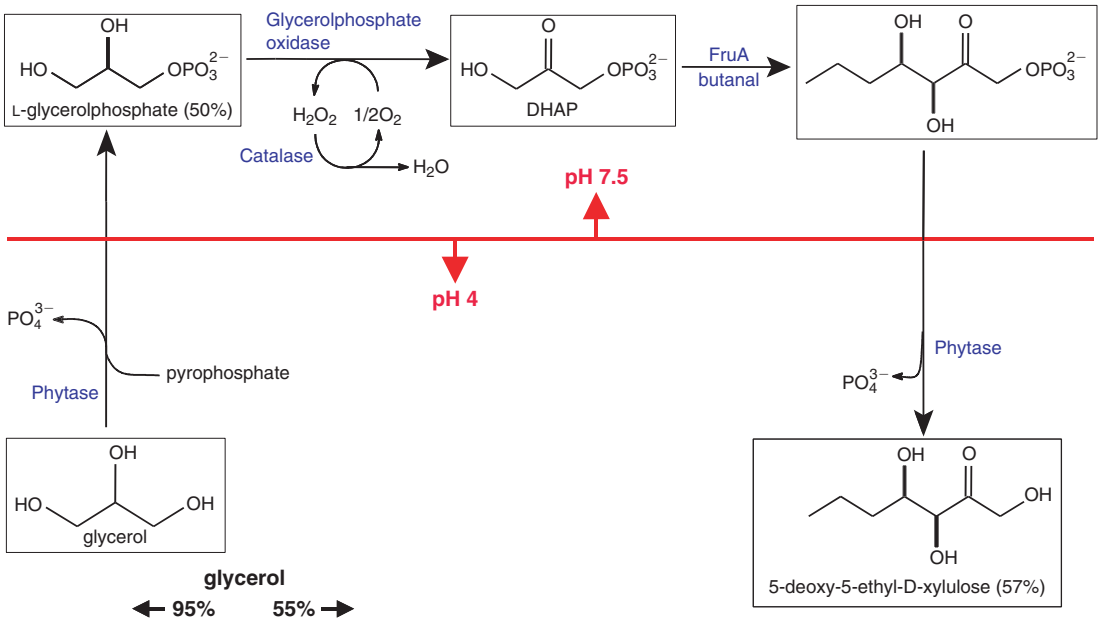
In the quest for processes for the manufacture of fine chemicals that are both economically and environmentally attractive, catalysis is expected to play an important role.[1] The application of catalysis can provide for processes that involve fewer steps, generate minimum waste, and can possibly be carried out in continuous operation. Fine chemical syntheses generally involve multi-step processes and the ultimate in efficiency is to combine these, preferably catalytic, steps into a one-pot, multi-step catalytic cascade process.[25] Indeed, this is truly emulating nature's catalysts. Metabolic pathways conducted in living cells involve an elegant orchestration of a series of biocatalytic steps into an exquisite multicatalyst cascade, without the need for separation of intermediates.

Catalytic cascade processes have numerous advantages. Fewer unit operations (process telescoping) are involved, which translates to less reactor volume, less solvents, shorter cycle times, and higher volumetric and space/time yields. In most cases this will also lead to less waste generation (lower *E*-factors). Another benefit is that by coupling steps together unfavourable equilibria can be driven in the desired direction. For example, the nitrilase-catalyzed conversion of an aldehyde and HCN to an α-hydroxy acid (see earlier) involves driving the equilibrium of the hydrocyanation by irreversible hydrolysis of the nitrile group.

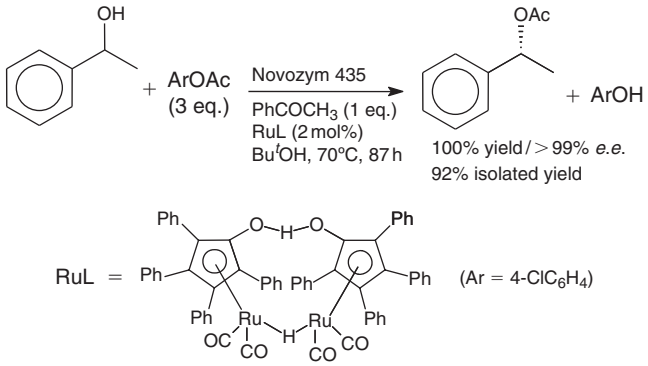
Notwithstanding the considerable benefits, catalytic cascade processes are fraught with several problems. Different catalysts, for example combinations of biocatalysts and chemocatalysts, are often incompatible. The rates may be very different and the optimum conditions for each catalyst may differ considerably. Complicated reaction mixtures, requiring complicated work-up procedures may result, and recycling of a complex mixture of catalysts will not be simple. How does nature cope with these problems in the living cell? Interference between the different biocatalytic steps is circumvented by compartmentalization in or behind membranes. Following nature's example, the key to compatibility would appear to be compartmentalization. This could be achieved, for example, by using immobilized (bio)catalysts (see later) or by performing the reaction in a liquid/liquid biphasic system.[26]

An example of a one-pot three-step catalytic cascade is shown in Scheme 12.[27] In the first step galactose oxidase catalyzes, in water at pH 7, the selective oxidation of the primary alcohol group to the corresponding aldehyde at the 6-position. Subsequently, the aldehyde, present as the hydrate, undergoes L-proline-catalyzed elimination of water (at 70°C), to give the corresponding unsaturated aldehyde. Finally, the latter is catalytically hydrogenated over Pd/C. The overall process constitutes a catalytic cascade for the conversion of galactose to the deoxy sugar.

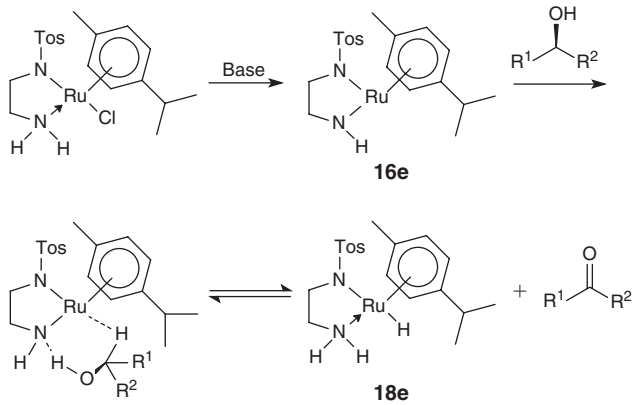
A biocatalytic, one-pot/four-enzyme process for the conversion of inexpensive, readily available glycerol to a non-natural carbohydrate is depicted in Scheme 13.[28] In the first step, glycerol undergoes phytase-catalyzed phosphorylation with pyrophosphate, affording a racemic mixture of glycerol phosphate. This is followed by L-glycerolphosphate oxidase-catalyzed oxidation to dihydroxyacetone phosphate (DHAP), the required co-substrate for DHAP-dependent aldolases. In the next step, the DHAP-dependent fructose bisphosphate aldolase catalyzes the aldol rection with *n*-butyraldehyde. Finally, the aldol adduct is dephosphorylated by the action of phytase to afford the non-natural carbohydrate, 5-deoxy-5-ethyl-D-xylulose in 57% yield. The key to performing the overall reaction is using a pH shift to switch enzymes on and off. The initial phosphorylation is conducted at pH 4, an optimum pH for phytase. This step is also conducted in 95% aqueous glycerol, to promote phosphorylation with regard to hydrolysis (the reverse reaction). The mixture is then diluted to 55% glycerol and the pH adjusted to 7.5, thereby 'switching off' the phytase. The subsequent oxidation and aldol reaction proceed smoothly at this pH. Catalase is present in order to decompose the hydrogen peroxide, formed in the oxidation step, which otherwise would have a detrimental effect on the enzymes. Finally, the pH is brought back to 4 and the phytase is reactivated for the dephosphorylation step. At the same time the unutilized D-glycerol phosphate is also dephosphorylated. The overall process constitutes an elegant catalytic cascade for the synthesis of a range of non-natural carbohydrates as the aldolase can accept a variety of aldehyde substrates.



Scheme 13. Cascade catalysis: one-pot/four-enzyme synthesis of a non-natural carbohydrate.



Scheme 14. Chemoenzymatic DKR of α-methylbenzyl alcohol.



Scheme 15. Mechanism of ruthenium-catalyzed racemization of secondary alcohols.

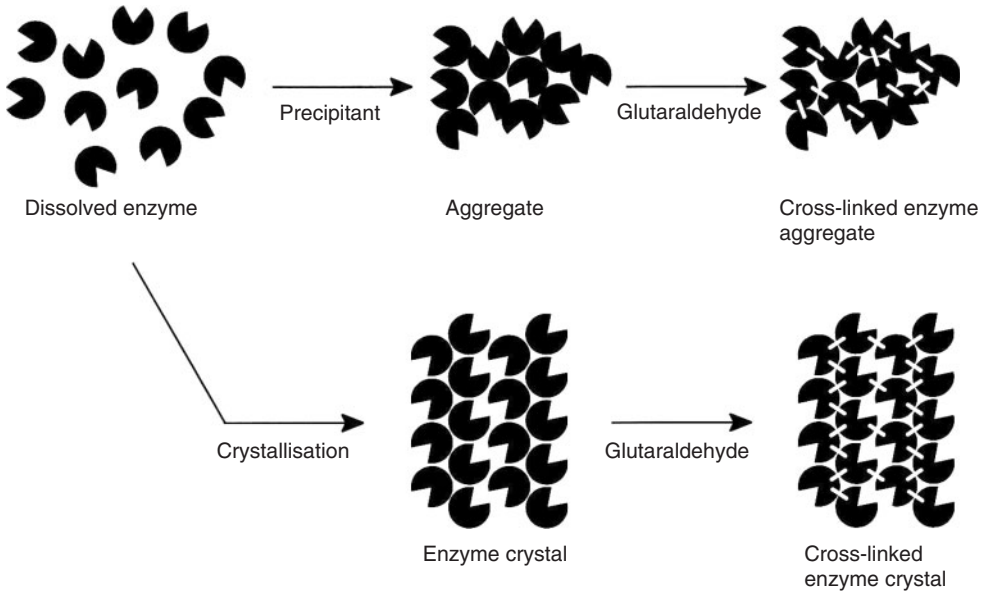
An example of a combination of a metal complex- and an enzyme-catalyzed reaction into a one-pot process is the chemoenzymatic dynamic kinetic resolution (DKR) of chiral secondary alcohols.[29] This combines the lipase-catalyzed enantioselective acylation of the alcohol with ruthenium-catalyzed in situ racemization of the unreacted enantiomer (Scheme 14). Bäckvall and co-workers were the first to describe the successful combination of ruthenium-catalyzed racemization with lipase-catalyzed[30] resolution. Subsequently, several groups have reported the use of various ruthenium complexes in this DKR.[29]

We showed[31] that a ruthenium(II) complex of the *p*-toluenesulfonamide of ethylenediamine catalyzes the racemization of optically active secondary alcohols in the presence of an added base. We propose that racemization involves the initial formation of a reactive 16-electron complex by base-mediated elimination of HCl (Scheme 15). This is followed by coordination of the alcohol substrate and reversible dehydrogenation. We propose that the latter involves non-classical, metal–ligand bifunctional catalysis as proposed by Noyori and coworkers for asymmetric hydrogen transfer between alcohols and ketones.[32]

Cross-Linked Enzyme Aggregates

As noted earlier the key to success in designing catalytic cascade processes may be, in emulation of nature, to design for compatilibity by compartmentalization. This can be achieved, for example, by immobilizing one or more of the catalysts involved. Obviously when two catalysts are immobilized, for example on a support, then they cannot interact with each other. Hence, reaction of a metal complex with an enzyme, for example, can be circumvented.

It is worth noting, in this context, that the economic viability of an enzyme-catalyzed process generally depends on the effective immobilization of the enzyme in order to increase its operational stability and facilitate its recovery and recycling (for example see penicillin acylase, mentioned earlier). Recently, we developed a simple and extremely effective method for enzyme immobilization as cross-linked enzyme aggregates.[33,34] Cross-linked enzyme crystals (CLECs), developed in the early 1990s,[35] represented a significant advance in enzyme immobilization technologies. CLECs

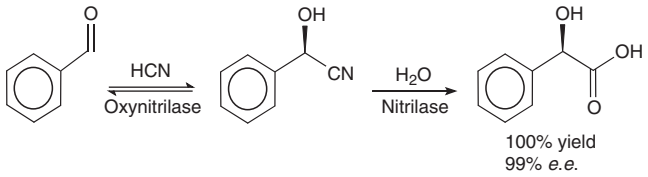


Scheme 16. CLEAs versus CLECs.

proved to be highly stable during operation and, because they are essentially 100% protein, to have high catalyst productivities (mass of product to mass of catalyst per hour) and space–time yields. An inherent disadvantage of the CLECs is the need to crystallize the enzyme, which is an often laborious procedure requiring a highly pure enzyme. Hence, we surmised that comparable results could possibly be obtained by precipitating the enzyme and cross-linking the resulting physical aggregates of enzyme molecules (Scheme 16). This led to the development of a new genus of immobilized enzymes which we called cross-linked enzyme aggregates (CLEAs).[36]

CLEAs have many advantages: there is no need for pure enzyme (the method essentially combines purification and immobilization in one step and we have generally not experienced problems in preparing CLEAs from impure samples of enzymes), thus the procedure is exquisitely simple, fast, and inexpensive. In common with CLECs, CLEAs are essentially 100% (active) protein, thus providing high catalyst and volumetric productivities. The high retention of activity, analogous to that observed with CLECs is attributed to the fact that the cross-linking agent, glutaraldehyde, reacts only with the amino groups of lysine residues on the external surface of the enzyme aggregates or crystals, respectively. This contrasts with the extensive denaturation that was often observed in the earlier technique of cross-linking in solution (CLE) where more vital lysine residues could be accessible to the glutaraldehyde. The method appears to be universally applicable and has already been applied to, for example, lipases, esterases, penicillin acylase, nitrilases, oxynitrilases, glucose oxidase, galactose oxidase, carbonyl reductases, formate dehydrogenase, catalase, and deoxyribose aldolase. In contrast with CLECs, combi-CLEAs can be prepared by co-precipitating two (or more) enzymes and cross-linking the resulting aggregates (see later for an example).

The initially developed method[36] involved a two-step procedure: precipitation and subsequent addition of a



Scheme 17. One-step conversion of benzaldehyde into (*R*)-mandelic acid with a combi-CLEA. Conditions: Enzymes (*R*)-oxynitrilase from *Prunus amygdalus*, nitrilase from *Pseudomonas fluorescens pap79*; benzaldehyde 1 mM, HCN 5 mM, 30°C, 15 min.

cross-linking agent, generally glutaraldehyde. Subsequent development led to a superior one-step procedure[37] in which the precipitant and cross-linking agent were added simultaneously to a solution of the enzyme in aqueous buffer. A further refinement involved the addition of an additive to the enzyme solution, for example a crown ether or a surfactant, which could modify the conformation of the enzyme, resulting in a higher activity and/or selectivity. After precipitation and cross-linking the additive could be washed out, with water or an organic solvent, affording a CLEA in which the enzyme is 'locked' in a more favourable conformation, conducive with higher activity and/or (enantio)selectivity. For example, lipase CLEAs were prepared exhibiting up to twelve times the activity of the native enzyme they were prepared from.[37]

A combi-CLEA was prepared from an (*R*)-oxynitrilase and a nitrilase [which can be (*R*)-selective or aselective] and used for the one-step conversion of benzaldehyde to (*R*)-mandelic acid.[38] The latter was obtained in 100% yield and 99% *e.e.* in 15 min at 30°C in aqueous buffer at pH 7.5 (Scheme 17). By combining the oxynitrilase- and nitrilase-catalyzed reactions into a one-step process the equilibrium of the first step is driven to the right. The enantioselectivity is obtained in the first step and, since both (*R*)- and (*S*)-oxynitrilases are readily available, the method can be applied to the production of (*R*)- or (*S*)-mandelic acids by using an aselective nitrilase.

Concluding Remarks

Hopefully this brief review has shown that biocatalysis has much to offer in the way of new methodologies for sustainable organic synthesis. Significant inroads have already been made with regard to the replacement of traditional organic syntheses of β-lactam antibiotics by greener, biocatalytic alternatives. The completion of the switch to a totally biocatalytic process is contingent on the development of a short enzymatic synthesis of the activated side-chain donor using a nitrile-converting enzyme. Indeed, the latter group of enzymes, comprising nitrile hydratases and nitrilases, has considerable potential for replacing traditional organic syntheses through nitrile intermediates.

The ultimate in greener catalytic chemistry is to emulate the 'cell factory' by coupling (bio)catalytic steps together in a catalytic cascade process. Recent developments in this area would seem to hold much promise for the future. A major issue in developing economically viable biocatalytic processes in general, and cascade processes in particular, is an effective immobilization of the enzyme(s). In this context the novel technique of immobilization via cross-linked enzyme aggregates has proven to be particularly effective. It is even possible to prepare multi-enzyme combi-CLEAs for cascade processes. Such developments will surely pave the way for a (fine) chemicals manufacturing industry based on sustainable organic synthesis.

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