Enzyme-Catalyzed Synthesis in Pharmaceutical Manufacturing

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Abstract. In recent years, with the progress of science and the rise of green chemistry, enzyme catalysis technology has been highly valued as an important branch of green chemistry. Due to the development of biotechnology, enzyme catalysis technology has been increasingly used in organic synthesis, especially asymmetric synthesis, the synthesis of optically active compounds and natural products. It has been applied in medicine, food and textiles industry. Among these application areas, the pharmaceutical field has been a hot topic in enzyme catalysis technology in recent years. The application of enzyme catalysis technology in the pharmaceutical industry is a means to promote sustainable and environmentally friendly practices. It helps to solve continuously upgrading environmental problems and promote the green development of the industry. Therefore, conducting research on enzyme-catalyzed synthesis in pharmaceutical manufacturing is of great significance. In this work, several practical examples of enzyme catalysis techniques used in the pharmaceutical industry are introduced and discussed.

Keywords: Pharmaceutical industry; synthesis methods; bio-enzyme catalysis.

1. Introduction

The dynamic interplay between chemical principles and biological enzymes is a cornerstone of modern biocatalysts, eliciting a series of intricate responses that are pivotal for the advancement of pharmaceutical manufacturing. The remarkable progress in the stability and flexibility of these biological catalysts cannot be overstated. This progress has largely been propelled by the revolutionary strides in genetic engineering, which have enabled the customization of enzymes to suit specific industrial needs.

Enhanced enzyme stability and flexibility mean that biocatalysts can now withstand harsh conditions that would normally denature traditional enzymes, such as extreme pH levels, high temperatures, and the presence of organic solvents. This resilience is revolutionizing pharmaceutical synthesis, providing a green alternative to chemical catalysts by reducing energy consumption and eliminating the need for toxic chemicals [1].

The key factors of this transformation are various catalysts, including oxidases, reactive enzymes, polycrystalline enzymes, and pigments. Each of them plays a pivotal role in pharmaceutical innovation. These catalysts are integral to synthesizing drug molecules and drug control substances, offering a more selective, efficient, and eco-friendly approach compared to traditional chemical synthesis [2]. Oxidases, for example, are employed for their ability to catalyze oxidation reactions with incredible specificity, while polycrystalline enzymes provide a structured and stable environment for reactions to occur, which enhances their reusability. Reactive enzymes have the unique ability to participate in and drive complex reactions, which is essential for the development of novel pharmaceutical compounds [3].

The catalytic prowess can be seen in the common oxidation and reduction reactions pivotal in drug manufacturing [4]. These reactions, facilitated by biocatalytic enzymes, are not only more environmentally benign but also offer unparalleled control over product stereochemistry. The ability to precisely manipulate molecular structures is critical in the production of pharmaceuticals, where the right structural configuration can mean the difference between a life-saving drug and an ineffective or even harmful compound [5].

The integration of these biotechnological advancements promises to reshape the pharmaceutical landscape by improving the production efficiency of current drugs and by offering new pathways for

drug discovery and development. This burgeoning field not only stands to enhance the pharmaceutical capabilities but also aligns with the global pursuit of sustainable industrial practices.

2. The process of oxidation reaction

The utilization of biological oxidation reactions in the pharmaceutical sector has gained significant traction, leading to an increased focus on exploring the potential of enzymes as catalysts in this context. In the realm of industrial manufacturing, notable enzymes alongside the principal metal dopamine ethanol, have been employed. Estrogen and turpentine have been widely discussed among medical researchers for their potential protective effects against many diseases. In Figure 1, the combination of vanillin oxidase with a bacterial enzyme, specifically an oxidase obtained through liquid mixing, is depicted.

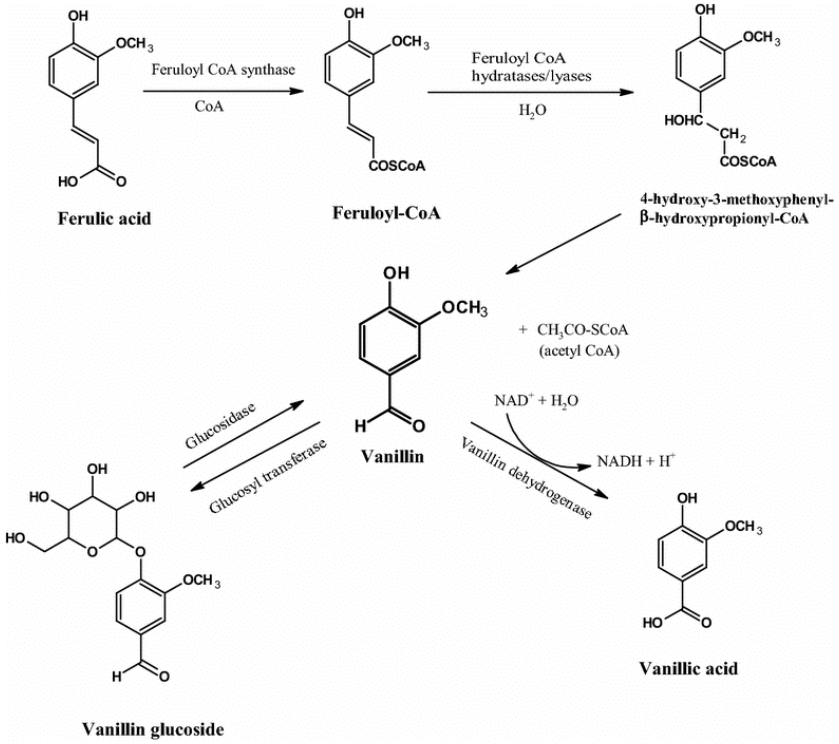

Fig. 1 the combination of vanillin oxidase with a bacterial enzyme [6]

As an alternative approach to modern biomanufacturing technology, bioelectrocatalysis fully combines the merits of both biocatalysts and electrocatalysis to realize the green, efficient production of target products from electricity. According to research reports, new microbial strains have been isolated from natural habitats using this technology [6]. The issue at hand pertains to the isolation of newly discovered microbial strains from their natural habitat. These bacteria exhibit a moderate level of acidity in their composition, along with a specific chemical makeup that includes characteristics such as temperature stability and the general receptiveness towards organic solvents by the wider scientific community.

Tert-butylhexamyanoyl (3R, 5R) dihydroxycaproate of optical alcohol, a key chiral precursor of Lipitor (atorvastatin calcium), is produced by reductase from tert-butyl hexamyanoyl (5R) hydroxy-3-oxo-caproate. A new carbonyl reductase, KlAKR, has been reported to be found in Kluyveromyces lactis, which is able to asymmetrically reduce hacyano (5R) hydroxy-3-butyl oxycaproate.

In addition, new biological enzyme catalysts can also be mined through the genome, such as the reported identification of NADPH-dependent carbonyl reductase YlCR2, which can bind ethyl 4-chloro-3, by analyzing the genome sequence of Yarrowia lipolytica ACA-DC 50109Ethyl oxybutyrate (COBE) is reduced to ethyl (S) -4-chloro-3-hydroxybutyrate ((S) -CHBE), and -CHBE (S) is a key intermediate in the synthesis of statins. It is worth mentioning that, using the co-substrate

mannitol or sorbitol, Y1CR2 can be used for both COBE reduction and NADPH automatic production. This submatter-coupled cofactor regeneration system using only one enzyme is much simpler than the traditional double-enzyme coupled system. Under optimized conditions, the synthesis of (S) - CHBE from E. coli (Escherichia broadcasting) cells overexpressing Y1CR2 can achieve 90% yield and 99%ee within 10 hours. The facilitation of novel enzyme development can be achieved by several methods, including the screening of new microorganisms, gene attraction, and the utilization of potent enzyme tools like as rational and irrational design, as well as online slimming. It is also crucial to identify an environment conducive to their activation and responsiveness.

The process of synthesizing tarin medications involves a concentrated collective synthesis, which serves as the fundamental mechanism for manufacturing these compounds. This synthesis is capable of constructing the intricate molecular structure characteristic of tarin pharmaceuticals, including their distinctive hands. Hence, chemical synthesis plays a crucial role in the ongoing production of high-quality pharmaceuticals that entail higher production costs. The production of synthetic selective enzymes has the potential to introduce novel poisons that can be substantially lowered in terms of cost and quantity by limiting the influence of external patents. The Tyco ketone extraction enzyme was produced once more, while for non-enzymes, as depicted in Figure 2.

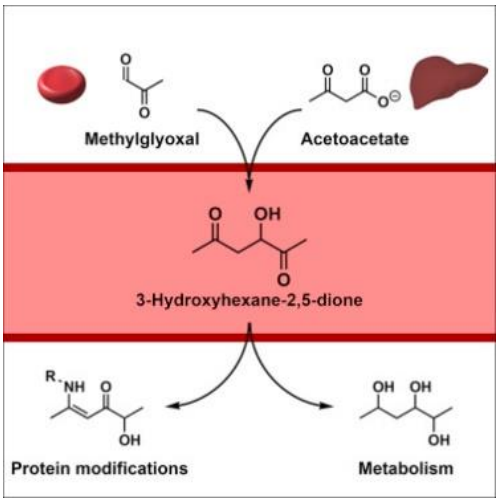

Fig. 2 Tyco ketone extraction enzyme [7]

The effective implementation of bio-enzyme technology facilitates the enhancement of crucial elements in pharmacological photosynthesis. It also contributes to the attainment of selected and ecologically sustainable technological benefits. The introduction of these new technologies has the potential to not only significantly decrease manufacturing expenses, but also enhance global competitiveness [8]. The practical utilization of these technologies can also extend to the manufacturing of potent enzymes, non-natural amino acids, medicines, and primary commodities.

3. Research progress

Biorthogonal mimetic catalysis is a technological approach to in situ activation of prodrugs for disease treatment, but its practical application requires consideration of the metabolic inactivation of in situ synthesised drugs and the selectivity of the catalyst. Qu Xiaogang's team proposed a two-drug activation strategy [8]. They designed and created a G-quadruplex aptamer-modified hydrogen-bonded organoframework catalytic platform with iron porphyrin as the ligand. The iron porphyrin ligand can be reduced to ferrous porphyrin by excess glutathione (GSH) in the tumour, and the catalytically active ferrous porphyrin further catalyzes the activation of two loaded prodrugs, 5-fluorouracil (5-FU) and 5-ethynyluracil (5-EU). The targeting of the G-quadruplex AS1411 aptamer and the GSH-triggered catalytic activation provided dual guarantees of a tumour-selective, biologically orthogonal, catalytic reaction. catalytic reaction, avoiding premature drug release and off-target toxicity. To address the metabolic inactivation of chemotherapeutic drugs, 5-ethynyluracil

synthesised by the two-drug activation strategy overcame the metabolic inactivation of 5-fluorouracil during treatment by inhibiting the activity of dihydropyrimidine dehydrogenase (DPD), which improved its bioavailability and therapeutic effect. The bioorthogonal enzyme mimetic catalytic strategy proposed in this study provides a versatile approach for in situ drug synthesis and reduction of drug side effects. Figure 3 depicted the synthesis of cephalotene.

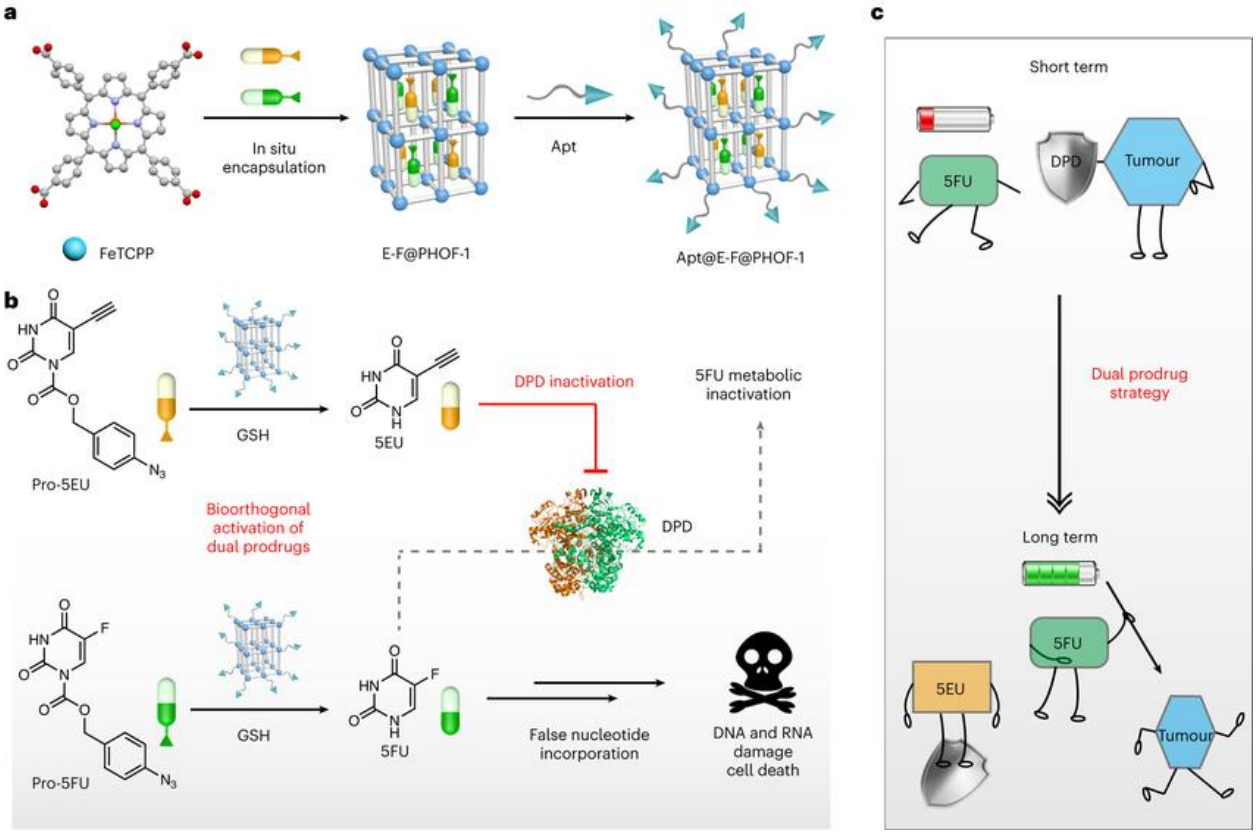

Fig. 3 Synthesis of Cephalotene, a precursor of Hainanese crude cephalotene lactone, catalysed by trichothecene synthase CsCTS [9]

Functional characterization and cyclization mechanism of a diterpene synthase catalyzing the skeleton formation of cephalotane-type diterpenoids is another example of enzyme catalysis technology used in the pharmaceutical industry. Cephalotaxolide has a rigidly parallel 6/6/5/7 tetracyclic diterpene skeleton, a six-membered lactone bridging ring and a tetrahydrofuran ring, with seven chiral centres in the molecule, which makes the skeleton novel and unique. Pharmacological studies revealed that it possesses a variety of biological activities, especially the IC50 value of 43 nm against human KB cells, which is very promising for research and development. However, the compound is found in plants at very low levels, and its complex structure makes it difficult to be chemically synthesised and its sources are limited. Biosynthesis may provide a new strategy to solve this challenge, however, studies on the biosynthesis of Cephalotaxus hainanus lactone have not been reported. The diterpene synthase, diterpene skeleton and its cyclisation mechanism remain enigmatic. In this study, a type I diterpene synthase from Cephalotaxus sinensis (C. sinensis) was found to be able to produce one main product 1 and four derailed products (M1-M4), and the structure of main product 1 was determined to be tricresyne by NMR, chemical derivatisation and X-ray single crystal diffraction analyses, which led to the identification of the enzyme's function and the naming of the enzyme as tricresyne synthase CsCTS (Cephalotaxus sinensis Cephalotene Synthase). In order to elucidate the catalytic mechanism of CsCTS, the authors identified the structures of the derailment products M1-M4 and combined with bioinformatics analyses and the catalytic properties of CsCTS, proposed three possible cyclisation mechanisms (pathways i-iii). The key hydrogen migration, methyl migration and cyclisation reactions in the proposed cyclisation mechanisms were further demonstrated by isotope labelling experiments; combined with density functional theory calculations,

it was elucidated that CsCTS catalyzes the formation of cephalotene via the pathway i cyclisation mechanism.

Further studies suggested that the homologous CsCTS/TbTS might have originated from the same ancestor, conferring similarity in their pre-cyclisation mechanisms; meanwhile, the mutation of key amino acid residues (F613/V610) during evolution led to divergence of their cyclisation mechanisms and the evolution of functionally branched diterpene synthases, which resulted in the formation of species-specific metabolites, demonstrating that the nature of plant- gene/enzyme-pathway-metabolite evolutionary correlation in nature. In this study, the diterpene synthases associated with the first step reaction of triterpene biosynthesis, such as Cephalotaxolide, were identified, and the catalytic mechanism was elucidated, which lays the foundation for deciphering and artificially constructing the complete biosynthetic pathway of triterpenes, such as cephalotaxolide, in Hainan Province. Figure 4 depicted the synthesis of cephalotene, a precursor of hainanese crude cephalotene lactone, catalysed by trichothecene synthase CsCTS.

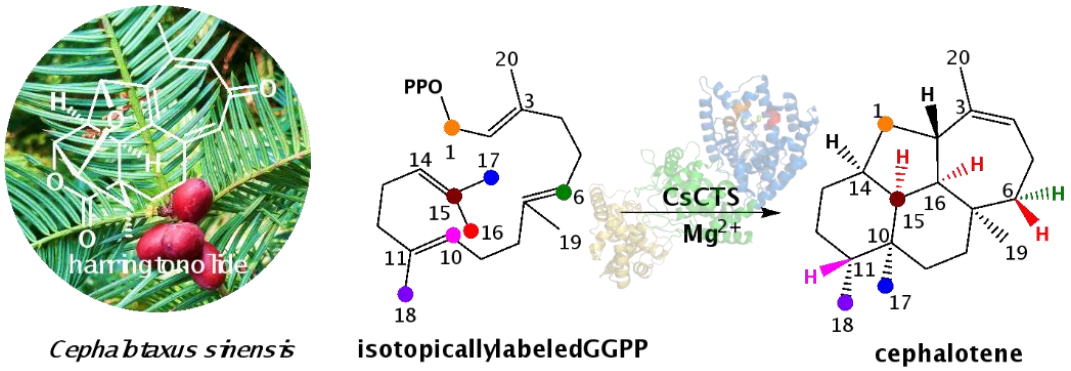

Fig. 4 Synthesis of cephalotene, a precursor of Hainanese crude cephalotene lactone, catalysed by trichothecene synthase CsCTS [10].

4. Conclusion

Over the past few decades, there have been significant advancements in biological enzymes that have played a crucial role as catalysts in delicate chemical and pharmaceutical procedures. Enzymes are the most proficient catalysts, offering much more competitive processes compared to chemical catalysts. The number of industrial applications for enzymes has exploded in recent years, mainly owing to advances in protein engineering technology and environmental and economic necessities. Moreover, these novel technologies exhibit a higher level of environmental friendliness and sustainability compared to conventional chemical synthesis methods. In the forthcoming era, biological enzymes are poised to assume the role of catalysts in the realm of medication synthesis, thereby offering extensive applications for patients and society at large.

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