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Recent examples of the use of biocatalysts with high accessibility and availability in natural product synthesis

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ABSTRACT

Utilization of biocatalysts with high accessibility and availability, which have recently been applied in the preparation of enantiomerically enriched starting materials and synthetic intermediates for natural product syntheses (mainly 2013–2017) are summarized in this review. The main contents are as follows: 1) recruitment of biocatalysts for the transformation of organic compounds; 2) special precautions for preparative-scale biocatalytic synthetic experiments; 3) asymmetric reduction of carbonyl substrates; 4) kinetic resolution of alcohol and carboxylate enantiomers; 5) desymmetrization of multifunctional alcohol and carboxylate substrates; and 6) recognition of remote and non-central chirality.

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1. Introduction

Biocatalytic processes and reactions have been utilized in the production of naturally occurring compounds, and pertinent overviews of this field have been published.[1,2] In this review, we focus on recent examples (mainly published between 2013 and 2017) of the use of biocatalysts with high accessibility and availability, that have been applied to the preparation of enantiomerically enriched starting materials and synthetic intermediates for natural product syntheses.

2. General remarks

2.1. Recruitment of biocatalysts

When pure synthetic organic chemists consider whether to use biocatalytic processes, the easy access and handling of reagents and real reaction processes are important factors. For these reasons, we will review achievements mediated by 1) commercially available whole-cell microorganisms such as bakers' yeast and wild-type microorganisms deposited in official institutes and by 2) commercially available isolated enzymes developed for industrial and reagent uses. We have omitted applications of specifically engineered enzymes with only limited availability, even if their catalytic activity and selectivity have been highly improved either by site-specific or random mutation.

Available microorganisms have classification numbers according to various institutes, such as ATCC (American Type Culture Collections),[3] CBS (CBS-KNAW Collections),[4] JCM (including IAM culture collections, Japan Collection of Microorganisms, RIKEN Institute),[5] NBRC (involving IFO culture collections, Biological Resource Center, NITE),[6] and NRRL (Agricultural Research Service Culture Collection: Northern Regional Research Laboratory).[7]

Even if microbial strains are readily available, researchers must pre-cultivate the microorganisms to obtain the enzymes; in some cases, co-factor recycling systems are also necessary. Pure synthetic chemists need the assistance of microbiologists to maintain "aseptic" conditions to avoid contamination by undesired indigenous microbes from the surroundings (*e.g.*, air, oral, skin), especially at the initial stage of cultivation.

Recent progress in screening and protein engineering has resulted in the commercial availability of overexpressed and purified carbonyl reductases. Several companies provide all-in-one kits for screening a variety of carbonyl reductases by combining the reducatases with co-factor (NADH or NADPH) recycling systems. Examples include SelectAzyme™ CREDs from Almac,[8] screening kits from Codexis Inc. (including BioCatalytics Inc.),[9] ChiralScreen OH® from Daicel Corp.,[10] and reductases from evoXX Technologies GmbH (including Evocatal GmbH).[11] These kits circumvent the tedious cultivation of microorganisms and isolation of enzymes, and trials for individual substrates can be initiated by merely 1) dissolving all kit ingredients in water, 2) adding a small amount of substrate, and 3) stirring the mixture at 30 °C. The progress and selectivity of the reaction can be easily evaluated after a suitable incubation time.

Many commercially available hydrolases, including lipases and esterases, were originally developed for the food and detergent industries and are now used for functional group transformations in organic synthesis. Some hydrolytic enzymes are also available from reagent companies or from manufacturers such as Amano Enzyme Inc.,[12] Meito Sangyo Co., Ltd.,[13] and Novozymes A/S[14] by way of proper reagent agencies. Lipases PS and AK are isolated from microorganisms such as *Burkholderia cepacia* and *Pseudomonas fluorescens* and are available from Amano Enzyme Inc., but show different substrate specificities, reactivities and selectivities depending on the structure of substrate.

Mergers and acquisitions (M & A) of companies and research groups, and the transfer of technologies, can result in original enzymes described in the literature becoming no longer unavailable. In this review, we use "updated" names of enzymes throughout the text and schemes.

2.2. Enantioselectivity and enantiomeric excess of the products in kinetic resolution

Selectivity between enantiomers in enzyme-catalyzed kinetic resolution is expressed in E (enantiomeric ratio) values.[15] The E value is calculated from any two of the following three experimental data: 1) enantiomeric excess (ee) of the unreacted substrate [ee(S)]; 2) ee of the product [ee(P)]; 3) conversion by the reaction. The enantioselectivity of a specific reaction can be compared to the enantioselectivities of other reactions. However, from ees and conversion obtained from the experiments, the calculation involves logarithmic computation. Even small difference of conversion brings about a large error. We agree the opinions from some researchers that upper limit of thus calculated E value is 200 in a practical manner, and the values over that should be unified to >200. In this review, we have omitted E values even if they were reported in the original papers.

Readers may wish to replicate examples cited in this review to prepare enantiomerically enriched starting materials. In this case, monitoring ees is very important, as the ees of unreacted starting materials and reaction products greatly depend on the conversion. It should also be noted that the E value is essentially obtained by the equation [V_{max} (fast isomer)/K_m (fast isomer)]/[V_{max} (slow isomer)/K_m (slow isomer)], and each parameter may change, depending on the reaction conditions. Also, in the case of desymmetrization, selectivity often depends on the reaction conditions.

2.3. Special precautions in the performance of biocatalytic transformations in aqueous phase

The risk of contamination when using cultivated whole-cell microorganisms decreases once exponential growth phase is reached but prolonged incubation should be avoided. Wild-type microorganisms may have multiple enzymes which can transform the substrates. Some are expressed in stationary phase (late stage of pre-cultivation) and are mainly used for biotransformation reactions. Given a prolonged reaction time, some of the grown cells will lyse, promoting re-growth of the remaining "seed" microorganisms. If other minor, but relevant enzymes are expressed at the early stage of growth, then the incubation broth will show different reactivity and selectivity depending on the growth stage.[16]

It should be noted that some microorganisms require elaborate