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A Review on 3-Hidroxy-3-Methylglutaryl-Coenzym A Reductase and Inhibitor: The Medies Potential of The Enzyme Inhibitor.

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ABSTRACT

The 3-Hidroxy-3-Methylglutaryl-Coenzym A Reductase (HMGR) enzim is a limiting factor to regulate cholesterol synthesis, especially in the formation mevalonic acid from Hidroxy Methylglutaryl-Coenzym A (HMG-CoA). The four amino acid residues that are key to the catalytic activity of HMGR are glutamate, lysine, aspartate and histidine. Catalytic mechanism changes HMG-CoA into mevalonic form occurs in 3 stages, the reduction of HMG-CoA into mevaldyl-Coa, decomposition mevaldyl-CoA to mevaldehyde, and reduction of mevaldehyde into mevalonic acid. Statins are the product metabolite which acts as HMGR inhibitors to limit the formation of mevalonic. Statin are the product by several microorganism such as *Aspergillus terreus, Penicillium citrinum, Streptomyces carbophilus, Actinomadura sp., and Bacillus megaterium.* The similarity in structure between the substrate lacton on statins and HMG-Coa allow the two molecules to compete also the HMG-CoA reductase enzyme. Statins are classified into 2 types: natural statins (microorganisms biosynthesis), i.e compactin, pravastatin, lovastatin simvastatin; and synthetic statin: atorfastatin, fluvastatin, and resuvastatin. The similarity between the structures of natural and synthetic statin are located in part which compete with HMG-CoA in the active side of HMGR. Some fermented food extract known can reduce HMGR activity i.e *narezushi, heshiko,* and *bekasam.*

Keywords: *cholesterol, HMG-CoA reductase, statin*

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INTODUCTION

Cholesterol is a molecule with 27 carbon atoms tetrasiclik that is essential to form lipid structures. Cholesterol has an essential function in bile acid synthesis. Cholesterol biosynthesis is a complex pathway involving 20 enzymes to construct 30 carbons of acetyl CoA to 27 carbon structures including a 4 ring. 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase or HMGR enzyme is one of the synthesis cholesterol enzyme complex that a unique characteristic in the regulation of early pathway caused by irreversible catalytic properties [1] HMG CoA reductase (HMGR) is found in many higher animals, eukaryotes and prokaryotes. HMGR catalyzes the conversion of HMG-CoA to mevalonic acid by the reduction 4 electrons of HMG-CoA. In the cell, the concentration of mevalonic acid is very strictly controlled through the activity of HMG-CoA reductase (HMGR) so the enzyme is a limiting factor of steps in the cholesterol and other isoprenoid synthesis [2].

The existence of the synthesis of cholesterol and intake of foods containing high cholesterol often leads to elevated levels of cholesterol in the blood. Increased cholesterol levels have been identified as the primary risk factor for coronary artery disease. Inhibition of HMGR significantly reduces cholesterol levels and may reduce the risk of stroke by 29% and reduce mortality by 22%. Statins compound are highly effective in lowering serum cholesterol levels and suppress hypercholesterolemia The similarity structure of HMGR and HMG-CoA cause specific inhibition of the synthesis of mevalonic acid. Therefore, structure of the 3-hygdroxy-3-methylglutaryl coenzim A (HMG-CoA) reductase enzyme and statins as its inhibitors have been widely conducted [2]. This article discusses the enzymologists of HMGR in mevalonic synthesis pathway and biosynthesis of statin as its inhibitors.

Structural and Characteristic of HMG-CoA Reductase (HMGR)

Different organisms have different of HMGR. The crystal structure for the HMGR Class I ($HMGR_H$) and form of the protein bound to the substrate HMG-CoA or coenzyme form that are NADH or NADPH or both [2]. Crystal structure for class II enzymes in *Pseudomonas mevalonii* ($HMGR_P$) had been reported by Friensen and Rodwell [3].

HMGR in humans has three major domains, they are catalytic, linker and anchor, whereas HMGR in *P. mevalonii* has only catalytic domain. Catalytic region is COOH terminal that composed of 338 amino acid residues. The linker domain is consisted of 110 amino acid residues and the anchor which is a NH_2 terminal consisted of 339 amino acid residues. Linker domain is the liaison of the catalytic and anchor domain (Panda and Devi, 2004). $HMGR_H$ have a dimer active side that residues are contributed by each monomer and non-Rossmann-type coenzim-binding site, which folds into three-dimensional structure that contains the nucleotide binding. It is found in many enzymes that use dinucleotida (NADH and NADPH) for catalysis. The core region contains the catalytic domain. The four amino acid residues that are key to the catalytic activity $HMGR_H$ is glutamate, lysine, aspartate and histidine [3].

The active site of HMG-CoA reductase is located at the interface between the first monomer nicotinamide dinucleotide binding site and the second monomer which contain HMG-CoA binding. In human $HMGR_H$, the catalytic lysine is found in monomer binding sits HMG-CoA that is derived from the cis-loop (the part that connects the HMG-CoA binding region with NADPH binding region). $HMGR_P$ use NADH as coenzyme, while $HMGR_H$ use NADPH, but alanine mutations of the residues Aspartyl of $HMGR_P$ typically limit the NADPH binding and may allow use of NADPH as a coenzyme although not optimal for $HMGR_P$ [3].

Catalytic Reaction Mechanism of HMG-CoA Reductase

The reaction mechanism of HMGR shows reduction of HMG-CoA to mevalonic with 2 molecules of NADPH that processed through 2 hydrida transfer. The reaction occurs in three stages, the first is reduction which results in the formation of *mevaldyl-CoA hemi thioacetat intermediate*; the second is decomposition of mevaldyl-CoA to mevaldehyde, and the third is transfer $NADP^+$ from the second NADPH to reduce mevaldehyde to mevalonic. In summary can be written as follows:

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(*S*)-HMG-CoA + 2 NADPH + 2 H^+ → (*R*)-mevalonate + 2 $NADP^+$ + CoA-SH.

More details on the relationship between the catalytic residues and mechanisms overhaul of HMG-CoA to mevalonic can be seen in Figure 1 below.

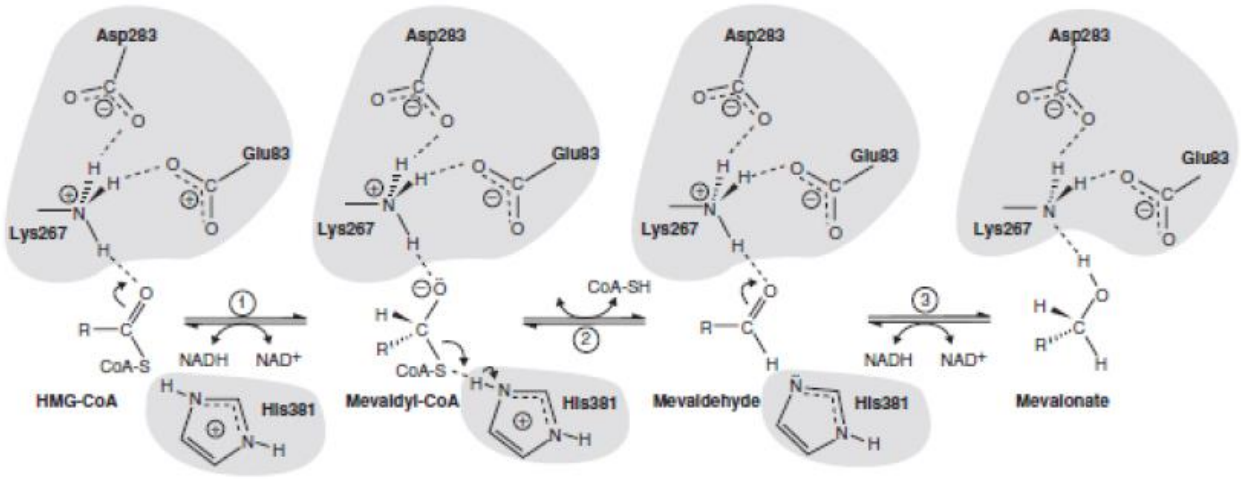

Figure 1: HMGR catalytic reaction mechanism (Lys267, Asp283, Glu83 and His381 and R as subtract and products). Source: Friensen dan Rodwell [3]

Binding Statin as Competitive Inhibitor

Statins are potential competitive inhibitors of HMGR. All statins play important role in the inhibition on the binding of HMG. Hydrophobic group bind to the enzyme HMG covalently bonded [4]. The CoA binding part (CoA binding pocket) are long and narrow so it is not suitable for the binding of cyclic shaped statins. Cyclic group statins mimicked nicotinamida ring of NADP (H) and the inhibitor utilize the binding pocket that statins can bind to the HMG binding site and nicotinamide binding site [2].

A comparison between the structures of the substrate (HMG-CoA) with statins cause rearrangement subtract binding pocket to accommodate the statin molecules. There are differences in the structure of proteins in the COOH terminal, where the formation of a complex with a statin, Gly 860 at the COOH terminal residues missing. Despite the changes in the structure of the complex with statin unpredictable, residues of COOH HMGR terminal are known as mobil element in HMGR protein. Flexibility COOH terminal HMGR exploited by statins to form the bond that a statin molecule inhibitor. All statins limit step of cholesterol biosynthesis by inhibiting the biosynthesis of isoprenoids and sterols.

Statin as a inhibitor of 3-Hydroxy-3-Methylglutaryl Coenzim A Reductase (HMGR)

Statins are a group of drugs that block cholesterol synthesis by inhibiting and restrictions of the enzyme 3-hydroxy-3-methylglutarycoenzyme A (HMG-CoA) reductase. Statins were first discovered by Endo in 1976 as a researchers from Sankyo Co.. Ltd. Japan which found components HMG-CoA reductase inhibitors (enzymes extracted from the liver), he found mevastatin and compactrin from *Penicillium citrinum*. However, mevastatin and compactin not used and traded, because the test further mentioned that both have negative effects for humans that cause tumors, muscle damage and even death in animals [5].

According to Barrios-Gonzalez and Miranda [5], scientists from Merck screened several fungi that can produce a statin. They retrieved Aspergillus terreus that is capable produced lovastatin. Lovastatin is known efficient in inhibiting HMG-CoA reductase. After lovastatin has been known can reduced LDL and safer, in 1987, the U.S. Food and Drug Administration (FDA) approved for trading by the name of Mevacor. Then Sankyo developed pravastatin which was derivated of compactin. Pravastatin known more efficient and secure than the compactin.

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Table 1: The kinds of statin and its production sources

No	Kinds	production sources
1	Mevastatin	*Penicillium citrinum*
2	Compactin	*Penicillium citrinum* *P. brevicompactum*
3	Lovastatin	*Aspergillus terreus; Monascus ruber; M. purpureus* *M. pilosus, M. vitreus*
4	Pravastatin Derivated product of compactin	*Streptomyces carbophilus; Actinomadura sp.* *Bacillus megaterium; Nocordia autrophica*
5	Simvastatin Derivated product of lovastatin	*Aspergillus tereus mutan*
6	Fluvastatin	Synthetic
7	Atorvastatin	Synthetic
8	Resorvastatin	Synthetic
9	Pitavastatin	Synthetic

Summarised from: Barrios-Gonzalez and Miranda [5]

Merck developed the "second generation of lovastatin semisynthetic derivated" i.e. simvastatin. Merck sponsored the use of simvastatin in Scandinavia in 2.221 patients of moderate hypercholesterolemia. The results showed that simvastatin efficiently reduce total cholesterol by 25% and lowered LDL by 35%, and even reduce mortality by 42%. Clinical trials are obtained encouraging results, leading to the development of the production of statins, thus developing a synthetic statin, that fluvastatin, atorvastatin (*Lipitor*), resurvastatin (*Crestor*), and Pitavastatin. The development of several studies to produce various types of statins from a variety of sources and the synthesis of microorganisms as listed in Table 1.

Statins are grouped into 2 types: Statins type 1, a statin that is naturally synthesized by a variety of microorganisms, which are included in this type are lovastatin, pravastatin, and simvastatin that resembles the structure of compactin. Statins Type 2, a statin full synthetic (artificial), which are included in this type are fluvastatin, cerivastatin, atorvastatin and resuvastatin. Structure of type 1 statins has different with type 2 statin. Only the part that is similar to the HMG-CoA reductase inhibitors are responsible as HMG-CoA reductase (HMGR) commonly owned by statins, both on the statin type 1 and type 2. The fundamental difference between lovastatin, simvastatin, compactin and pravastatin that were classified statin type 1 located on the side chain groups R1 and R2 [4]. The difference in the structure of statins can be seen in Figure 2 below.

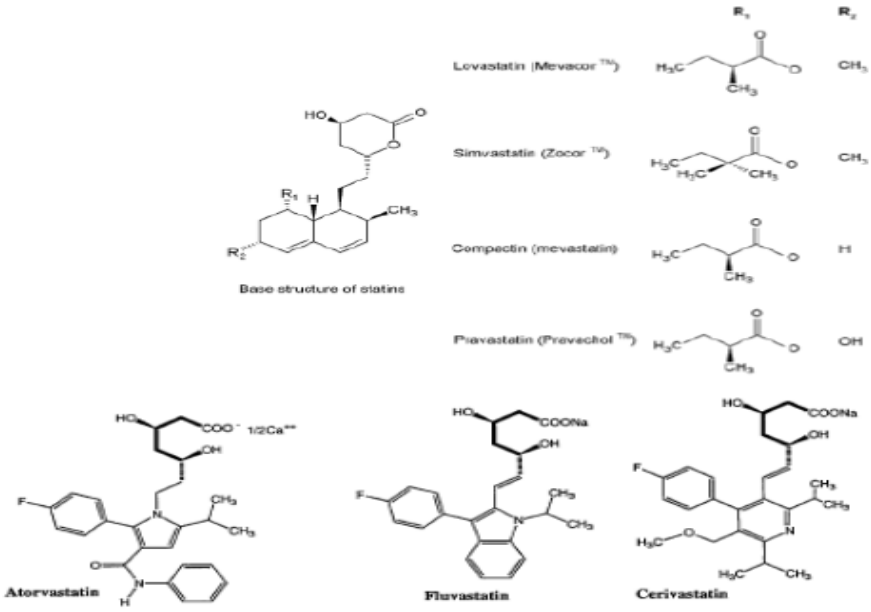

Figure 2: The basic structure and differences in the various statins. Sources: Barrios-Gonzales dan Miranda [5], Dansette [6] dan Tobert [7].

Statins are synthesized by various microorganisms, both fungi and bacteria. Some studies say that they have similarities statin biosynthetic pathway. Studies on *Penicillium citrinum* and *Monascus ruber* indicate the same formation of lovastatin and compactin that is through polyketide pathway. Some statins are derivatives produced by bacteria. pravastatin is derivatives produced from compactin and simvastatin from lovastatin.

Lovastatin Biosynthesis

Lovastatin (mevinolin/monacolin K/Mevacor) is one of the effective inhibitors of HMGR. This is caused by condition when the lactone ring form of lovastatin in the open condition (occurs in the human heart), its structure is similar to the HMG-CoA. This shows that lovastatin and monacolin other is a specific competitive inhibitor of reductase to convert HMG-CoA, which reduce serum cholesterol levels by inhibiting cholesterol biosynthesis [8].

Biosynthesis of lovastatin starting from acetate units (length 4- 8 carbons) are bonded to each other to form two polyketide chain. Methyl groups located in the side chain or in the C6 that derivative of methionine. Oxygen atoms are in the main chain is included later by using the aerobic oxidation of precursor deoxygenation Monacolin L which is an intermediate product in the biosynthesis of lovastatin molecules formed from 9 acetate. Monacolin L had hydroxylation to monacolin J. Through a monacolin X , it is done esterification for conversion into lovastatin [8]. Investigation of several studies showed that enzymatic kinetics Along with the regulation and expression of genes involved in the biosynthesis of lovastatin in *Aspergillus terreus*. Genetics lovastatin biosynthesis associated with 2 polyketide chain. Polyketide synthase system (PKSs) and lovastatin nonaketide synthase (LNKS) involved in catalysis polyketide chain to form a ring and a diketide hexahydro nephtalene synthase (LDKS) involved in the transfer of side chains metylbutyryl to monacolin J. The structure of lovastatin (Fig. 3) consists metilbutirik side chain (R1) and a 6-α methyl group (R2).

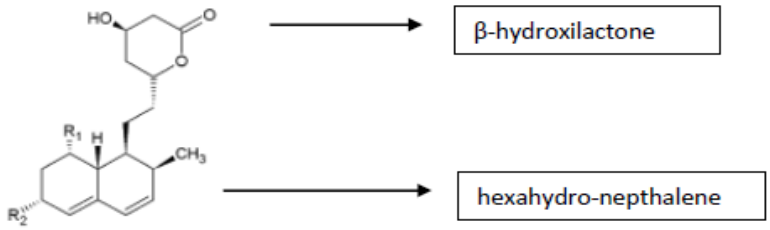

Figure 3: Structure of Lovastatin. Source: Seenivasan [8]; Barrios- Gonzales and Miranda [5]

LNKS producing gene lovB and lovC to catalyze the reaction in the first stage of biosynthetic pathways that initiate dihydromonacolin L. LovA change dihydromonacolin L to monacolin L. In the final stages of the formation of lovastatin, LDKS made by section 2 methylbutiric lovF catalyzing acid to monacolin J, monacolin derived from L. The next, Lovd change monacolin J to lovastatin. Some microorganisms that can synthesize lovastatin is *Aspergillus terreus, Monascus ruber, M. purpureus, M. pilosus, M. vitreous, M. pubigerus, M. numbers, Paecilomyces viridis, Penicillium citrium* and *Lactobacillus acidophilus* [5, 9, 10, 11, 12, 13, 14].

Several physical and chemical factors can affect the production of lovastatin. Optimization of the production of lovastatin in *Aspergillus terreus* by treatment with 8 days of incubation, the media pH 8.5, the addition of methionine 2 g/L, and Sodium acetate 20 g/L, yielding 188.3 mg lovastatin/mL compared with a control condition with 7 days of incubation treatment, media with pH 7, methionine 0 g/L, and Sodium acetate 10 g/L, which only produce lovastatin 54.5 microg/mL. Incubation with non-aerated conditions produce lovastatin higher than the aeration (160:120) at 8 days incubation, media with pH 8.5, the addition of methionine 2 g/L, sodium acetate and 20 g/L, which produces lovastatin 188.3 ug/mL [15, 16].

Compactin Biosynthesis

Compactin ($C_{23}H_{34}O_5$) also known as mevastatin or ML-236B is a member of the statin class that includes a group of polyketides. Polyketides are a rich source of pharmaceuticals including antibiotics, anti-cancer drugs, cholesterol-lowering drugs and other therapies [17]. Although compactin not be used as a drug, but compactin is needed in the biosynthesis of pravastatin. Compactin (C23H34O5) has a molecular weight of

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390. Compactin structure can be divided into two fragments, namely hexahydro-napthalene unit at the bottom and at the top of the lactone unit (Fig 4).

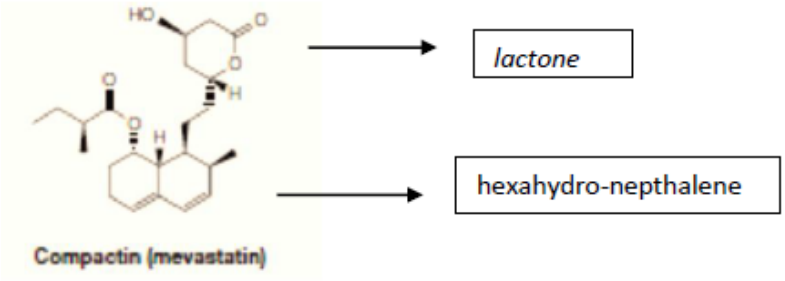

Figure 4: The structure of compactin. Source: Tobert [7]

There are the main line of the same in the formation of lovastatin and compactin. Compactin biosynthesis was first reported by Endo et al. 1985, which reported that the main lines are compactin polyketide formation of acetate. Substituting 2-methyl butyl acetate prepared by the addition of a methyl group at C-19 and C-24 are derived from methionine. Based on testing using NMR, linear relationship between the addition of acetate and folding forms can be known [5]. there are 9 genes (MLCA - H and mlcR) that play a role in the synthesis of compactin, including two genes for polyketide synthesis and 1 gene regulator. The results of the analysis of 9 genes homologous between compactin and lovastatin, which is *mlcA* and *lovB* (59% homologous); *mlcB* and *lovF* (61% homologous); *mlcC* and *LovA* (72% homologous); *mlcD* and *orf8* (63% homologous); *mlcE* and *orf10* (70% homologous); *mlcF* and *orf5* (57% homologous); *mlcG* and *lovC* (70% homologous); *mlcH* and *LovD* (75% homologous), finally *mlcR* and *lovE* (34% homologous) as transcription factor [17].

Polyketide as a compactin synthesis pathway is a large group of secondary metabolites with diverse structures produced by bacteria, fungi, and plants. Key enzyme associated with polyketide biosynthesis defined as polyketide syntheses (PKSs), which consists of type I PKSs in fungi and type II PKSs in bacteria. Ooptimization production of the compactin with the differences treatment specifically in the fermentation time, carbon source, a source of inorganic nitrogen, organic nitrogen sources, and the difference in pH. The optimum fermentation time devoted to fermentation for 168 h with a glycerol as a carbon source at 22%, and pH 6.5. While the addition of nitrogen sources (organic and nonorganic) didn't affect the production of compactin [18].

Pravastatin Biosynthesis

Compactin (ML-236B) is used as a substrate for microorganisms convert to pravastatin sodium. Pravastatin sodium ($C_{23}H_{35}O_7Na$) is widely used as a pharmaceutical drug in hypercholesterolemia. Pravastatin sodium is sodium compactin hydroxylation product 6β position (Fig. 5).

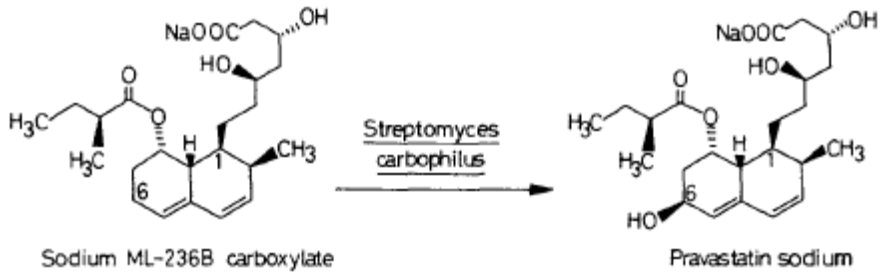

Figure 5: The Hydroxylation of compactin to pravastatin. Source: Matsuoka [19].

Pravastatin was first discovered as a minor urinary metabolite in dogs that fed intake compactin which supported the role of cytochrom P450 in the liver. When tested on mikroorganiseme that has

cytochrom P450 occurred hydroxylation of compactin. Over the past 2 decades known that cytochrom P450 system in prokaryotes is hydroxylation of fatty acids and steroids, such as *Bacillus megaterium* [19].

Protein hepatic microsomal cytochrom P450 are generally induced by various drugs or xenobiotics and plays an important role in detoxification. A large number of microorganisms in the soil, including *Streptomices* also known to detoxify and degrade various xenobiotics. Therefore, some microorganisms are known to perform hydroxylation of compactin to pravastatin [19]. In early development, the pravastatin was derived from compactin by chemical synthesis, but It was the high cost and produced stereoisomers, after that, compactin hydroxylation developed by microorganisms. Industrial production of pravastatin with microorganisms have more efficient with higher average conversion of compactin to pravastatin than chemical synthesis [11].

All this time, bioconversion of compactin to pravastatin done by many mold/fungi. However, fungi are generally intolerant of an increasing number of compactin that were added to the medium. This condition is caused by the presence of compactin as a antifungal properties. *Pseudonocardia autotrophia* included in gram-positive bacteria are known to be capable of changing compactin into pravastatin. These strains were isolated based on the ability of resistance to compactin. Some microorganisms that can do bioconversion compactin into pravastatin, they are *Streptomyces roseochromogenus, S. carbophilus, S. halstedii* (low bioconversion compactin to pravastatin) and *Actinomadura sp., Nonomuraea recticatena, Streptomyces sp.* (high bioconversion compactin to pravastatin (> 50%)) [20, 21].

Lactobacillus delburuecki known as probiotic of lactic acid bacteria that have a cytochrom P450 which capable to do xenobiotics metabolism by hydroxylation, such as the nicotine and aflatoxin. *Lactobacillus rhamnosus strain GG* reduces aflatoxin by metabolism via the expression of P450 cytochrom [22]. *Lactobacillus kefir* produce tert-butyl (3R, 5S) 6-chloro-dihydroxyhexanoate known as HMG-CoA reductase inhibitor [23]. Some lactic acid bacteria isolated from fermented fish products in Japan are kaburazushi and narezushi has the ability as a radical scavenger. This condition is identical to the function of pravastatin which can also be as a radical scavenger in the case of atherosclerosis. Therefore, further study of pravastatin in the presence of fermented products as a result of the bioconversion of microorganisms during the fermentation process needs to be done.

Benefits and Role of Statins

Statins have three (3) mechanism in the control of blood cholesterol, first, Effect lowering LDL cholesterol with increased catabolism of LDL mechanism, enhance the removal / disposal of LDL and reduced production of VLDL; second, lower triglycerides; third, increasing HDL. Inhibition of cholesterol synthesis by statins is done by limiting the synthesis of mevalonic as a cascade in the formation of cholesterol. Mevalonic synthesized from acetoacetyl CoA with the help of the enzyme 3-hydroxy-3-methylglutaryl CoA (HMG-CoA) reductase. Inhibition of activity by statins to act as a competitive inhibitor of the enzyme HMG-CoA reductase leads to changes acetoacetyl CoA to mevalonic be inhibited resulting in the formation of cholesterol, respectively [17].

The presence structural similarities between the structure of lacton/hydroxilacton on statins to shape the structure of the substrate HMG-CoA reductase in cholesterol synthesis causes statins act as a competitive inhibitor in HMGR (Fig. 6).

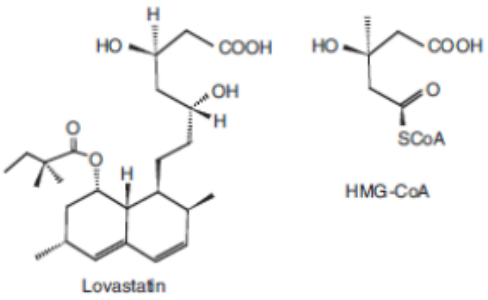

Figure 6: Similarities part lacton of statin and HMG-CoA. Source from Friensen dan Rodwell [3]

Lovastatin and simvastatin are an inactive lactone that is hydrolyzed in the gastrointestinal tract into derivative active hydroxyl-β, whereas pravastatin has an active lactone ring open. Atorvastatin, cerivastatin, and fluvastatin contain fluorine, which is active when ingested. Absorption against doses may vary from about 40% to 75% with the exception of fluvastatin, which is almost completely absorbed. Most of the absorbed dose is excreted in the bile; approximately 5-20% is excreted in the urine. The half-life of the drug ranged from 1 to 3 hours but the half-life of atorvastatin is 14 hours.

Blocking to the HMG-CoA reductase can inhibit cholesterol synthesis in the liver and this will lower plasma LDL levels. Decreased cholesterol levels will cause changes related to the potential of this drug. But obviously reductase inhibitors induce an increase in LDL receptor with high affinity. These effects increase the fractional catabolic rate of LDL and LDL precursors extraction by the liver (VLDL remnant), thereby reducing plasma LDL deposits. A slight decrease in plasma triglycerides and a modest increase in HDL cholesterol levels also occurred during treatment. Statins lower cholesterol by increasing the number of LDL receptors, so the catabolism of cholesterol happening more and more. Thus, these drugs can lower cholesterol (LDL). Lovastatin and pravastatin are a result traded biosynthesis microorganisms as generic drugs. Therefore the demand is high lovastatin and pravastatin compared with simvastatin (a statin results biosinteisi other microorganisms) and synthetic statins (atorvastatin, fluvastatin and resuvastatin). The high demand as lovastatin and pravastatin cholesterol lowering drugs to support opportunities to explore the many microorganisms that can synthesize lovastatin and pravastatin. Therefore the study of the sources that allow for producing lovastatin and pravastatin needs to be done.

Inhibitor HMGR from Food extract

Some fermented food extract known can reduce cholesterol level in serum. Japanese *heshiko* and *narezushi* were reported capable of reducing cholesterol by inhibition of HMG-CoA reductase, an enzyme responsible in the first step of cholesterol biosynthesis. The fractionation of *heshiko* and *narezushi* extract produced peptides and non peptide fractions found as inhibitor of HMG-CoA reductase [24, 25]. HMG-CoA reductase activity was inhibited by statins (> 80%) and peptides (> 40%) (Barrios-Gonzalles et al. 2010; Kato et al. 2009). Extract of *Bekasam* (Indonesia traditional fermented fish) can reduce the activity of HMG-CoA reductase enzyme [26].

CONCLUSION

The HMG-CoA reductase (HMGR) enzyme is a limiting factor to regulate cholesterol synthesis in the mevalonic formation from HMG-CoA. Statins are the product metabolite microorganisms that are HMGR inhibitors to limit the formation of mevalonic. The similarity in structure between the substrate lacton on statins and HMG-Coa cause they are competitively on the HMGR. Some fermented food extract known can reduce HMGR activity i.e *narezushi, heshiko,* and *bekasam.*

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