

REVIEW

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Enzyme promiscuity: using the dark side of enzyme specificity in white biotechnology

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Abstract

Enzyme promiscuity can be classified into substrate promiscuity, condition promiscuity and catalytic promiscuity. Enzyme promiscuity results in far larger ranges of organic compounds which can be obtained by biocatalysis. While early examples mostly involved use of lipases, more recent literature shows that catalytic promiscuity occurs more widely and many other classes of enzymes can be used to obtain diverse kinds of molecules. This is of immense relevance in the context of white biotechnology as enzyme catalysed reactions use greener conditions.

Keywords: Enzyme specificity, Catalytic promiscuity, Enzymes in organic synthesis, Enantioselectivity, Green chemistry

"I suspect they put Socrates to death because there is something terribly unattractive, alienating and non-human in thinking with too much clarity."

The Bed of Procrustes by Nassim Nicholas Taleb

Introduction

The basic tenet of white biotechnology is to minimize damage to the environment rather than taking recourse to remediation as an "end of the pipe" solution [1,2]. Enzymes either in isolated form or in the form of whole cells can play an important role because of their two well known virtues. The biocatalysts normally do not require high temperature or other conditions which involve high consumption of energy. The biocatalysts are believed to be fairly specific, which would mean less number of side reactions. Side reactions lead to side products which lower the atom economy of the reactions. These side reactions hence lower the yield of the desired product (making the catalysis less efficient) and necessitate complicated downstream processing.

This review discusses the paradigm shifts over the years in our understanding of the enzyme specificity. It also explains why so called lack of specificity is also a good news as far as the usefulness of enzymes in biotechnology

is concerned. The most dramatic departure from the classical concept of enzyme specificity is seen in the phenomenon of catalytic promiscuity. This refers to the same enzyme catalyzing very different kinds of biotransformations [3-6].

Classification of enzymes and enzyme specificity

Most of the people have forgotten (understandable since it disappeared from the standard texts many decades back!) that initially proteins were classified according to their solubility in various solvents. The five classes of proteins were albumins (soluble in water and salt solutions), globulins (sparingly soluble in water but soluble in salt solutions), prolamines (soluble in 70-80% ethanol), glutenins (soluble in acid or alkali) and scleroproteins (insoluble in aqueous solvents) [7]. As our knowledge of proteins grew, the distinction between these various classes became fluid and in fact confusing. The enzymes, in the early phase of biochemistry became the most important and most studied class of proteins. These were named in a random fashion just as people name buildings, streets and monuments. Many of these names persist e.g. catalase, trypsin, lysozyme etc. Many of these names were based upon the nature of their catalytic activity. Lysozyme is named so as it lyses cells. So, the nomenclature and catalytic specificity have a long history in the area of biocatalysis.

The idea of a particular structure being absolutely related to a biological activity has been considered the hallmark of biology for many decades. Hence, it is not surprising that enzyme commission classification was based upon the type

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of reaction the enzyme catalyzes –hydrolysis, isomerisation or redox reactions etc. [8]. This in retrospect may not have been such a wise move as we believed till now.

In 1955–56, International Commission of enzymes was set up and that is how the EC classification came into being [8]. As we know, this classification provides a number with four figures. It is worth noting that all the four figures relate to the details of the specificity of the enzyme. The firm belief was that if one is able to pin down complete details of the specificity, one has described the enzyme and that is its identity.

Various kinds of enzyme promiscuity

Nothing promotes a scientific direction as much as coining a new term. System biology, nanotechnology and synthetic biology are illustrative examples. Enzyme promiscuity is also a case in point. As Hult and Berglund [9] pointed out, promiscuous catalysis by pyruvate decarboxylase of formation of C-C bonds was reported in 1921 and forms the basis of a current industrial process. Khersonsky and Tawfik [4] have also cited examples of few other well known enzymes which, many decades back, were reported to catalyse reactions “in addition to the ones for which they are physiologically specialized or evolve...”. As Babbie et al. [10] highlighted “Promiscuous activities are generally considerably less efficient than the primary function of an enzyme, but second order rate constants (k_{cat}/K_M) as high as $10^5 \text{ M}^{-1} \text{ s}^{-1}$ and rate accelerations ($(k_{\text{cat}}/K_M)/k_2$) up to 10^{18} have been reported: these values match or exceed those for many native enzymatic reactions.”. After initial loose usages of the terms (associated with promiscuous behaviour of enzymes), there is clarity that broad specificity of an enzyme in terms of catalysis of the same reaction with range of substrates should be called substrate promiscuity. Instances when an enzyme catalyses a different reaction (which is not believed to be physiologically relevant at that point in evolution) with a different transition state should be termed as examples of catalytic promiscuity [9,10].

Hult and Berglund [9] go a step further and suggest that reverse reactions catalysed by enzymes in many non-aqueous media or co-solvents can be classified as condition promiscuity. In such cases, the transition state may be same as in normal reactions. So, this may be little confusing.

Enzyme promiscuity started as being perceived as “associated with unwanted side effects, poor catalytic properties and errors in biological function” [11]. Today, it is increasingly being perceived as immensely useful phenomenon which can dramatically enhance utility of biocatalysis in biotechnology.

Babbie et al. [10] have provided a good discussion on how catalytic promiscuity may operate. Apart from the different amino acids of the active site involved in a qualitative or quantitative fashion during binding of the

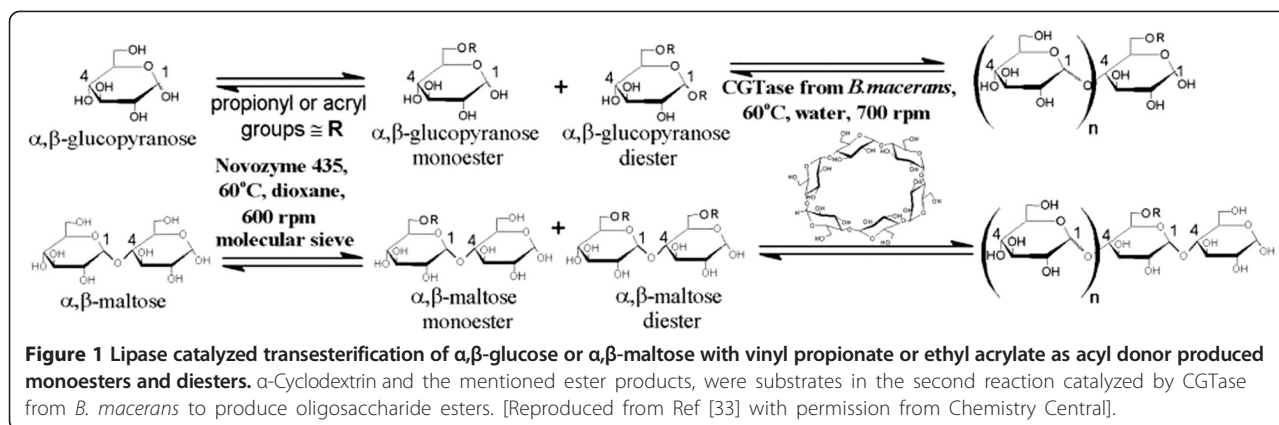
“alternative substrates”, hydrophobic interactions may have an important role to play. Not only during binding, even during catalytic steps, the contributions of the active site residues may differ qualitatively and/or quantitatively. This “accidental promiscuity” observed in wild forms of the enzymes should be distinguished from “induced promiscuity” exhibited by mutants obtained by protein engineering or directed evolution [12]. Many excellent reviews (other than those already quoted above) which include evolutionary aspects of promiscuity are available [3,13,14].

Condition promiscuity of enzymes

Hydrolases hydrolyse substrates. What happens if water is nearly absent? Advent of non-aqueous enzymology showed that exploring this possibility led to carrying out biocatalysis in nearly anhydrous organic solvents [15,16], reverse micelles [17,18], ionic liquids [19–22] etc. So, a lipase in low water containing organic solvent synthesizes an ester bond and is obviously not functioning as a hydrolase [23].

This has been a very useful discovery. It has been also very important as it opened up huge possibilities of using enzymes in biotransformations [16,24] [Figure 1]. Not only it makes it possible to use inexpensive hydrolases like proteases and lipases in organic synthesis, it provides an additional handle of medium engineering [25,26]. Changing the organic solvent may change the enantioselectivity [27,28]. The other possibilities emerged e.g. using organic solvents as co-solvents [29] or using biphasic systems consisting of aqueous medium and water immiscible organic solvents [30]. One can use organic solvent phase to dissolve substrates and aqueous phase can contain the enzyme. This makes the catalytic process very efficient in case the substrate inhibition is involved [31]. If the product also goes back to the organic phase, shifting of the equilibrium (in favour of product formation) and overcoming product inhibition (if involved) are both achieved. More new possibilities continue to emerge. As Dordick's group showed few years back, nanotube mediated assembly of enzymes at the interface of aqueous and organic media overcomes the transport limitations typical of such biphasic systems [32].

It incidentally also meant that one could use green solvents for biocatalysis such as glycerol, ethanol, succinate esters, lactate esters, limonene and supercritical fluids [34]. The case of glycerol is especially relevant in the context of white biotechnology as massive amounts of glycerol are generated during biodiesel production and hence fits in well with the biorefinery concept. The biodiesel as such can be obtained from wastes and can be a good example of waste valorization [35–37] [Figure 2]. Many of the above mentioned solvents also, in principle, can be obtained from non food biomass or waste food materials



[35]. All this points out to the emerging contours of a chemical industry based upon sustainable practices.

These possibilities have emerged as our classical concept of hydrolases will be always hydrolases turned out to be not absolutely valid. If you change the reaction condition, enzymes show condition promiscuity. That has not turned out to be bad at all from the perspective of white biotechnology.

Catalytic promiscuity: recent results

As many examples of catalytic promiscuity have been covered in quite a few recent reviews [5,38,39], we will mostly focus on literature pertaining to last couple of years. Rather than aiming at being comprehensive, the thrust of this

review is on illustrating how catalytic promiscuity can be exploited to create novel possibilities in the area of biocatalysis driving the growth of white biotechnology. Some examples of reactions carried out using catalytic promiscuity for the synthesis of organic compounds in the authors' laboratory are illustrated in Table 1. Also, we have mostly limited ourselves to examples of accidental promiscuity rather than including ever growing number of examples where catalytic promiscuity has been tailored by using protein engineering or directed evolution.

Catalytic promiscuity of lipases

Single pot multicomponent reactions are an efficient synthetic strategy especially if accompanied by good atom

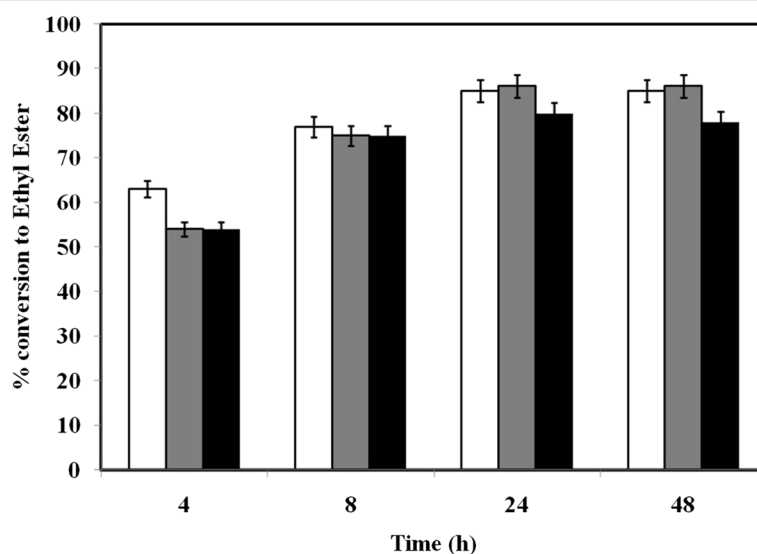
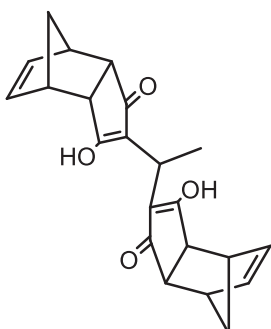
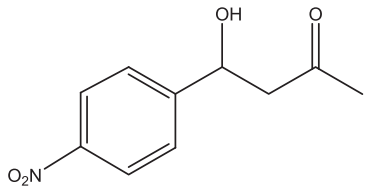
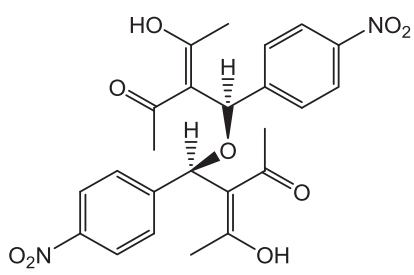
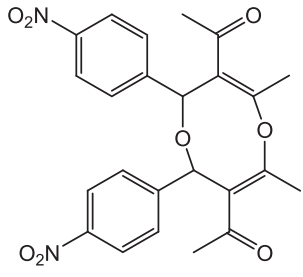
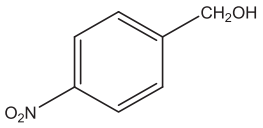
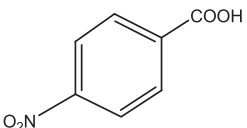


Figure 2 Biodiesel conversion with clean oil using Novozym 435 + RMIM. Reactions were performed taking 0.5 g coffee oil. Ethanol was added in molar ratio 4:1 (ethanol: oil). Novozym 435 + RMIM = 3:1 (White Bars); 37.5 mg of enzyme Novozym 435 (enzyme load being 7.5% w/w of oil) and 12.5 mg of enzyme RMIM (enzyme load being 2.5% w/w of oil) was added. Novozym 435 + RMIM = 1:1 (Grey Bars); 25 mg of enzyme Novozym 435 (enzyme load being 5% w/w of oil) and 25 mg of enzyme RMIM (enzyme load being 5% w/w of oil) was added. Novozym 435 + RMIM = 1:3 (Black bars); 12.5 mg of enzyme Novozym 435 (enzyme load being 2.5% w/w of oil) and 37.5 mg of enzyme RMIM (enzyme load being 7.5% w/w of oil) was added. [Reproduced from Ref [37] with permission from Chemistry Central].

Table 1 Some interesting examples of organic compounds synthesized in the authors' laboratory exploiting catalytic promiscuity

Structure of the compound	Substrate and reaction conditions	Reference
	Substrate: 5-Hydroxy-endo tricyclo [5.2.1.0 ^{2,6}] deca-4,8-dien-3-one and vinyl acetate Biocatalyst: Novozyme 435 Solvent: Vinyl acetate (substrate) containing 10 % (v/v) pyridine/DMF	[40]
	Substrate: <i>p</i> -nitrobenzaldehyde and ethyl acetoacetate Biocatalyst: Lipases from <i>Candida antarctica</i> lipase B, <i>Mucor javanicus</i> , <i>Mucor miehei</i> , <i>Candida rugosa</i> and Novozyme 435 Solvent: Aqueous organic co-solvent mixtures were used as the solvent	[41]
	Substrate: <i>p</i> -nitrobenzaldehyde and acetyl acetone Biocatalyst: Lipases from <i>Candida antarctica</i> lipase B, <i>Mucor javanicus</i> , <i>Mucor miehei</i> , <i>Burkholderia cepacia</i> and Novozyme 435 Solvent: Aqueous organic co-solvent mixtures	[42]
		
	Substrate: <i>p</i> -nitrobenzaldehyde Biocatalyst: Novozyme 435 Solvent: Aqueous buffer	[43]
		

economy. Zhang [44] showed that porcine pancreatic lipase (PPL) could be used to synthesize a number of 2, 4-disubstituted thiazoles with methanol as the organic solvent. Their interest in synthesis of these compounds was due to their many biological activities. There are several noteworthy features of this work. Use of organic solvents (as compared to water or aqueous-organic co-solvent mixtures) has been underexploited in the context of catalytic promiscuity of enzymes. More important, some amylase preparations have also been shown to be catalysing this reaction. It is very likely that enzymes used in this work were industrial grade preparations and may have been contaminated by lipase activities. Earlier, it took several decades before Nakajima's group could show that lipases (contrary to an earlier claim) if purified do not catalyse peptide synthesis in organic solvents [45]. As we explore more and more catalytic promiscuity of enzymes, it is necessary that we keep in mind that some of these may be caused by contaminating proteins. Another important point is that methanol as a solvent was far superior to ethanol. That is unfortunate as ethanol, unlike methanol, is a green solvent. So, from sustainability point of view, perhaps we need to separately screen out a panel of green solvents and determine which one is the best, even if not as good as another non green solvent.

Ugi reaction is one of the more well known multicomponent reactions [46]. The classic Ugi reaction involves condensation of a primary amine, a carbonyl compound, carboxylic acid and isocyanide. Kzossowski et al. [47] have shown that Novozym 435 could form a peptide bond in organic solvents like toluene and chloroform. An important objective of white biotechnology has been to be able to operate chemical processes starting with simple set of compounds [34] which can be obtained from renewable resources. In that respect, peptide bond formation starting with an amine, aldehyde and isocyanide (rather than two amino acids) is a good advancement and shows the wide ranging promise and potential of catalytic promiscuity.

Catalytic promiscuity: beyond lipases

The phenomenon of catalytic promiscuity has turned out to be far more widespread than initially believed. While earlier, there was a strong focus on the use of lipases for C-C bond formation reactions [38,40,41,48,49], use of other enzymes in catalyzing diverse kinds of reactions is being increasingly demonstrated. Liu et al. [50] have reported asymmetric aldol reactions between isatin derivatives with cyclic ketones catalyzed by nuclease p1 from *Penicillium citrinum*. This example of catalytic promiscuity provides a green approach for the synthesis of pharmaceutically active compounds. Li et al. [51] had earlier used the same enzyme for catalyzing asymmetric aldol reactions between aromatic aldehydes and cyclic ketones

under solvent-free conditions. The catalytic promiscuity of *Escherichia coli* BioH esterase was recently exploited for the synthesis of 3, 4-dihydropyran derivatives [52]. The authors used a series of substituted benzalacetones and 1, 3-cyclic diketones as reactants in anhydrous DMF, with yields as high as 76% being obtained in some cases. Recently, ficin from fig tree latex (a plant cysteine proteinase) was shown to catalyze the direct asymmetric aldol reactions of nitrogen, oxygen or sulphur containing heterocyclic ketones with aromatic aldehydes in organic medium [53]. Earlier work from the same group showed that commercially available papain (from *Carica papaya* latex) can be used as an efficient catalyst for Knoevenagel reactions involving a wide range of substrates [54]. Zheng et al. [55] developed the trypsin catalyzed one-pot multicomponent synthesis of 4-thiazolidinones as a novel strategy for the synthesis of this important group of heterocyclic compounds. The derivatives of 2H-1-benzopyran-2-one form the scaffold of many pharmaceuticals [56,57]. Wang et al. [58] have used an alkaline protease from *B. licheniformis* to obtain these by domino Knoevenagel/intramolecular transesterification reactions in low water containing organic solvents.

An interesting example of exploiting catalytic promiscuity for carrying out domino, single-pot reactions was reported by Zhou et al. [59]. The authors used α -amylase from *Bacillus subtilis* for catalyzing the oxa-Michael/aldol condensation for the synthesis of substituted chromene derivatives. Gao et al. [60] described Henry reactions catalyzed by glucoamylase from *Aspergillus niger*. The reactions carried out in mixed solvents of ethanol and water, formed the β -nitroalcohols in yields upto 99%.

One emerging concern has been the bacteria developing resistance towards existing antibiotics like in the case of aminoglycosides which are broad spectrum antibiotics [61]. The catalytic promiscuity of aminoglycoside acetyl transferases was utilized for chemoenzymatic synthesis of variety of novel molecules including acylated aminoglycosides which show promise in circumventing bacterial resistance towards existing antibiotics. The synthetic strategy avoids longer multistep processes. Werneburg et al. [62] have used the polyketide synthetase for preparative scale synthesis of 15 new aureothin (a shikimate-polyketide with antimicrobial and antitumor activities) analogs, many with less cytotoxicity but improved anti-proliferative action. It may be noted that engineered mutants of the organism were used. So, it is an example of metabolic engineering wherein the strategy was based upon the catalytic promiscuity of the enzyme.

Alcohol dehydrogenases (ADHs) are enzymes which catalyze the transformation of ketones/aldehydes to the corresponding alcohols and vice versa at the expense of a nicotinamide cofactor that acts as hydride donor and acceptor respectively [63]. Of the many approaches

available for co-factor recycling, a simple way is to use a co-substrate such as propanol [64,65]. Gotor's group [66] has used ADHs from *L. brevis*, *R. ruber* and *Thermoanaerobacter* sp. to carry out regioselective and stereoselective reductions of 1, 2- and 1,3- diketones to obtain enantio-pure hydroxyketones or diols. Some cyclic diketones were also reduced. The co-substrate propanol was used for co-factor regeneration in this case as well. While the reaction carried out was essentially a normal one, the binding modes of the substrates were different with different ADHs and show how different parts of the active site of the enzymes can be involved in binding. This is a good illustration of substrate promiscuity being exploited for designing useful synthetic strategies. An interesting example of catalytic chemo-promiscuity of alcohol dehydrogenase was reported by Ferreira-Silve et al. [67]. The authors observed that some alcohol dehydrogenases transformed phenylacetaldoxime to the primary alcohol via the imine and aldehyde intermediates, suggesting that the hydride of the co-factor was transferred to the N-atom of the oxime moiety rather than the C-atom.

Alanine racemase is a key PLP-dependant enzyme in cyclosporine (a well known immunosuppressant drug) biosynthesis. Di Salvo et al. [68] reported that just like serine hydroxymethyl transferase and threonine aldolase, the cloned racemase was able to carry out both retroaldol cleavage and transamination reactions. It is a strong evidence that these three enzymes illustrate divergent evolution from a common ancestor which perhaps singly performed all these individual reactions. So, the specialised functions evolved but the enzymes retained features which are responsible for the promiscuous behaviour.

A novel protocol for the D-aminoacylase catalyzed double Michael addition was developed by Chen et al. [69]. The reactions, which were used for the synthesis of (hetero)spiro[5.5] undecane derivatives produced the *cis* isomers in all the cases. Grulich et al. [70] provide a useful summary of the applications of Penicillin G acylases which are known to catalyse transesterifications, Markonikov additions or Henry reactions. Unfortunately, the catalytic efficiency reported so far in these promiscuous reactions is far from satisfactory for any biotechnological applications. However, as Nobeli et al. [11] had pointed out, such results lay the foundation of subsequent efforts using protein engineering/ directed evolution to improve upon these catalytic activities. Large number of successful results which prove this are already available in the literature [12,71-73].

He et al. [74] reported for the first time that hen egg white lysozyme (HEWL) efficiently promotes the one-pot, three-component aza-Diels-Alder reaction of aromatic aldehydes, aromatic amine and 2-cyclohexen-1-one. Under optimised conditions, yields up to 98% and stereoselectivity of *endo/exo* ratios up to 90:10 were obtained.

Similarly, Baas et al. [75] have reviewed the enzyme promiscuity in five members of the tautomerase super family. These enzymes show diverse catalytic activities encompassing C-H, C-C, C-O and carbon-halogen bonds. The review of Baas et al. [75] discusses how looking at promiscuous activities helps in understanding of mechanism of normal activities of the enzymes. It is interesting that all enzymes have N-terminal proline as a key catalytic residue. It is very well established that some amino acids as such catalyse diverse kinds of reactions on their own [76,77]. Obviously, the catalytic promiscuity of enzymes has its origin in confluence of different factors [11]. Table 2 summarizes the usefulness of catalytic promiscuity in biocatalysis (Table 2).

Archae constitute the oldest organisms on our earth. Given the harsher conditions prevalent in those days, archae are prominent examples of extremophiles. The specialised functions of the enzymes (part of the evolved metabolism) in more complex organism have evolved from the small number of enzymes which these ancestors had. So, these organisms constitute valuable systems to track extensive promiscuity shown by the early enzymes. Jia et al. have detailed both promiscuity as well as moonlighting shown by archae enzymes [82]. Archae enzymes are rich in intrinsic disorder. Jia et al. [82] point out that capacity for the structure to survive under harsh conditions and multitasking (which is seen as promiscuity and moonlighting) required conformational pliability. It is interesting that many "hub proteins" important in metabolic regulations in many organisms have been found to be intrinsically disordered proteins (IDPs) [83]. So, promiscuity is one facet of the evolutionary design of enzymes. As Skolnick et al. [84] state, promiscuous behaviour is the biochemical noise (low level, ligand-protein interactions) which was nearly impossible to eliminate as enzyme molecules evolved to assume specialised catalytic roles.

The example of glutathione transferase is especially interesting. Atkins group [85] have discussed that two isoforms of glutathione transferases in humans vastly differ in their catalytic promiscuity. This highlights the conceptual relationship between the phenomenon of iso-enzymes to promiscuous behaviour as pointed out by one of us few years back [5]. The A-class GSTA1-1 showed the fairly wide substrate specificity as a detoxification enzyme. On the other hand, GSTA4-4 acted upon lipid peroxidation products. This was one of the early reports to point out that "conformational plasticity" is inbuilt to achieve catalytic promiscuity at the cost of stability [85]. More recent work from the same group [86] concludes that smooth barrier free transitions within the local conformational landscape of the active site is associated with the more promiscuous GST. Furthermore, local molten globule behaviour optimizes the catalytic function of the GST in detoxification [87]. So, catalytic promiscuity may not be

Table 2 Applications of catalytic promiscuity for useful biotransformations

Biotransformations	Substrate (s)	Enzyme (s)	References
Regio- and stereoselective reductions	Diketones	ADHs from <i>Lactobacillus kefir</i> , <i>Rhodococcus ruber</i> , <i>Thermoanaerobacter</i> etc.	[66]
Reduction reaction	Phenylacetaldoxime	ADHs from <i>Rhodococcus ruber</i> , <i>Ralstonia sp.</i> , <i>Thermoanaerobacter</i> etc.	[67]
Aza-Diels-Alder reaction	4-chlorobenzaldehyde, cyclohexenone, 4-anisidine	Hen egg white lysozyme (HEWL)	[74]
Domino Knoevenagel/intramolecular transesterification	Salicylaldehyde, ethyl acetoacetate	Alkaline protease from <i>Bacillus licheniformis</i> (BLAP)	[58]
Transesterification reaction	Guaifenesin, vinyl acetate	Penicillin G acylase	[78]
Knoevenagel reaction	Aromatic aldehyde, acetyl acetone/Ethyl acetoacetate	Papain from <i>Carica papaya</i> latex	[54]
Ugi reaction for peptide synthesis (MCR)*	Aldehyde, amine, isocyanide	Novozyme 435 (commercially available, immobilized <i>Candida antarctica</i> lipase B)	[47]
Asymmetric aldol reaction	Aromatic aldehyde, cyclic ketone	Nuclease p1 from <i>Penicillium citrinum</i>	[51]
Asymmetric aldol reaction	Heterocyclic ketone, aromatic aldehyde	Ficin from fig tree latex	[53]
Synthesis of 4-thiazolidinones	Aromatic aldehyde, benzyl amine, mercaptoacetic acid	Trypsin from porcine pancreas	[55]
Domino oxa-Michael/aldol condensations	Salicylaldehyde, methyl vinyl ketone	α -amylase from <i>Bacillus subtilis</i>	[59]
Henry reaction	4-cyanobenzaldehyde, nitromethane	Glucoamylase from <i>Aspergillus niger</i> (AnGA)	[60]
Retro aldol and transamination reactions	L-threonine and L- <i>allo</i> -threonine (for retro aldol reaction); D- and L-alanine (for transamination reaction)	Alanine racemase from <i>Tolypocladium inflatum</i>	[68]
Double Michael addition reaction	Cyclohexane-1,3-dione and (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one	D-amino acylase	[69]
Enantioselective aldol reaction	Isatin derivatives, cyclic ketones	Nuclease p1 from <i>Penicillium citrinum</i>	[50]
Synthesis of 2,4-disubstituted thiazoles (MCR)*	Benzylamine, isobutyraldehyde, thioacetic acid, methyl 3-(dimethylamino)-2-isocyanoacrylate	Porcine pancreatic lipase (PPL)	[44]
Michael addition-cyclization cascade reaction	Substituted benzalacetones and 1,3-cyclic diketones	<i>E.coli</i> BioH esterase	[52]
Synthesis of substituted 2H-chromenes (MCR)*	Salicylaldehyde, acetophenone, methanol	Porcine pancreatic lipase	[79]
Baylis-Hillman reaction	<i>p</i> -nitrobenzaldehyde and methyl vinyl ketone	<i>E.coli</i> BioH esterase	[80]
Biginelli reaction (MCR)*	Urea, ethyl acetoacetate, vinyl acetate	Trypsin from porcine pancreas	[81]

*stands for Multi-component reaction.

just accidental “noise” [84] but part of an intentional biocatalytic design.

Labrou's group [88] have recently reported a GST from *A. tumefaciens* which represents a novel class of bacterial GST superfamily. Its active site is quite different from cytosolic GST reported so far. It may be interesting to see the unravelling of its specificity in the years to come. Finally, as pointed out by them, GSTs are also possibly involved in the storage and transportation of wide variety of biological molecules and thus are also moonlighting proteins. This three way correlation between isoenzymes, promiscuous enzymes and moonlighting proteins have been pointed out earlier [5]. To sum up, the conformational pliability may be local or global in protein structure as the cause behind promiscuity.

Conclusions

Few trends are clear. While during early few years, examples of catalytic promiscuity were mostly concerned

with applications of lipases; last few years have seen other classes of enzymes (other hydrolases, oxidoreductases, transferases etc.) being equally capable of showing catalytic promiscuity.

The shift from our belief in enzyme specificity to the realization that these biocatalysts are fairly promiscuous has not been gradual. Some key milestones can be identified. We accepted the idea of broad specificity (lately called relaxed specificity) long ago. Isoforms or isoenzymes, the enzymes from the same organism, carrying similar catalytic activity but with different specificity and kinetic behaviour have been again known since several decades [5]. The catalysis starts with molecular recognition of the “substrate” by the enzyme. The binding site, part of the active centre was known to show promiscuous behaviour when textile dyes emerged as powerful affinity ligands [89]. The catalytic promiscuity largely arises because many different “substrates” can interact with different side chains of amino acids to result in different transition states.

This is akin to a general practitioner becoming a specialist in medical science but retaining enough knowledge of how to treat many diseases. In the beginning, we had RNA world [90]. Then came early enzymes. As complex metabolisms were required with evolution of more complex organisms, more specialised enzymes emerged. These enzymes did not forget entirely what their ancestors were capable of.

White biotechnology can definitely profit by exploiting many of the catalytic powers which enzymes did not entirely “forget”. This overview has looked at the proverbial “tip of the iceberg”. Hopefully, it will draw attention of many biotechnologists to look at the huge iceberg of potential application of these green catalysts to nurture sustainable approaches in chemical industries.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

BA played a large role in the search for the current literature. All authors participated in drafting of the text, read and approved the final manuscript.

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