Chemoenzymatic and photobiocatalytic strategies for chemical synthesis

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Chapter 1

Introduction

The pursuit of building complex molecules from simpler organic compounds has been at the center of discoveries made in the field of organic chemistry. The use of biocatalysis for chemical synthesis has emerged as an effective alternate tool, offering several advantages over traditional chemocatalytic methods. These include the inherent properties usually associated with enzymatic catalysis, such as, the use of environmentally friendlier reaction conditions, the ease of using enzyme catalysts and disposal of enzymatic waste, and the magnitude of control provided in terms of chemo-, regio-, and enantioselectivity. Importantly, the prospects of enzyme evolution for expanding substrate and reaction scope, make them worthy of both academic and industrial pursuits.[1] Moreover, despite several challenges, the prospects of biocatalytic systems to perform in synergy with chemo- or photocatalytic systems creates immense opportunities for more robust, rapid, efficient and cleaner access to important target molecules.[9, 10] In this thesis, we demonstrate the use of EDDS lyase and nitroreductases, in combination with chemo- and photocatalysis, to provide access to important pharmaceutical building blocks.

Part 1: EDDS-lyase catalyzed asymmetric synthesis of *N*-containing heterocycles and non-canonical amino acids.

Nitrogen-containing compounds are ubiquitous in biological systems and constitute almost 60% of FDA approved small-molecule drugs.[11] Therefore, the development of efficient and sustainable synthetic strategies for the inclusion of nitrogen into organic molecules is of great interest. Biocatalysis offers an effective addition or alternative to traditional chemocatalytic routes for the synthesis of *N*-containing molecules. The development of C-N bond forming enzymes, including transaminases,[12] reductive aminases,[13] and C-N lyases,[14] has enabled significant progress in the incorporation of nitrogen into organic molecules. C-N lyases have particularly emerged as an important class of enzymes for the synthesis of chiral *N*-containing compounds. This is attributed to their ability to utilize readily available alkenes or related starting materials in asymmetric hydroamination reactions, the absence of external cofactors, high atom economy and remarkable selectivity. In addition to these characteristic advantages, the ability of C-N lyase to naturally catalyze the synthesis of chiral amino acids make them amenable to be used for the synthesis of complex non-canonical amino-acid derivatives, many of which exhibit biological activity.

C-N lyases can be divided into three major subclasses, based on the chemical nature of the products they are able to cleave.[14] Among them, the most relevant and well-studied constitute aspartate ammonia lyase (aspartase),[15] 3-methylaspartate ammonia lyase (MAL),[16] histidine[17] and phenyalanine ammonia lyase[18] (HAL and PAL, respectively), and EDDS lyase.[19]

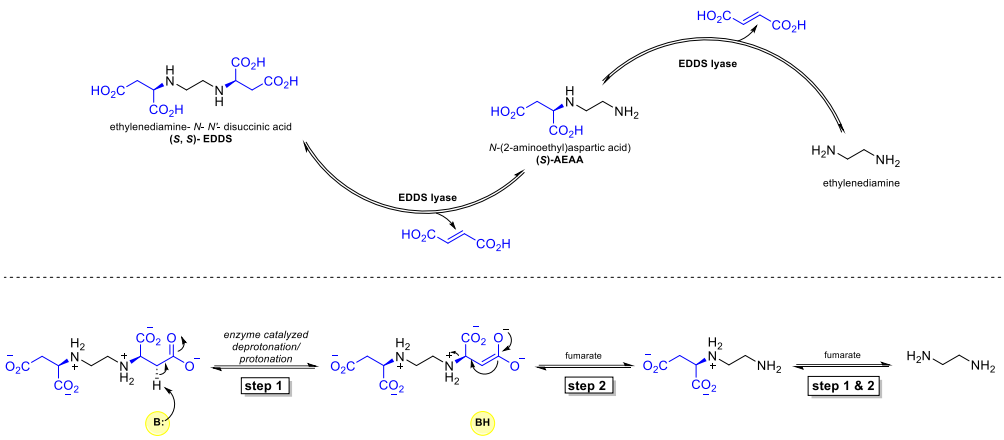

Figure 1. Reaction naturally catalyzed by EDDS lyase involving the reversible breakdown of (*S*,*S*)-EDDS (top) and proposed catalytic mechanism (bottom).

The reaction that EDDS lyase naturally catalyzes is the breakdown of ethylenediamine-*N*-*N*′-disuccinic acid (*S*,*S*- EDDS) into two molecules of fumaric acid and one molecule of ethylene diamine (Figure 1). The EDDS lyase relevant for the work described in this thesis was recently identified in our group from *Chelativorans* sp. BNC1 and has been well characterized.[19] Crystal structures of the enzyme in an unliganded state and in complex with fumarate and (*S*,*S*)-EDDS reveal a tertiary and quaternary fold that is characteristic of the aspartase/fumarase superfamily and support a mechanism that involves general base-catalyzed, sequential two-step deamination of (*S*,*S*)-EDDS (Figure 1).[19] Briefly, (*S*,*S*)-EDDS tightly binds and is activated in the enzyme active site, making the Cβ-hydrogen prone to deprotonation by a catalytic base (serine) present in the enzyme active site. This is followed by electronic rearrangement and cleavage of the C-N bond to yield (*S*)-AEAA and a molecule of fumarate (Figure 1, steps 1 and 2). The initial product (*S*)-AEAA then re-enters the enzyme active site to undergo another deprotonation and C-N bond-cleavage step to yield ethylenediamine and another molecule of fumarate (Figure 1, step 1 & 2). The reversible nature of this enzymatic reaction allows the biocatalytic synthesis of (*S*,*S*)-EDDS, which finds use as an important metal-chelating agent, widely applied in industrial processes such as waste-water treatment and soil-remediation.[20] Interestingly, substrate and enzyme engineering enabled the preparation of various high-value chiral aspartic acid derivatives, including aliphatic aspartic acid derivatives,[5, 21] *N*-cycloalkyl-substituted aspartic acids,[22] *N*-arylated aspartic acids, and pyrazolidinones.[23] To further demonstrate the synthetic usefulness of EDDS lyase, we present the use of chemoenzymatic approaches for the asymmetric synthesis of complex *N*-containing heterocycles (**Chapter 2**) and aminopolycarboxylic acids (**Chapter 3**).

Part 2: Nitroreductase catalyzed synthesis of chiral alcohols, aliphatic amines, and amino-, azoxy- and azo-aromatics

Nitroreductases are NAD(P)H-dependent flavoenzymes that reduce nitro-containing compounds into the corresponding amines via nitroso (NO) and hydroxylamine (NHOH) intermediates.[24] They often form homodimeric structures and are divided into oxygen-sensitive and oxygen-insensitive enzymes, predominantly found in bacteria.[25] The nitro reduction is achieved by repetitive rounds of Hydride Transfer (HT) following a general ping-pong bi-bi redox mechanism. Briefly, NAD(P)H binds to the enzyme active site and reduces the FMN_{ox} prosthetic group to FMN_{hq} (step **I**), this is followed by release of $NAD(P)^+$ from the active site and subsequent replacement by a nitro-containing substrate (step **II**), and finally the two-electron reduction of the substrate with the help of FMN_{hq} (step **III**). The cycle consisting of step **I** - **III** is repeated until full reduction to the amine product is achieved (Figure 2). The ability of nitroreductase to bind substrates for sequential delivery of electrons and protons (HT), makes them ideal candidates for photobiocatalysis.[26]

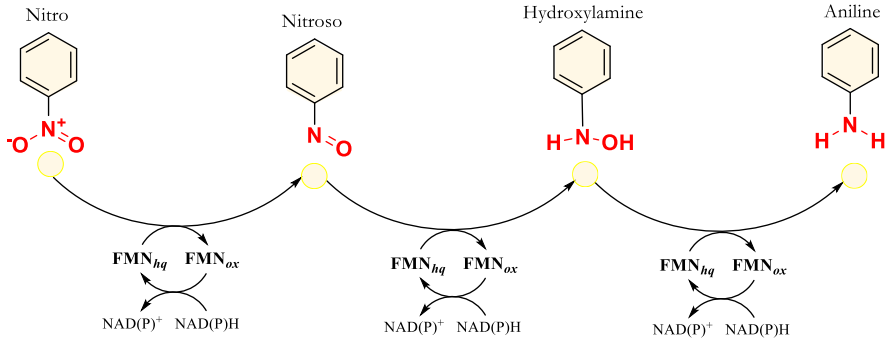

Figure 2. General catalytic ping-pong bi-bi mechanism of nitroreductases involving the step-wise reduction of nitrobenzene (NO_2), via nitroso (NO) and hydroxylamine (NHOH) intermediates, to yield the final product aniline (NH_2).

Irradiation of a photocatalyst or photoactive compound with visible light can be used to initiate radical reactions for organic synthesis.[6] If a radical reaction mechanism is executed within the active site of an enzyme, exquisite chemo-, regio- and stereoselectivity can often be achieved.[8] Moreover, the integration of photocatalytic and biocatalytic systems enables the use of water-compatible substrates for discovering completely new reactivities not seen in small-molecule catalysts, as well as protein engineering for tailoring the activity, selectivity and stability of the photoenzymatic systems. Hence, photobiocatalysis can be employed for challenging redox chemistry, offering several advantages such as ease of handling and engineering, as well as altered selectivity profiles, as opposed to more traditional methods.[8]

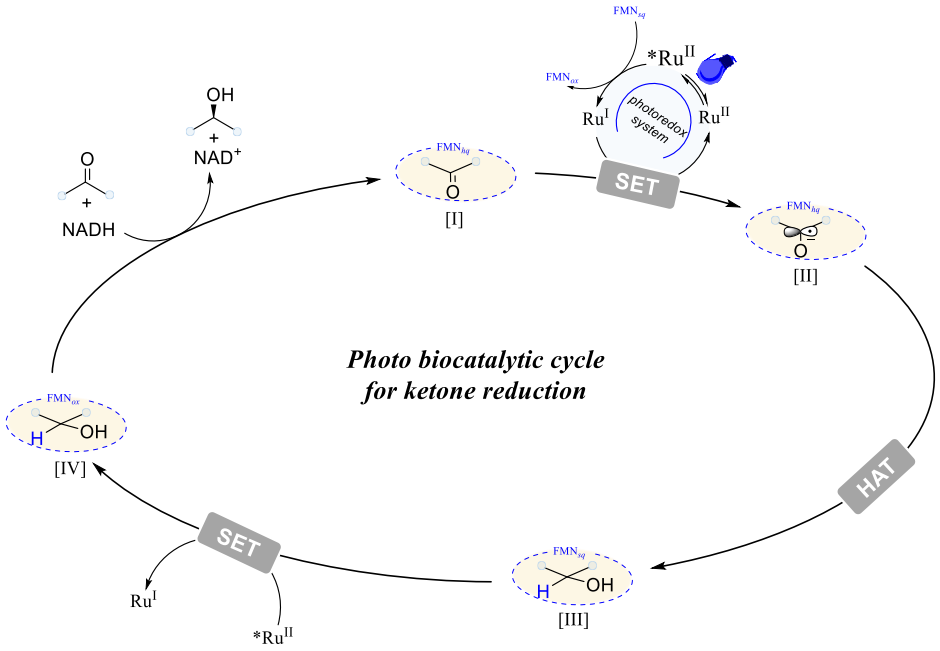

Figure 3. Proposed mechanism for photobiocatalytic reduction of a ketone in the presence of blue-light irradiation.

A general proposed mechanism for the asymmetric photobiocatalytic reduction of a ketone in presence of blue-light irradiation is highlighted in Figure 3. Briefly, the ketone [**I**] enters the enzyme active site, and is stabilized and activated by hydrogen-bond and hydrophobic interactions. Simultaneously, the photo-redox system (Ru^{II} - $*Ru^{II}$ – Ru^{I}) performs a Single Electron Transfer (SET) to give a free radical (ketyl) intermediate [**II**]. At this stage, a Hydrogen Atom Transfer (HAT) is assisted by FMN_{hq} present in the active site of the enzyme, giving access to an asymmetric alcohol [III]. Finally, SET allows the FMN_{sq} to return back to FMN_{ox} and the nicotinamide cofactor is regenerated by a glucose dehydrogenase (GDH) recycling system.[27] This general photobiocatalytic reduction mechanism inspired us to develop a new photoenzymatic system for the chemo- and enantioselective reduction of α,ß-unsaturated ketones to give the desired enantioenriched alcohols without reducing the C=C or C≡C bond (**Chapter 4**). We further explored the use of a widely abundant and benign photocatalyst (chlorophyll) and different nitroreductases to develop new photoenzymatic systems for the selective reduction of aliphatic and aromatic nitro compounds into aliphatic amines and amino-, azoxy- and azo-aromatics (**Chapter 5**).

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