Evolving enzyme technology for pharmaceutical applications: case studies

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The case studies focus on two types of enzyme applications for pharmaceutical development. Demethylmacrocin O-methyltransferase, macrocin O-methyltransferase (both putatively rate-limiting) and tylosin reductase were purified from Streptomyces fradiae, characterized and the genes manipulated for increasing tylosin biosynthesis in S. fradiae. The rate-limiting enzyme, deacetoxycephalosporin C (DAOC) synthase/hydroxylase (expandase/hydroxylase), was purified from Cephalosporium acremonium, its gene over-expressed, and cephalosporin C biosynthesis improved in C. acremonium. Also, heterologous expression of penicillin N epimerase and DAOC synthase (expandase) genes of Streptomyces clavuligerus in Penicillium chrysogenum permitted DAOC production in the fungal strain. Second, serine hydroxymethyltransferase of Escherichia coli and phthalyl amidase of Xanthobacter agilis were employed in chemo-enzymatic synthesis of carbacephem. Similarly, echinocandin B deacylase of Actinoplanes utahensis was used in the second-type synthesis of the ECB antifungal agent.

Keywords: biosynthesis; tylosin; cephalosporin C; biocatalysis; carbacephem; ECB antifungal agent

Introduction

Case studies in enzyme technology can trace their roots to toluene dioxygenase from Pseudomonas putida. The monooxygenase with cytochrome p450 was the only recognized bacterial three-component oxygenation system in the early 1970s. The multi-component nature of toluene dioxygenase was not realized at the time, and this made its purification quite challenging. The subsequent resolution of the dioxygenase as a three-component system [30] was a significant milestone for this unique bacterial enzyme. The three-component system [24–26] was not only academically interesting but also industrially useful. The evolution of toluene dioxygenase was intimately connected with the evolution of multiple degradative enzymes of the β-ketoadipate pathway [28]. The metabolic evolution gave rise to practical enzyme technology in the areas of antibiotic biosynthesis, antibiotic biocatalysis and drug discovery.

The advances in enzyme technology have affected different aspects of enzyme biochemistry including: enzyme assay and purification, reaction optimization, enzyme characterization, substrate specificity, enzyme kinetics and mechanism, enzyme refolding and metabolic engineering. Examples of two types of enzyme technology in a number of these aspects of enzyme biochemistry are described below.

Results and discussion

Antibiotic biosynthesis

Antibiotic biosynthetic pathways in microorganisms are frequently complex and involve many enzymatic interconversions. Our primary focus has been the biosynthesis of the macrolide antibiotic, tylosin, and that of the beta-lactam compound, cephalosporin C.

Tylosin biosynthesis: Tylosin is an animal health product used agriculturally as a growth promotant. It is a secondary metabolite produced by the soil bacterium, Streptomyces fradiae. The tylosin biosynthetic pathway is shown in Figure 1 [3,12]. Our interest was focused on the two O-methyltransferases catalyzing the final two steps in the biosynthesis of tylosin, and on the aldehyde reductase mediating the conversion of tylosin to the less active compound, relomycin. Demethylmacrocin O-methyltransferase (DMOMT) catalyzes the 2'-O-methylation of the deoxyadipate moiety forming macrocin, which in turn is the substrate for the 3'-O-methyltransferase (MOMT) that methylates the 3'-hydroxyl position of the same moiety to yield tylosin (Figure 1) [22]. Because of substrate (demethylmacrocin/macrocin) accumulation by high tylosin-producing strains, both enzymatic reactions were rate-limiting for tylosin biosynthesis [22]. Tylosin reductase (TR) catalyzes reduction of tylosin to relomycin, resulting in poor recovery of the tylosin product [12].

Through the efforts of several persons over a number of years, the three enzymes (DMOMT, MOMT and TR) were purified to homogeneity and characterized [4,12,16]. The physical, catalytic and kinetic properties of the three enzymes are listed in Table 1 [4,12,16]. DMOMT and MOMT catalyze two consecutive O-methylations in tylosin biosynthesis, and they are distinctive O-methyltransferases [4,16]. Both DMOMT and MOMT exhibit a low Km (ie, high affinity) and a high Vmax for their substrates and a very narrow substrate specificity (Table 1) [4,16], thus both enzymes may be specific to tylosin biosynthesis [12]. Interestingly, based on the Km values of the macroline inhibitors [4,16], the two O-methyltransferases are inhibited independently by different metabolites of the pathway, DMOMT...
Figure 1  Tylosin biosynthesis in *S. fradiae*. A solid arrow indicates a known metabolic step and a dashed arrow indicates a shunt route.