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Research review paper

Lovastatin production: From molecular basis to industrial process optimization

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ABSTRACT

Lovastatin, composed of secondary metabolites produced by filamentous fungi, is the most frequently used drug for hypercholesterolemia treatment due to the fact that lovastatin is a competitive inhibitor of HMG-CoA reductase. Moreover, recent studies have shown several important applications for lovastatin including antimicrobial agents and treatments for cancers and bone diseases. Studies regarding the lovastatin biosynthetic pathway have also demonstrated that lovastatin is synthesized from two-chain reactions using acetate and malonyl-CoA as a substrate. It is also known that there are two key enzymes involved in the biosynthetic pathway called polyketide synthases (PKS). Those are characterized as multifunctional enzymes and are encoded by specific genes organized in clusters on the fungal genome. Since it is a secondary metabolite, cultivation process optimization for lovastatin biosynthesis has included nitrogen limitation and non-fermentable carbon sources such as lactose and glycerol. Additionally, the influences of temperature, pH, agitation/aeration, and particle and inoculum size on lovastatin production have been also described. Although many reviews have been published covering different aspects of lovastatin production, this review brings, for the first time, complete information about the genetic basis for lovastatin production, detection and quantification, strain screening and cultivation process optimization. Moreover, this review covers all the information available from patent databases covering each protected aspect during lovastatin bio-production.

Contents

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1. Introduction

The World Health Organization (WHO) reported that cardiovascular disease is the leading cause of death worldwide. In 2008, about 17.3 million people died from cardiovascular disease, accounting for 30% of total world deaths. This number is expected to increase 34% by 2030 (www.who.int, 2013). One of the factors leading to cardiovascular disease is hypercholesterolemia, which represents high blood cholesterol levels (>200 mg/dL). In the U.S. one in every six Americans has high blood cholesterol levels (www.cdc.gov, 2012), and a study performed in Brazil showed that about 40% of its population has high blood cholesterol levels (Martinez et al., 2003).

Statins are the most widely used drugs for hypercholesterolemia treatment. These compounds inhibit the enzyme hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase, the first enzyme in the cholesterol biosynthesis pathway that catalyzes the reduction of HMG-CoA to mevalonate with concomitant oxidation of 2NADPH molecules. Statin treatment reduces cholesterol synthesis, preventing the buildup of plaque inside the arteries (Barrios-González and Miranda, 2010). Nowadays, it is one of the best sold drugs in the U.S. with sales totaling US$11.6 billion by 2011 (www.drugs.com). In addition to cholesterol reduction, statins have been reported to show other effects including nitric-oxide-mediated blood vessel growth (Shuto et al., 2011), femoral osteolyses (Lubbeke et al., 2012), modification of low-density lipoprotein quantity (Bojadzievski et al., 2012), and also anti-inflammatory activity (Khanicheh et al., 2013). Recently lovastatin was also considered as a candidate to inhibit methanogenic archea present in ruminants (Jahromi et al., 2013a,b). Archeas present in ruminant intestine are responsible for about 20% of methane production, one of the main gases responsible for the greenhouse effect. Those microorganisms synthesize isoprenoid chains to be incorporated into its membrane cell walls. Thus HMG-CoA reductase plays an essential role in isoprenoid biosynthesis. Thus its inhibition by lovastatin leads to reduction of methanogenic archea. Therefore *Aspergillus terreus* strains were used to hydrolyze rice straw improving the quality of ruminant feed (Jahromi et al., 2013a,b). Moreover ruminants feed with this hydrolizate was shown to significantly reduce methane production. In a similar study, lovastatin was shown to inhibit growth rate of *Methanobrevibacter smithii*, one of the methanogen archeas present in ruminant intestine (Jahromi et al., 2013a). Thus besides its medical application, lovastatin may play an important role in feed preparation maximizing biomass utilization.

The first reported statin, mevastatin, also known as compactin, was first discovered in 1976 and was isolated from a *Penicillum citrinum* strain using a screening assay of 6000 fungal extracts for cholesterol biosynthesis inhibitors (Endo et al., 1976a,b). This molecule has a similar structure to the substrate HMG-CoA and thus acts as a competitive inhibitor of HMG-CoA reductase. Mevastatin has been assayed in cell cultures (Brown et al., 1978) and in vivo (Endo et al., 1979); however, when it entered into clinical trials, high dosages led to side effects such as lymphoma formation in dogs. In addition, the parallel discovery of other statins impaired commercialization of mevastatin (Endo, 2010). In the beginning of the 1970s, Merck started a program for isolating new antihypercholesterolemic compounds that resulted in the characterization of mevinolin (Stossel, 2008). This molecule was found in the supernadant of *A. terreus* culture and it was shown to have a higher inhibitory action than mevastatin (Alberts et al., 1980). Indeed its corresponding acid form has an even strong inhibition action and it has been called lovastatin. Thus in this manuscript mevinolinic acid is called lovastatin. Lovastatin is the active ingredient of Mevacor and is the precursor for simvastatin, the active principle in Zocor. Since the discovery of natural statins, filamentous fungi extracts have been patented to be used as food additives, mainly in oriental diets as cholesterol reducers (Hajjaj et al., 2003; Hong et al., 2003).

Statins can be produced via microbial or chemical synthesis. Among the ones produced via microbial synthesis, lovastatin is the most studied. Fig. 1 illustrates the main research focus in developing a bioprocess for microbial lovastatin production. To date, many reviews have covered different aspects of lovastatin including its discovery (Alberts et al., 1980; Manzoni and Rollini, 2002; Tobert, 2003), metabolic pathways involved in its production (Manzoni and Rollini, 2002), genomic organization and regulation of lovastatin biosynthetic clusters (Barrios-González and Miranda, 2010; Brakhage, 2013; Keller et al., 2005; Manzoni and Rollini, 2002), process optimization for development of cultivation medium, and establishment of fermentation modes (Bizukojc and Ledakowicz, 2009; Radha and Lakshmanan, 2013). Nevertheless, none of them compile all the information available on lovastatin biosynthesis. Therefore, this review brings a complete overview of the mechanisms in which lovastatin inhibits active sites of HMG-CoA reductase, the genetic basis for lovastatin production, detection and quantification protocols, the different strain screening assays in addition to a complete vision on what has been done during optimization of cultivation conditions. Moreover, from a commercial standpoint, this review covers all the information available from patent databases covering all protected aspects regarding lovastatin bioproduction (Table 1).

2. Lovastatin interactions in HGM-CoA reductase active sites

HMG-CoA reductase is the third enzyme in the cholesterol biosynthetic pathway and the first rate-limiting step within this pathway since the previous two reactions are reversible. It catalyzes the four-electron reductive HMG-CoA into mevalonate with the concomitant oxidation of 2NADPH molecules and the release of CoA-SH. Overall, statins compete with HMG-CoA by binding to the active sites of HMG-CoA reductase while keeping the NADPH binding site untouched. As can be seen in Fig. 2A, lovastatin hydrophobic-ring structure contacts residues from the helical structures at the enzyme's large domain. Furthermore, it has been described that HMG-CoA reductase is arranged in a strongly associated tetramer with bipartite active sites (Istvan and Deisenhofer, 2001). When the HMG-binding pocket is expanded in view (Fig. 2B), it can be observed that it is characterized by a cis-loop formed by residues 682–694. Since lovastatin is a competitive inhibitor, it seems that its HMG-like moieties bind to HMG-binding active sites. Nevertheless, in this complexation mode, their bulky hydrophobic groups collide with amino acid residues that compose the fine pocket, accommodating the pantothenic acid moiety of CoA (Istvan and Deisenhofer, 2001). Finally, it can be seen that no portion of the elongated NADP(H) binding site is occupied by lovastatin, clearly showing competitive inhibition.

Multiple polar interactions are also formed between the HMG-moieties and amino acid residues situated at cis loops (Asp690, Lys691, Lys692) (Fig. 2B). Lys691 also coordinates the hydrogen-bonding network formation with Glu559, Asp767 and the statin O5-hydroxyl.

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