Review Article

Squalene – biochemistry, molecular biology, process biotechnology, and applications

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Squalene is a natural triterpene and an important intermediate of sterol and hopanoid biosynthesis in various types of cell from bacteria to human. Synthesis and further conversion of squalene are key steps in the metabolism of sterols and related components. Here we summarize the recent knowledge of squalene biochemistry, its molecular properties, and its physiological effects. We compare squalene biosynthetic pathways in different cell types and describe biotechnological strategies to isolate this lipid. Finally, applications of squalene in nutrition, pharmacy, and medicine are discussed.

1 Introduction

Squalene (2,6,10,15,19,23-hexamethyl-6,6,10,14,18,20-tetracosahexane) is a triterpenic hydrocarbon. It is widely present in nature, and substantial amounts are found in olive oil, palm oil, wheat-germ oil, amaranth oil, and rice bran oil. The richest source of squalene (SQ), however, is shark liver oil (60 wt%) which has been traditionally used as source of this lipid. In humans, SQ is present at its highest concentration in sebum (∼13%). Squalene is synthesized in all types of cells because it is a key intermediate in the formation of eukaryotic sterols and bacterial hopanoids. Squalene and its related compounds, oxidosqualene and bis-oxidosqualene, are precursors of nearly 200 different triterpenes [1]. Some microorganisms, e.g., bacteria, are also able to utilize SQ as a carbon source.

Squalene has several beneficial properties. It is a natural antioxidant [2], serves in skin hydration [3] and has been used as emollient in adjuvants for vaccines [4]. As a compound of olive oil, it also has a preventive effect on breast cancer, possesses tumor-protective, and cardio-protective properties [5–7] and decreases the serum cholesterol level [8, 9]. Moreover, squalenoylation has become a common method for delivering prodrugs into cells [10–12].

During the last decades many efforts were made to isolate SQ from new sources. Distillate residues from olive oil, soybean oil, rice bran oil, or amaranth oil became attractive alternatives for shark liver oil. Isolation of SQ from microorganisms is still under development and investigated at present only at a scientific level. Initial studies of this kind were performed with bacteria, yeast, and with microalgae.

In this review, we will describe biochemical and biophysical properties of SQ and then address biosynthesis of this compound in different cell types with emphasis on SQ forming enzymes. Methods of molecular biology set the stage to identify genes and gene products involved in SQ metabolism from mammalian cells, plants, and microorganisms. We will discuss differences of SQ synthetic pathways in different cells and describe specific properties of enzymes catalyzing key steps in squalene formation and conversion. Finally, we will briefly describe technological processes to isolate squalene from various sources and discuss new developments to utilize squalene in nutrition, pharmacy, medicine, and cosmetics.

2 Biochemical and biophysical properties of squalene

Squalene is a polyunsaturated triterpene, which contains six isoprene units (Fig. 1). At RT, it is a liquid with pleasant,

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Abbreviations: DMAPP, dimethylallyl diphosphate; **DXP,** 1-deoxy-D-xylulose-5-phosphate; **DXR,** DXP reductoisomerase; **DXS,** DXP synthase; **ER,** endoplasmic reticulum; **FPP,** farnesyl diphosphate; **FPS,** FPP synthase; **G-3-P,** glycerol-3-phosphate; **GPP,** geranyl diphosphate; **HMG-CoA,** 3-hydroxy-3-methyl-glutaryl-CoA; **HMGR,** HMG-CoA reductase; **IDI,** isopentenyl diphosphate isomerase; **IPP,** isopentenyl diphosphate; **MEP,** 2-C-methyl-D-erythritol 4-phosphate; **MVA,** mevalonate; **SQ,** squalene; **SQS,** squalene synthase; **SQE,** squalene epoxidase; **TAG,** triacylglycerol
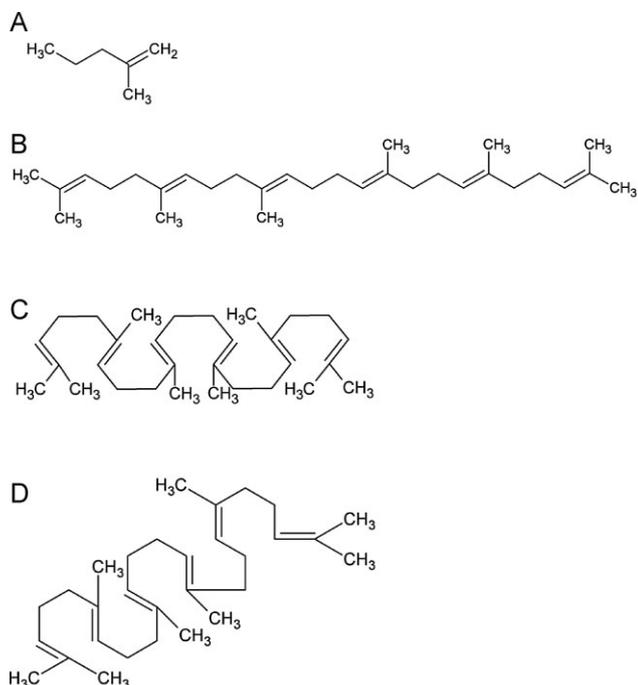
Figure 1. Chemical structure of squalene and its precursor. A, Chemical structure of isoprene. Different structures of squalene are: B, stretched form; C, coiled form; and D, "sterol-like" form.

bland taste. In Table 1, some properties of squalene such as viscosity, density, and solubility are summarized. These data underline the strong hydrophobic nature of this molecule. Due to its chemical structure, especially the high degree of unsaturation, squalene is not very stable and gets easily oxidized. In complex mixtures such as olive oil, however, its stability is improved. Vice versa, squalene was found to contribute to virgin olive oil stability under light exposure [13, 14]. Nevertheless, Manzi et al. [15] observed decomposition of squalene in the range of 26–47% in olive oil after 6 months storage in the dark and at RT. Other studies described a maximum of 20% degradation even under more severe conditions or during pan-frying [13, 14, 16–18].

Due to its non-polar nature incorporation of squalene into biological membranes is limited. As a consequence, squalene rather accumulates in lipid storage compartments. Kalvodova [19] showed that phagocytes when treated with squalene

Table 1. Physical properties of squalene

Properties	Values	Ref.
Octanol/water partitioning coefficient (log P)	10.67	[302]
Solubility of squalene in water	0.124 mg/L	[302]
Viscosity	~11 cP	[303–305]
Surface tension	~32 mN/m	[303–305]
Density	0.858 g/mL	[303–305]

containing oil-in-water emulsions accumulated this lipid together with other components in so-called lipid droplets. In Schwann cells, squalene was also found mainly in lipid droplets [20]. Similarly, yeast squalene was detected in the highly hydrophobic core of lipid particles/droplets, and only at small amounts in cellular membranes [21, 22]. As can be seen from Fig. 1, double bonds allow squalene to occur in several conformations, e.g., in a symmetric, stretched, or coiled form [23]. Most interestingly, squalene can also be organized in the shape of a sterol which may allow accommodation in a membrane. Hauss et al. [24] showed that squalane, a hydrogenated relative of squalene, was horizontally inserted in a phospholipid bilayer membrane. These authors argued that such a topology caused protection against proton leakage and affected transmembrane proton flux. Lohner et al. [25] demonstrated that squalene at a concentration of 6 mol% in artificial phospholipid vesicles altered the lamellar-to-inverse-hexagonal phase transition by increasing the size of inverse hexagonal phase tubes. It was assumed that in such a situation squalene was rather coiled and stored in the most disordered region of the membrane bilayer. Experiments from our own laboratory (Spanova et al., unpublished data) using biological membranes from the yeast and model membranes extended this model. We concluded from these experiments that squalene in the endoplasmic reticulum (ER) may rather adapt to a conformation close to ergosterol, whereas in the plasma membrane the coiled conformation may be predominant.

3 Squalene in animal and human cells

In the mammalian organism, squalene is one of the most important lipids of skin cells. It is synthesized in sebaceous glands where it accounts for 13% of total lipids [26]. Its total concentration in the skin [27, 28] and the squalene to cholesterol ratio [29] vary with the skin site. Secretion of squalene was found to depend on the individual in a range from 125 to 475 mg per day [29]. Interestingly, very little squalene produced in sebaceous cells is further converted to cholesterol. This effect may be caused either by overexpression of squalene synthase (SQS), enhanced activity of the enzyme, or by down-regulation of oxidosqualene cyclase (SQE) which converts squalene to oxidosqualene and channels the intermediate to the cholesterol biosynthetic pathway. Both enzymes of sebaceous glands are sensitive to environmental conditions and subject to transcriptional regulation [26].

Squalene of mammalian cells originates partly from endogenous biosynthesis and partly from dietary sources. The intracellular pool of squalene appears to be in an equilibrium with the pool in the plasma [30]. About 60–85% of dietary squalene is absorbed and transported in the serum, mostly together with VLDL, and then distributed to various tissues. Only a very small amount of squalene taken up as nutrient is converted to cholesterol, and even higher consumption of squalene does not change the cholesterol level

[31]. Increased amounts of squalene in the serum are safe, beneficial, and exhibit chemo-preventive and hypocholesterolemic properties [32, 33].

Squalene at very high concentration can be found in the bodies of cartilaginous fish which lack a swim bladder and must therefore reduce their body density with fats and oils. Squalene is highly abundant in sharks (*Squallus spp, Centrophorus squamosus*) and whales (*Physeter macrocephalus*) [34–36]. In shark liver oil, the amount of squalene reaches 40–70% by weight. This extraordinary high concentration of squalene resulted in intense shark hunting to use this lipid as a basis for health care products. However, environmental and marine protection concerns became a strong motivation to search for alternative squalene sources.

3.1 Biosynthesis of squalene and its regulation in mammalian cells

Sterols are essential structural and regulatory components of eukaryotic cell membranes. Squalene plays an important role as an intermediate in the sterol biosynthesis. Synthesis of squalene is similar in all organisms, although properties of enzymes involved in its formation can differ. In some cases, reactions leading to squalene formation are catalyzed by single enzymes, whereas in other cases more enzymes (isoenzymes) are involved.

In animal and human cells, cholesterol can be synthesized via de novo mevalonate (MVA)/isoprenoid pathway or taken up through LDL. These lipoproteins enter the cell via LDL receptors on the cell surface, get transported to lysosomes, and hydrolyzed in this compartment. There is a balance between internal and external cholesterol sources which is governed by feedback control of biosynthetic and uptake pathways. Major players in feedback control mechanisms are 3-hydroxy-3-methyl-glutaryl-CoA reductase (HMGR) and LDL receptors. To prevent cholesterol accumulation, HMGR activity can be reduced by more than 90%, and the number of LDL receptors can be decreased [37], respectively.

In animal cells, de novo synthesis of cholesterol (Fig. 2) occurs through the mevalonate/isoprenoid pathway. This pathway starts with acetyl-CoA which is converted to 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) and then reduced by HMGR to MVA. As mentioned above, this step is rate limiting and highly regulated. Regulation is maintained by activating or degrading HMGR. HMGR, an integral protein of ER membranes contains a transmembrane sterol-sensing domain which plays an important role in the degradation of the enzyme by the proteasome. Recent studies showed direct and indirect stimulation of degradation by cholesterol, lanosterol and oxysterols [38, 39]. After phosphorylation and decarboxylation of mevalonate, isopentenyl diphosphate (IPP) and dimethylallyl diphosphate (DMAPP) are formed, and the latter component serves as a precursor of all polyprenyl compounds. Subsequently, condensation with
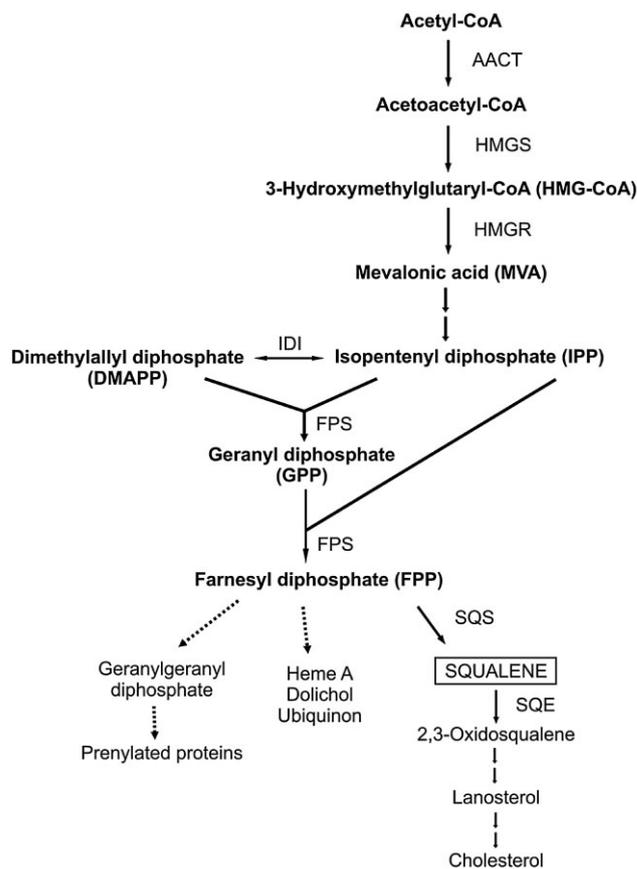

Figure 2. Squalene synthesis via MVA in mammalian cells. AACT, acetoacetyl-CoA thiolase; FPS, FPP synthase; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; IDI, isopentenyl diphosphate isomerase; SQS, squalene synthase; SQE, squalene epoxidase.

another IPP molecule yields farnesyl pyrophosphate (FPP), which can be either converted to squalene and sterols or directed toward the synthesis of isoprenylated cellular metabolites such as heme, dolichols, and ubiquinone [40–42]. FPP is also involved in farnesylation and geranylgeranylation of proteins including small GTP-binding proteins like Rho, Ras, and Rac [43–45]. In the subsequent step of the pathway, SQS combines two FPP molecules to form squalene and thus directs FPP toward cholesterol synthesis [46–48]. SQS competes with other enzymes for the FPP substrate and responds to the cellular sterol content in a similar manner as HMGR [48]. SQS affects synthesis of other essential non-sterol isoprenoids by triggering FPP to their pathways [49, 50]. In the following enzymatic step, epoxidation of squalene to 2,3-oxidosqualene catalyzed by SQE (squalene monooxygenase) occurs [51, 52]). For the activity of this enzyme, a cytosolic (S105) fraction, molecular oxygen, NADPH-cytochrome c reductase, NADPH and FAD are required [53–56]. This enzymatic step has been well studied, since inhibitors (allyl-

amines) of this reaction have beneficial effects such as lowering LDL bound cholesterol [57]. SQE is affected by sterols in a feedback mechanism, but not by non-sterol intermediates of the MVA pathway [58]. The product of the SQE reaction, 2,3-oxidosqualene is then further converted to cholesterol in several steps which will not be discussed here.

Subcellular localization of enzymes involved in the pre-squalene biosynthetic pathway of sterol formation has been a matter of dispute for a long time [59–62]. Reactions are distributed in the cytosol and the ER [61], but early steps of isoprenoid formation are also associated with peroxisomes [63]. It appears that acetyl-CoA derived from peroxisomal beta-oxidation can be preferentially channeled to isoprenoid formation in this compartment.

4   Squalene in plants

Besides shark liver oil several plants became valuable sources for the isolation of squalene. Highest enrichment of squalene was detected in olive oil and amaranth oil, but smaller amounts are also present in palm oil, wheat germ oil, peanut oil, and rice brain oil [5, 64]. In olive oil squalene accumulates at a yield of 7 mg per g oil [5]. In combination with oleic acid (72%) and polyphenols squalene was found to be beneficial with a more pronounced effect on prevention than treatment [5, 65, 66].

4.1   Biosynthesis of squalene in plants and its regulation

In plants, the biosynthetic pathway of sterols is slightly different from animal cells and fungi. Biosynthetic reactions from squalene to phytosterols result in formation of various sterols, such as sitosterol, stigmasterol, campesterol, and isofucosterol. In plants, squalene is oxidized to 2,3-oxidosqualene and then converted to cycloartenol (9β,19-cyclo-24-lanosten-3β-ol) instead of lanosterol as known from in animals and fungi, which is further metabolized to the end product of this biosynthetic cascade, sitosterol [67–69]. Phytosterols synthesized in the ER are transported mostly to the plasma membrane; a minor amount of squalene is retained to the Golgi [70–72].

Isoprenoids of plants can be synthesized via mevalonate pathway (MVA) in the cytosol leading to the formation of sterols and brassinosteroids or in mitochondria where side chains of ubiquinone are formed. Alternatively, the 2C-methyl-D-erythritol-4-phosphate (MEP) pathway, formerly known as non-mevalonate or 1-deoxy-D-xylulose-5-phosphate (DXP) pathway [73], located to plastids leads to the synthesis of carotenoids, the side chains of chlorophylls, plastoquinones, and isoprenoid-type phytohormones [73, 74] (Fig. 3). The MVA pathway forms only IPP, whereas the MEP pathway generates IPP and DMAPP. Exchange of isoprenoids between cytosol and plastids is rather inefficient [69].
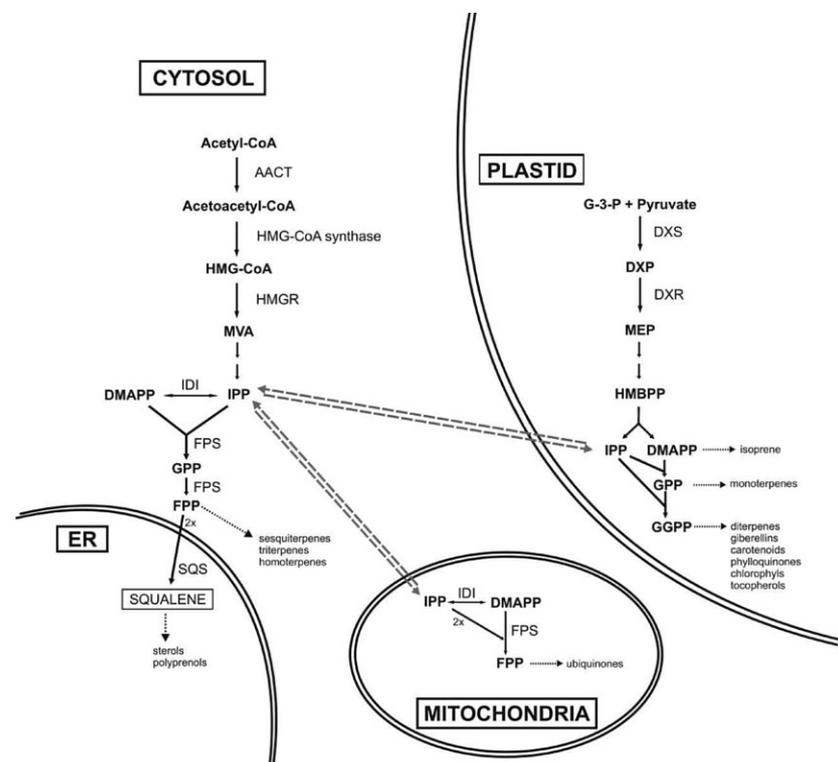

Figure 3. Squalene synthesis in plants via mevalonate (MVA) pathway in cytosol and methylerythritol phosphate (MEP) pathway in plastids. The product of MVA pathway, IPP, is further metabolized to FPP. FPP either forms sterols and polyprenols via squalene in the ER or is metabolized to sesquiterpenes, triterpenes and homoterpenes. In mitochondria, IPP condensates with DMAPP yielding ubiquinons. MEP pathway products are monoterpenes, diterpenes, tocopherols, carotenoids etc. in plastids. Updated and simplified from [299]. AACT, acetoacetyl-CoA thiolase; DMAPP, dimethylallyl diphosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; DXR, DXP reducto-isomerase; DXS, DXP synthase; FPP, farnesyl diphosphate; FPS, FPP synthase; G-3-P, glycerol-3-phosphate; GPP, geranyl diphosphate; GGPP, geranylgeranyl diphosphate; HMBPP, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; HMGS, HMG-CoA synthase; HMGR, HMG-CoA reductase; IDI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; MVA, mevalonate; SQS, squalene synthase; ER, endoplasmic reticulum.

The MVA pathway and squalene synthesis in plants are similar to vertebrates and fungi (see Fig. 3) with three important steps catalyzed by HMGR, farnesyl pyrophosphate synthase (FPS), and SQS. The number of genes encoding HMGR varies from two as described for *Arabidopsis thaliana* [75, 76] to at least eleven in potato [77, 78]. HMGR was found to be the crosstalk enzyme for sphingolipid and sterol biosynthesis [79]. An *hmg1* mutation in *Arabidopsis* exhibited dwarfism, early senescence, and male sterility. In contrast, *hmg2* had no visible phenotype [80] but complete deletion was lethal for male gametophytes [81]. As in mammalian cells, HMGR is controlled through feedback regulation in response to selective depletion of endogenous sterols [82]. In tobacco cells, up-regulation of HMGR led to overproduction of sterols [83], which were stored together with fatty acids in the form of steryl esters in lipid droplets, called sterolosomes. Overexpression of other enzymes of the pathway, e.g., FPS1S [84], did not exhibit any or only a minor effect on the total amount of sterols.

The MEP pathway starts with condensation of glyceraldehyde-3-phosphate and pyruvate to form DXP catalyzed by DXP synthase (DXS, formerly CLA1). DXP serves as a precursor in the thiamine and pyridoxol biosynthesis in bacteria and plastids. DXS is the feedback regulation point of this pathway [84, 85]. Deletions in this pathway resulted in seedling-lethal albino phenotype which was rescued by addition of 1-deoxy-D-xylulose, declaring the MEP pathway essential for plants [86–88]. Two additional DXS-like (DXL) genes were found in green siliques (*DXL1*, formerly *DXS2*) and roots (*DXL2*, formerly *DXS3*) to encode DXS. Since DXL genes did no rescue a *cla1/dxs-1* deletion they are most likely functionally unrelated to DXS. The other steps of the MEP pathway seem to be encoded by single genes, each. MEP is formed via reductive isomerization catalyzed by 1-deoxy-D-xylulose 5-phosphate reductoisomerase (DXR). MEP is further converted to IPP and DMAPP via several steps. It is noteworthy that almost all null mutants of this pathway exhibited the albino phenotype suggesting that chloroplast development of these mutants is arrested at early stages [86, 89–91].

Condensation of two molecules of IPP with DMAPP producing FPP is catalyzed by FPS. In plants, FPP serves as substrate for the synthesis of phytosterols, dolichols, ubiquinones, heme *a*, sesquiterpenoid, phytoalexins, or abscisic acid. *Arabidopsis thaliana* contains three FPS isoenzymes, namely mitochondrial *FPS1L*, and cytosolic *FPS1S* and *FPS2* [92, 93]. *FPS1L* and *FPS1S* differ only at the N-terminus. *FPS1S* and *FPS2* are differently expressed. While *FPS1S* is expressed in most plant organs and during the whole plant cycle, *FPS2* is strongly expressed during seed development [92, 94]. Single FPS mutations did not show any major effect because isoenzymes compensated for the defect. Lack of *FPS2* causes HMGR upregulation in seeds which compensates for the low expression of *FPS1* during seed development. The *fps1fps2* double mutant was viable,

but resulted in arrested embryo development at the preglobular stage [94]. The effect of FPS overexpression is not yet clear. Whereas overexpression of *FPS1S* in transgenic *Arabidopsis* did not exhibit any or only a minor effect on the total amount of sterols [95], overexpression of yeast *FPS1* in tobacco cells increased the amount of sterols [96]. Interestingly, overexpression of *FPS1S* in *Arabidopsis* showed other effects such as induction of a cell death/senescence-like response and reduction of the cytokinin level [95]

In the last step of plant squalene synthesis two molecules of FPP condense and form squalene via presqualene diphosphate catalyzed by *SQS* [97]. In *Arabidopsis thaliana*, *SQS1*, and *SQS2* encode two SQS, but only the gene product of *SQS1* shows enzymatic activity [98]. *SQS1* is expressed in all plant tissues and targeted to the ER membrane [99]. It can be specifically inhibited by squalestatin, also called zaragozic acid, which has frequently been used to investigate the isoprenoid pathway in plants. In the presence of zaragozic acid, FPP gets redirected toward the non-sterol isoprenoid pathway [100].

SQE catalyzes conversion of squalene to 2,3-oxidosqualene. In contrast to mammals and yeast, plants have multiple genes which were predicted to encode *SQE*. One of six putative *Arabidopsis SQE* genes, *SQE1*, is essential for normal plant development and regulates root and hypocotyl elongation [101, 102]. It is involved in drought tolerance and regulates the amount of ROS [102]. Mutants deleted of *SQE1* accumulate squalene, have elongation defects and are not able to create viable seeds. The gene product of *SQE2* produces primary 2,3-oxidosqualene, whereas gene products of *SQE1* and *SQE3* can also synthesize 2,3:22,23-dioxidosqualene [101]. Triterpenoid synthesis may also be associated with mitochondria, since *SQE2* and one putative SQE from rice have predicted mitochondrial targeting sequences. Moreover, *Arabidopsis FPS1* is a mitochondrial protein [93, 101]. *SQE4*, *SQE5*, and *SQE6* have specific although hypothetic functions, e.g., in plant defensive mechanism of rice [103, 104]. Inhibition of SQE with terbinafine leads to accumulation of squalene which is stored mostly in lipid droplets from where it can be mobilized when needed [82].

5 Squalene in microorganisms

Microbial squalene production has become a promising alternative to other sources of this lipid mentioned above. Although microorganisms do not accumulate as much squalene as plants or shark liver, their advantage is fast and massive growth. Squalene isolation from yeast [105], especially *Saccharomyces* [106–109], *Torulaspora delbrueckii* [110], *Pseudomonas* [111], *Candida* [112], the algae *Euglena* [113] and the microalgae *Traustochytrium* [110, 114], *Schizochytrium mangrovei* [115, 116], and *Botryococcus braunii* [117] has been reported.

5.1 Squalene synthesis in prokaryotes

The synthesis of squalene in bacteria differs depending on species [118]. The squalene precursors IPP and dimethylallyl diphosphate (DMAPP) are synthesized either via MVA, MEP, or both pathways [119]. The MEP pathway occurs mostly in eubacteria and cyanobacteria, whereas MVA was found in archaea and a few eubacteria [41]. Obligate parasitic eubacteria such as *Rickettsia* or *Mycoplasma* do not use any of these mechanisms and obtain their isoprenoids most likely from host cells [120, 121].

The eubacterial MEP pathway (Fig. 4) is similar to plants. Enzymes of the bacterial MEP pathway were identified and characterized [74, 89, 118, 119, 122, 123]. The pacemaker in this pathway is DXS which plays a limiting role in the isoprenoid pathway of prokaryotes [124–128]. Instead of glycerol-3-phosphate (G-3-P) and pyruvate, DXS can also utilize other substrates such as sugar phosphates and short aldehydes as acceptors, and the α-ketoacids hydroxypyruvate and α-oxobutyrate as donor substrates [129]. Recently, conversion of DXP to MEP catalyzed by a new family of DXR was reported in the bacterium *Brucella abortus* [130]. Some bacteria lack DXR, but have DRL (DXR-like) enzymes which perform the same reaction. In some bacteria both types of enzymes were detected [130].

Ershov et al. [131] reported that inhibition of DXR from cyanobacteria did not affect isoprenoid biosynthesis under photosynthetic conditions. These authors proposed alternative substrates from the pentose phosphate cycle which might enter the MEP pathway downstream of MEP. When isopentenyl diphosphate isomerase (IDI) type II was inactivated also DMAPP was synthesized through an alternative pathway [132] different from the typical MEP pathway found in *E. coli*. In contrast to plants, many bacteria harbor two types of IDI, namely IDI-type I and type II [133–135]. IDI-type I depends on divalent cations whereas IDI-type II requires metal ions, FMN, and NADPH under anaerobic conditions.

The MVA pathway is used by most archaebacteria, e.g., *Halobacterium cutirubrum* or *Caldariella acidophilus*, for the synthesis of their membrane ether-linked isoprenoid lipids [136, 137]. Three enzymes of the MVA pathway, namely HMGS, HMGR and mevalonate kinase were identified. Moreover, IDI type II was detected in archaea. In some *Streptomyces* species, the complete MEP and MVA pathways were found [138]. It was shown that the MEP pathway forms primary metabolites whereas the MVA pathway played a nonessential role in synthesizing secondary metabolites [139, 140]. Based on these observations and on results of lateral gene transfer it was concluded that the MEP pathway is older than the MVA pathway [123].

IPP and DMAPP formed in bacteria as described above are condensed to FPP, and FPP is then further metabolized to squalene by the action of SQS. The sequence of SQS from *Thermosynechococcus elongatus* BP-1 has only 30% similarity with eukaryotic SQS, but the isolated protein showed similar
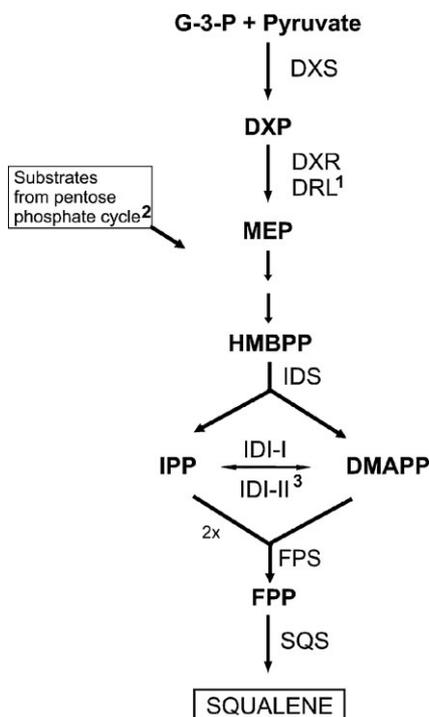

Figure 4. Squalene synthesized via methylerythritol phosphate (MEP) pathway in *E. coli*. DMAPP, dimethylallyl diphosphate; DXS, DXP synthase; DXR, DXP reductoisomerase; FPP, farnesyl diphosphate; FPS, FPP synthase; G-3-P, glycerol-3-phosphate; HMBPP, (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; IDI, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; SQS, squalene synthase. [1] DRL, DXR-like protein found in *B. abortus* [130]. [2] Cyanobacteria can utilize substrates from pentose phosphate cycle derived from photosynthesis [300]. [3] IPP isomerase type II was found in cyanobacteria [301] and in actinomycetes *Streptomyces sp.* (in MVA pathway) [133].

biochemical properties such as the same pH dependence, metal ion dependence, kinetic behavior, and inhibition by zaragozic acid [141].

In prokaryotes, the conversion of squalene to other compounds varies. In bacteria, a class of triterpenoids, the pentacyclic hopanoids, are formed as building blocks for membrane biogenesis [142, 143]. Hopanoids are most abundant in aerobic bacteria (cyanobacteria, methanotrophs, and heterotrophs) and in some anaerobic bacteria, but not in archaea. Hopanoids play a role in maintaining membrane integrity and permeability [144] and cope with external stress such as ethanol tolerance [145], oxygen diffusion [146], and prevention of water diffusion into spores [147]. Synthesis of hopanoids starts from squalene and is catalyzed by squalene-hopene cyclase. The existence of this reaction, however, does not exclude synthesis of steroids by S-2,3-oxidosqualene cyclase. Interestingly, both enzyme activities were demonstrated in *Methylococcus capsulatus* [148]. Bode et al. [149]

who screened for squalene and steroid production in my-xobacteria concluded that steroid patterns are species and strain specific and not affected by inhibitors of the steroid biosynthetic pathways of eukaryotes. Recently, Lamb et al. [150] showed the presence of a post-squalene lanosterol biosynthetic enzyme complex acting as a component of the prokaryotic sterol biosynthesis pathway. Thus, some bacteria may indeed have the ability to synthesize sterols. These results could be a key to understand the phylogenetic development of sterol and steroid synthesis.

Interestingly, squalene can be used as a carbon source by some bacteria such as *Corynebacterium sp.* [151, 152], *Corynebacterium terpenotabidum sp. nov.* [153], *Rhodococcus sp.* [154], *Pseudomonas sp.* [155], or *Arthrobacter sp.* [156, 157]. Mechanisms involved in this process appear to include (i) oxidation of the terminal methyl groups and formation of the corresponding α,ω-diodic acid [152], (ii) hydration of the double bonds resulting in tertiary alcohols [151], or (iii) cleavage of internal double bonds catalyzed by an oxygenase leading to geranylacetone and 5,9,13-trimethyltetradec-4E,8E,12-trienoic acid [156, 157]. *Marinobacter sp.* (2sq31) is able to degrade squalene under aerobic and anaerobic conditions [158]. The proposed model for the anaerobic process is hydration of squalene to methyl ketones and alcohols, which are carboxylated to isoprenoid acids and further metabolized via β-oxidation and β-decarboxymethylation [155, 159–161]. Aerobic degradation starts with cleavage of C10/C11 or C14/C15 double bonds in addition to steps of the anaerobic pathway [156].

Recently, studies to improve squalene production in *E. coli* were published. Ghimire et al. [162] introduced and overexpressed the putative genes of hopanoid synthesis, *hopA*, *hopB* (encoding squalene/phytoenol synthase), and *hopD* (encoding farnesyl diphosphate synthase) from *Streptomyces peucetius* in *E. coli*. The yield of squalene was increased from ∼4 to ∼12 mg/L when genes encoding deoxyxylulose phosphate synthase and IPP isomerase were also expressed.

5.2 Squalene synthesis in yeast

Many studies defining the role of sterols in eukaryotic cells were performed with the yeast as a eukaryotic model system. Effects of sterols on membrane fluidity [163], membrane permeability [164, 165], energy source utilization [166], and the activity of membrane-bound ATPase [167] were investigated using yeast mutants bearing defects in the ergosterol biosynthetic pathway. As in other eukaryotic cells, the formation of sterols in yeast can be divided into two parts. The first part named mevalonate or isoprenoid pathway (Fig. 5) starts with acetyl-CoA and leads to the formation of farnesyl pyrophosphate (FPP) which is used as a substrate for further biosynthetic routes, e.g., synthesis of heme [168], quinones [169], and dolichols [170]. Mutations affecting these steps of the sterol biosynthetic pathway are lethal.
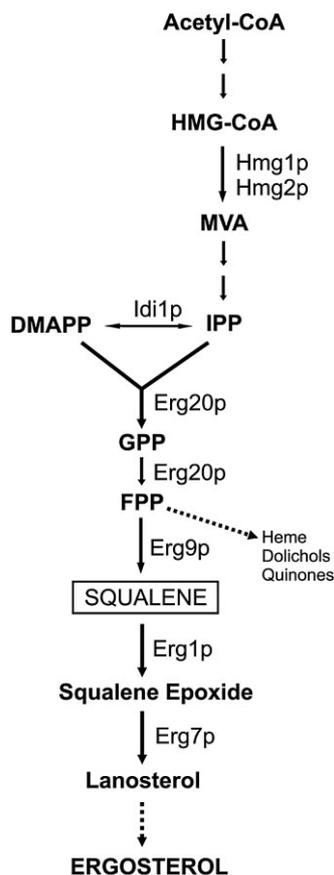

Figure 5. Squalene synthesis in yeast. DMAPP, dimethylallyl diphosphate; Erg1p, SQE; Erg7p, lanosterol synthase; Erg9p, SQS; Erg20p, FPP synthase; FPP, farnesyl diphosphate; GPP, geranyl diphosphate; HMG-CoA, 3-hydroxy-3-methyl-glutaryl-CoA; Hmg1p, Hmg2p, HMG-CoA reductase; Idi1p, isopentenyl diphosphate isomerase; IPP, isopentenyl diphosphate; MVA, mevalonate.

The best-studied enzyme of the yeast MVA pathway is HMGR, the first control point of regulation. Actually, yeast cells harbor two HMGR enzymes encoded by *HMG1* and *HMG2*, respectively [171]. It has been shown that overexpression of truncated *HMG1* leads to an approximately 40-fold increase of HMG-CoA reductase (HMGR) activity, higher yield of the dry matter and accumulation of squalene [172]. The enzyme shows feedback inhibition similar to animal and plant cells in the presence of ergosterol [173] and is subject to catabolic repression [174]. Recently, Garza et al. [175] reported that stability of Hmg2p is regulated by geranylgeranyl diphosphate. Although Hmg1p and Hmg2p are similar in function, regulation of their expression is different. Thorsness et al. [176] reported that expression of *HMG1* was stimulated whereas expression of *HMG2* was repressed by heme. Deleting *HMG1* a *HMG2* rendered yeast cells non-viable in the absence of mevalonate feeding because they could not form mevalonate [171]. In contrast to

Saccharomyces cerevisiae, *Schizosaccharomyces pombe* contains only one HMGR gene [177].

Conversion of FPP to the end-product of the pathway, the yeast specific ergosterol includes eleven reactions. The three initial steps are essential and well characterized points of regulation. Fusion of two FPP molecules yielding one molecule of squalene is catalyzed by the essential SQS Erg9p [178]. Similar to HMGR, *ERG9* is subject to transcriptional regulation [179]. In the yeast, squalene does not accumulate within the cell under normal growth conditions because it is efficiently converted to ergosterol. In wild type, a minor amount of squalene was detected in lipid droplets together with TAGs and steryl esters [22]. When squalene accumulates under anaerobiosis or in *hem1* mutant cells, over 70% of its cellular amount is accumulated in lipid droplets. Small amounts of squalene were also found in membranes [21, 180]. It was shown that squalene accumulation did not cause a lipotoxic effect [21].

In the ergosterol biosynthetic pathway, squalene formed through reactions described above is further converted to squalene epoxide by the SQE Erg1p [181]). This step requires oxygen making ergosterol synthesis strictly aerobic [182]. Erg1p is dually localized in the yeast, namely to the ER and lipid particles/droplets [183]. In vitro, only SQE from the ER but not from isolated lipid droplets is enzymatically active [183]. A reductase required for this reaction and localized exclusively to the ER may be responsible for this effect. The subsequent step of ergosterol synthesis is cyclization of 2,3-oxidosqualene and formation of the first sterol, lanosterol. This reaction is catalyzed by lanosterol synthase (oxidosqualene cyclase) encoded by the *ERG7* gene [184, 185]. The remaining steps of the ergosterol biosynthetic pathway include modifications of the sterol ring system and of the side chain as summarized in various review articles [186–188].

Several biosynthetic pathways of the yeast including synthesis of heme, sterols, and unsaturated fatty acids require molecular oxygen [189–192]. Therefore, ergosterol and unsaturated fatty acids are required as supplements to yeast cultures grown anaerobically [191, 192]. Under these conditions, squalene accumulates at a maximum yield of ~41 mg/kg dry weight (DW) [110, 114]. Variation of culture conditions and inoculum size further increases the yield of squalene [193]. It has to be taken into account, however, that strictly anaerobic cultivation of yeast is difficult. Jahnke and Klein [182] observed that SQE (Erg1p) activity increased to almost half of its maximal value in anaerobic yeast cells after adding as little as 0.03% oxygen and rapid synthesis of ergosterol from squalene occurred. This problem may be overcome by deletions of *ERG1* or *ERG7*.

Squalene also accumulates under heme-deficiency [21]. Blocking the synthesis of heme in the yeast leads to accumulation of lanosterol since sterol-14-α-demethylase (Erg11p) is the first NADPH-heme-dependent cytochrome P450 protein in the ergosterol biosynthetic pathway [194]. At the same time, however, squalene accumulates at substantial amounts. It has to be noted that only under anaerobiosis, in heme-deficient strain and sterol uptake mutants ergosterol can be properly incorporated into the yeast [195, 196].

Under aerobic conditions, squalene accumulation can also be achieved by increasing the flux through the early part of the MVA pathway, e.g., by increasing the activity of HMGR [172, 197]. Temperature shift is another possibility to accumulate squalene in the yeast. Loertscher et al. [198] showed that an *hmg1* mutant grown at 16°C produced approximately four times more squalene than cells grown at 30°C. Another experimental strategy to increase squalene was reported by Mantzouridou and Tsimidou [199, 200]. These authors showed that the stable Hmg2p induced a strong increase in squalene (18.5 mg/g) and a smaller increase in lanosterol under semi-anaerobic conditions. Combined Hmg2p stabilization and *ERG6* deletion did not further enhance squalene production, since lack of ergosterol feedback inhibition led to an elevated transfer of surplus squalene into C27 sterols.

5.3 Squalene synthesis in microalgae

Another microbial source for the production of squalene are microalgae, which are microscopic algae, typically found in fresh water and marine systems [201]. They are widely used for the production of various compounds such as polysaccharides, proteins and carotenoids, or as a source for renewable energy due to their ability to transform sewage and waste into valuable biomass. Typical representatives of this family of microorganisms are *Scenedesmus obliquus*, *Chlamydomonas reinhardtii*, *Chlorella fusca*, and *Botryococcus braunii* which belong to the group of photosynthetic algae. Some microalgae can also accumulate reasonable amounts of squalene [115]. Traustochytrid *Aurantiochytrium sp.* (formerly known as *Schizochytrium*) is an efficient producer of squalene. This microorganism grows rapidly and produces large amounts of squalene under heterotrophic conditions, because it lacks the photosynthetic apparatus for carbon fixation [202, 203]. This property eliminated the usual problem of microalgae, the light limitation in closed culture systems. Optimization of culture ingredients led to increase of content and yield of squalene [204]. Jiang et al. [115] reported that the squalene level also depends on the cultivation time. While the squalene level reached ~0.16 mg/g dry weight after 3 days, only ~0.04 mg/g dry weight were found after 5 days in cultures of *Schizochytrium mangrovei*. Treatment of cells with the inhibitor terbinafine led to a squalene content of ~0.5 mg/g biomass [205]. *Botryococcus braunii* was also found to accumulate squalene, but this microorganism did not grow well under heterotrophic conditions making this system less attractive for biotechnological production [117]. Chen et al. [202, 204] optimized the nitrogen source which enhanced squalene production in *Aurantiochytrium sp.*
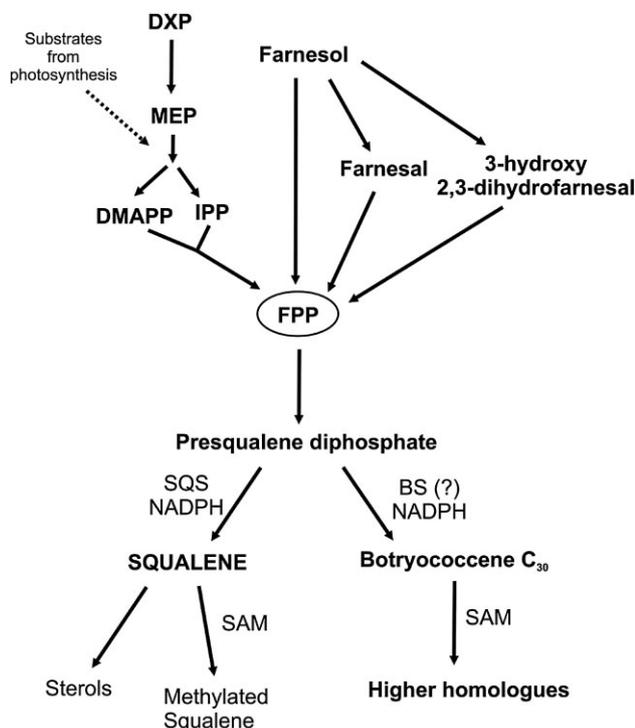
Figure 6. Squalene synthesized via methylerythritol phosphate (MEP) pathway in green algae *B. braunii*. IPP and DMAPP are synthesized via MEP pathway. Substrates derived from photosynthesis also might contribute to produce IPP. Condensation of IPP and DMAPP yields FPP. Other substrates for FPP can be farnesol or its derivates farnesal and 3-hydroxy 2,3-dihydrofarnesal. FPP is then condensed into presqualene diphosphate. SQS creates linkage 1′-1 of two farnesyl moieties leading to squalene, while botryococcene synthase (BS) creates linkage 1′-3 yielding botryococcene [214]. Squalene and botryococcenes can then be methylated by non-specific methylases of S-Adenosylmethionine (SAM). BS, botryococcene synthase; DMAPP, dimethylallyl diphosphate; FPP, farnesyl diphosphate; DXP, 1-deoxy-D-xylulose-5-phosphate; DMAPP, dimethylallyl diphosphate; IPP, isopentenyl diphosphate; MEP, 2-C-methyl-D-erythritol 4-phosphate; NADPH, nicotinamide adenine dinucleotide phosphate; SQS, squalene synthase; SAM, S-adenosylmethionine.

Botryococcus braunii 3 classes A, B, and L produce different types of hydrocarbons [206]. Type A forms hydrocarbons C_{25} to C_{31}, odd-numbered n-alkadienes, and alkatrienes. Type B synthesizes triterpenoids such as methylated squalene and hydrocarbons botryococcenes, whereas type L produces only lycopadiene $C_{40}H_{78}$ [207]. In contrast to fungi, IPP of microalgae is synthesized through the MEP pathway (Fig. 6) [208] or from substrates formed by photosynthetic reactions [209]. Condensation of two IPP and one DMAPP molecules yields FPP. Interestingly, farnesol or its derivatives farnesal and 3-hydroxy 2,3-dihydrofarnesal can also serve as substrates for FPP synthesis [210, 211]. Condensation of two molecules FPP yields presqualene

diphosphate. Cleavage of the rearranged cyclopropane catalyzed by SQS leads to squalene, but direct cleavage of the cyclopropane ring yields polyunsaturated C_{30}-C_{37} triterpenoid hydrocarbons termed botryococcenes. A SQS has been identified, but it is still unclear whether this enzyme catalyzes either squalene or botryococcene synthesis, or two individual enzymes are responsible for each reaction [212, 213]. C_{30} Botryococcenes are further methylated to higher homologs. The portion of squalene which is not used for sterol synthesis is often methylated by non-specific methylases and stored together with other hydrocarbons [214]. Methylated squalene can be further metabolized to produce botryoxanthins [215], braunixanthins [216], and tetramethylsqualene epoxides [217].

6 Process biotechnology of squalene production

Isolation of squalene is carried out preferentially from plant or animal sources as starting material. Due to environmental concerns squalene production from shark liver oil has recently been challenged, and other sources such as plants and microorganisms became more important. Extraction of squalene from olive oil deodorization distillate (OODD) became very popular because the squalene concentration in this source is high and reaches 10–30%. Alternatively, amaranth grains [218], leaves of the tree *Terminalia catappa* [219] or lotus (*Nelumbo nucifera* Gaertn) bee pollen [220, 221] are used as a source of squalene. Attempts of large scale production of squalene from microorganism and algae are still in their infancy.

Squalene is thermolabile due to its unsaturated linear chain. Thus, distillation, e.g., from vegetable oils is not a suitable process for separation and isolation. Moreover, thermal degradation of other compounds from oils such as TAGs may occur as well. There are, however, alternative strategies to isolate squalene from natural sources, such as solvent extraction or supercritical fluid extraction (SFE). Solvent extraction is a most efficient process, and squalene as a non-polar lipid can be extracted using organic solvents like hexane. This method, however, is largely restricted to research laboratories due to regulatory, financial, and safety concerns (toxicity and flammability). The method used more frequently in industry is short-path distillation, a high vacuum process. Condensers are positioned close to the evaporator surface, and the feed liquid flows on the evaporator surface as a thin falling film. Different temperatures and vacuum settings can be used to distill the compound which is required [222]. The third extraction method, SFE, is most preferred in industry. As the usual solvent, supercritical carbon dioxide (SC-CO_2) is used because of its inertness, non-toxicity, high volatility, and low cost. CO_2 gets fluid when it reaches a temperature of 31.1°C at a pressure of 7.38 MPa. Due to the near-ambient critical temperature of CO_2, SC-CO_2 is suitable for extraction of thermolabile natural prod-

ucts. This extraction method has several advantages such as high purity of the product and combination of extraction and concentration of components in one step. This process is not very expensive and yields squalene at natural quality without usage of solvents. The efficiency of this extraction method can be improved by modification of conditions. As an example, 10–15% ethanol can be used as a co-solvent to increase the yield and to extract also polar components. Disadvantages of this method, however, are complexity of equipment, precise maintenance of high pressure (∼10 MPa), and presence of solvent residues in extracted samples.

As mentioned above, squalene is mostly produced from its most abundant source, the shark liver oil [223]. The critical point and the limitation of this process is the presence of environmental pollutants such as PCB, dioxins, and heavy metals in shark liver [224, 225]. Moreover, shark liver oil contains 0.1% pristane, low volatile TAGs, and glyceryl ethers. Especially removal of pristane ($C_{19}H_{40}$) is needed since it is a skin irritant. Under optimum processing conditions (25 MPa and 60°C) squalene can be obtained at 95% purity by weight without using reflux and at 99% purity with reflux [34]. Recent investigations using short-path distillation led even to the isolation of odor-free 97% pure squalene [226].

Olive oil deodorization distillate residues (OODD) are by-products of the oil refining process and contain 10–30% squalene and 30% free fatty acids (FFA) by weight as well as smaller amounts of sterols and tocopherols. Usually, these samples also contain olive oil neutralization by-products and hence have a low market value. The technical problem of counter-current packed column extraction with SC-CO2 is separation of squalene from FFA because of very similar solubility in SC-CO2. Thus, additional steps are needed for the purification of squalene. Ruivo et al. [227] studied this problem with model mixtures of squalene and oleic acid by introducing nanofiltration with various membranes taking into account the different molecular weights of squalene and oleic acid. The idea was that FFA as smaller molecules with higher diffusivity in SC-CO2 would permeate membranes and squalene would concentrate in the retentate. Surprisingly, the opposite effect was observed and squalene permeated membranes better than oleic acid. This finding was explained by specific interactions of the permeating molecules and the active layer of the membrane. The highest selectivity was obtained using polydimethyl siloxane and polyamide AD membranes, but the former membrane material showed a low efflux [227]. Another strategy was used by Bondioli et al. [228] who separated squalene from glyceride and non-glyceride substances found in olive oil. FFA, fatty acid methyl and ethyl esters were converted to their corresponding TAGs prior to SFE and then easily separated as suggested for TAG/oleic acid mixture in pilot experiments [229]. This separation process which required zinc for catalytic esterification and high pressure yielded highly enriched squalene.

An alternative to counter-current packed column separation is static mixers. As an example, static mixers are used for the removal of caffeine from SC-CO2 by water after SFE decaffeination [230]. Low costs, short residence times, and minimal space requirements compared to the packed column led Catchpole et al. [231] to focus on supercritical extraction of lipids in a static mixer at laboratory and pilot-scale. Although the separation factor of squalene and other major components did not reach values achieved in packed column for mixtures which are easy to fractionate such as shark liver oil, the separation efficiency was similar in static mixer and in packed column with mixtures difficult to be fractionate.

Another plant source used for squalene isolation is amaranth seed oil (*Amaranthus cruneus*). Oil from amaranth grains contains 6–8% of squalene [218, 232, 233]. As an isolation method short-path distillation was employed (180°C, 3 mtorr vacuum) resulting in 76% recovery of squalene in the distillate [234]. Squalene was also found in leaves but not in seeds of *Terminalia catappa*, a tropical and sub-tropical tree used in folk medicine for its antipyretic and hemostatic properties and prevention of hepatitis and hepatoma [235, 236]. The squalene content in leaves increases during maturation. Using SFE a squalene yield of ∼12 mg/g and a content in extracts of ∼12% were obtained [219].

The deodorization distillate of rice bran oil contains 8% squalene as another possible source of this lipid. Several isolation methods for squalene or squalane from such deodorization distillates include saponification, solvent fractionation, distillation, hydrogenation, and finally molecular distillation [237–239]. Sugihara et al. [240] reported recently a new fractionation method of squalene and phytosterols which is based on a combination of solvent fractionation and supercritical fluid chromatography using silica gel after SFE of the deodorization distillate. This method had many advantages such as fewer operation steps, time-saving, no oxidative rancidity, and continuous production of the two functional components.

Soybean oil deodorizer distillate residues do not contain much squalene but can still be used as a reasonable source. This material contains 3.5% squalene, 13-14% tocopherols (vitamin E), 26% sterols, FFA, TAGs, DAGs, and MAGs [241]. Wang et al. [242] reported an improved isolation procedure of squalene by introducing an additional step of pressure swing adsorption in SC-CO2. Pressure swing adsorption is based on the fact that fluids tend to be adsorbed to solid surfaces under pressure. α-Tocopherol was selectively adsorbed on an octadecylsilica adsorbent at high pressure and squalene was collected at high purity. α-Tocopherol was then eluted in the desorption step by reducing the pressure.

Production of squalene from microbial sources is still under investigation. As mentioned before, microorganisms have a great potential to become reasonable sources for squalene isolation. Advantages as rapid and massive growth, however, still do not compensate for the low yield of squalene.

Current investigations are focused on improving the content of squalene in microorganism. *S. cerevisiae* as one of the best-studied eukaryotic organisms produces squalene only at low yield. Genetic manipulations (see previous Sections) may be a strategy for improvement. Other microorganisms showed a more promising accumulation of squalene. *Torulaspora delbrueckii* isolated from molasses accumulated ~240 mg squalene/kg dry weight [110], and *Pseudozyma sp.* produced up to ~5 g/L biomass and ~340 mg/L squalene [243]. Some marine bacteria such as *Rubritalea squalenifaciens* sp. nov. [244], *R. sabuli* sp.nov. [245], *R. spongiae* [246], and *R. tangerine* [246] were also found to accumulate squalene, but are not yet used for large scale isolation of this lipid.

7 Applications of squalene

Applications of squalene have been recently reviewed in some detail [5, 32, 247]. In the following section, we will address some of these applications focusing on the use of squalene for therapeutic, pharmacological, and cosmetic purposes.

7.1 Role of squalene as antioxidant

Previous studies have shown that squalene can act as a highly efficient singlet oxygen scavenging agent [2, 248]. Therefore, squalene was considered to exhibit antioxidant properties. Since oxidative stress and increase in ROS may induce cancer [249], squalene was also regarded as a potential anti-cancer component [5]. However, the scavenging capacity of squalene has not been studied in much detail and was challenged by recent studies. Warleta et al. [250] reported that antioxidant activity of squalene against 2,2-diphenyl-1-picrylhydrazil stable radicals, 2,2′-azino-bis(3-ethylbenzthiazoline-6-sulphonic acid) cation radicals, or 2,2′-azobis (2-methylpropinamid) dihydrochloride-induced peroxyl radicals was not observed even at high concentration. Similar observations were published before [16, 251] concluding that squalene antioxidant activity is extremely low. Conforti et al. [252], however, described an antioxidant effect of squalene with an IC_{50} value of 0.023 mg/mL. Squalene reduced the rate of oxidation in a crocin bleaching assay where it might act as a competitive compound to tocopherol and sitosterol [253]. A weak antioxidant activity of squalene was also observed in olive oil [251], which may, however, be due to the competitive oxidation of the various other lipids present in such samples. Dessi et al. [114] studied the effect of squalene on the oxidative stability of PUFA and reported antioxidant properties of squalene as a peroxyl radical scavenger in mild UVA-mediated PUFA oxidation. Combination of PUFA and squalene led to decreased lipid peroxidation in heart tissue of rats [254].

Interestingly, different antioxidant effects of squalene were observed in different types of cells. Squalene showed antioxidant activity in vitro only in mammary epithelial and bone marrow cells, but not in human breast cancer and neuroblastoma cells [250, 255], although reference antioxidants were efficient in all these cells. Warleta et al. [250] concluded that the squalene antioxidant selectivity depends either (i) on the ''glutathione paradox'', where squalene increases the amount of glutathione in normal cells [256]; (ii) on differences in squalene uptake, utilization, and accumulation [255]; or (iii) on deregulation of antioxidant systems in tumor cells [257]. Thus, squalene may act rather on prevention than direct treatment of cancer.

7.2 Effects of squalene as a dietary supplement

Consumption of squalene, which is an intermediate in sterol biosynthesis, did not increase the amount of cholesterol in human serum [6, 31, 258]. Even high daily squalene consumption did not enhance the cholesterol level, although squalene uptake was efficient and squalene levels in the serum were high [31]. A diet containing 850 mg squalene per day for 20 wk rather decreased levels of total cholesterol for approximately 17%, LDL- cholesterol for 22% and TAG for 5% in patients suffering from hypercholesterolemia [258].

Hyperleptinemia (elevated plasma leptin levels) is considered as a high risk factor of obesity and hypertension [259–261]. Squalene was suggested to be a possible component for the treatment of cardiovascular diseases, because it reduces the blood levels of cholesterol and TAG and decreases plasma leptin [262–264]. Moreover, high dosage squalene treatment decreased body fat and blood level of glucose in dogs and rats [265–267]. Mechanisms of these squalene effects are still not clear. It could either be a direct effect of squalene or indirect assistance through lowering TAGs and thus enhancing body sensitivity to leptin [262, 267]. Squalene was also shown to increase testicular weights and testosterone levels in dogs and rats [265–267], improved the reproductive performance of meat-type male chicken and increased the serum testosterone level and semen collection volume [268]. Squalene did not affect the egg fertile rate in an artificial insemination model but increased this rate in a natural mating model. Finally, Motawi et al. [269] studied the role of squalene on oxidative cardiac, urotoxic, and testicular damage induced by cyclophosphamid in male Wistar rats. These authors found that squalene treatment had a cytoprotective effect and attenuated cyclophosphamid-induced pathological alterations.

High dose of squalene (>13.5 g/day) significantly decreased wrinkles in aged human skin, increased type I procollagen and decreased UV-induced DNA damage in vivo but was associated with transient adverse effects such as loose stool [270]. Squalene exhibited antitumor activity against colon, skin, sarcoma, and lung cancer in rodents [6, 33, 271, 272]. As an example, olive oil consumption decreased incidence of breast cancer [66, 273], but squalene did not induce death of breast tumor cells and thus may be ineffective once breast cancer has established [250]. The mixture of lipid components in olive oil appears to contribute more to prevention than treatment as well [5, 65, 66]. Decreased risk of

breast, skin, and colon cancer [66, 274], a chemo- and cardio-protective effect [5, 6, 66], an antihypertensive effect [275, 276], anti-inflammatory action [277], and prevention of atherosclerotic plaque formation [278] were reported. The protective effect depends on the amount and time period of olive oil consumption. Interestingly, annual olive oil consumption per person can be up to 15 kg [66].

The mechanism proposed for the antitumor effect of squalene is inhibition of HMGR catalytic activity. It has been shown that squalene feeding in rats (1% in the diet for 5 days) inhibited HMGR activity (about 80%) in hepatic microsomes [279]. Inhibition of HMGR leads to inhibition of other intermediates of the cholesterol pathway, such as mevalonate or FPP. This affects farnesylation of oncogene Ras p21, signal transduction, and cellular proliferation [5]. A novel facet of squalene antitumor activity has been suggested by Newmark and collaborators [6] based on results presented by Strandberg et al. [279]. These authors showed that feeding of squalene resulted in a 20-fold increase in the serum and a 30-fold total increase of methyl sterols including lanosterol, 14-desmethyl lanosterol, and 14-monomethylated sterols. Katdare et al. [280] tested lanosterol and other metabolites of squalene as potential antitumor inhibitors. They concluded that squalene metabolites or precursor substances for posttranslational modifications of Ras p21 oncogenes showed stronger chemopreventive effect than squalene itself.

7.3 Use of squalene in human medical treatment

Squalene has been frequently used as an additive to lipid emulsions as drug carrier in pharmaceutical and vaccine applications (for reviews see [3, 4, 247]). Such emulsions are able to incorporate poorly soluble drugs within their dispersal phase, which is beneficial for increased drug and vaccine uptake, minimizing side effects through contact of drug and body fluid, decreasing the release of the drugs and other multiple adjuvant effects [4]. Squalene and squalane form very stable and viscose emulsions to solubilize lipophilic drugs, adjuvants, and vaccines with highly potent transfection activity [281, 282] and small droplet size [283]. The effect of some of these emulsions, e.g., SAF, MF59®, DETOX®, and PROVAX®, as vaccine adjuvant have been well studied [4]. As an example, squalene together with the detergents Tween® 80 and Span® 85 forms the adjuvant MF59® (Novartis), an oil-in-water microemulsion approved for human use [284, 285]. MF59® has been shown to be a potent and safe adjuvant with several vaccines, e.g., against hepatitis B and C, herpes simplex virus, HIV-1, and influenza (vaccine Fluad®).

However, utilization of squalene for vaccination is still a matter of dispute [286]. After the Gulf war, veterans showed multiply syndromes, such as rashes, headaches, arthralgias, memory loss, increased allergies, sensitivities, and neurological abnormalities [287]. Squalene was suggested to be a cause of these Gulf war symptoms since it was added as adjuvant to

anthrax vaccine [288]. Anti-squalene antibodies were found in gulf war syndrome-like patients [289]. Later, it was concluded that anti-squalene antibodies occur naturally in humans, non-correlated with anthrax vaccination [290]. MF59 emulsion adjuvant in vaccines did neither induce the level of anti-squalene antibodies nor enhanced the titers of pre-existing anti-squalene antibodies [291].

Recently, a prodrug strategy for improved delivery of nucleotide analogs became a research focus. Nucleotide analogs act a potent inhibitors of DNA synthesis and have been used as antiviral and anticancer therapeutics [292, 293]. Transport of these drugs into the cell is limited due to high hydrophobicity or poor in vivo stability. Squalenoylation of the nucleotide analogs may help to overcome slow diffusion. Conjugation of squalene to the drugs created nanoassembly without using surfactants [294]. As an example, interaction of the lipophilic prodrug gemcitabine-squalene with biomembranes was improved compared to free gemcitabine [11, 12]. Sarpietro et al. [295] studied the prodrug combination of squalene-acyclovir. The absorption to artificial membranes was improved and it was suggested that the squalene moiety stays in the membrane environment whereas the acyclovir moiety protrudes into the aqueous phase with only a small contribution of the prodrug to the phospholipid thermotropic behavior. Recently, a new strategy of squalenoylation was reported [10]. After entering the cell, nucleotide analogs get activated by phosphorylation yielding nucleoside-monophosphate. This step, however, is rate limiting because nucleotide analogs are poor substrates for cellular kinases. Therefore, it was suggested to use nucleoside monophosphate as drugs. Unfortunately, negatively charged phosphate groups of nucleoside monophosphates were not able to enter the cell [296]. To solve this problem, squalenoylation of the phosphate moiety was performed creating an amphiphilic molecule which self-assembled in water in the form of particles of 100–300 nm size. Assembling might be caused by compact and highly coiled conformation of the squalene moiety in the aqueous environment [297]. Such particles are able to enter the cell [10]. As an example, nanoassemblies of 4-(N)-1,1′,2-trisnor-squalenoyldideoxycytidine monophosphate showed improved anti-HIV activity, and 4-(N)-1,1′,2-trisnor-squalenoylgemcitabine monophosphate improved anticancer activity on leukemia cells compared to non-squalenoylated analogs [10, 294, 298].

8 Summary and conclusions

In this review article, we summarized our recent knowledge about squalene, an isoprenoid lipid and intermediate of sterol synthesis. Synthesis of squalene is slightly different in microorganisms, plants, and mammalian cells. Also further metabolic conversion of squalene varies in different cell system. In this article, we addressed biochemical and biophysical properties of squalene which are unique and mainly due to its highly hydrophobic structure. Cell biological effects of

squalene appear to be not dramatic, although this lipid may act as a mild modulator of membrane stability. These largely inert properties of squalene, however, may be regarded as beneficial for usage in nutrition, pharmacy, cosmetics, and medicine.

For reasons described above, different sources of squalene and processes to isolate this compound became of interest. It appears that besides shark liver oil olive oil deodorizer distillate will be used in the future as most efficient source of squalene. The advantage of olive oil deodorizer distillate is that it contains a relatively high amount of squalene and is a ''waste'' of olive oil raffination. Nevertheless, other sources of squalene such as microorganisms may also become important. For large scale isolation of squalane, various methods of process biotechnology were developed and applied. The challenge for these processes is efficiency, especially when samples with low squalene concentration have to be used.

Recent developments showed that squalene can became a useful component in nutrition, health care, and cosmetics. As a biological supplement to the diet and as an additive to drugs it appears to have beneficial properties. In summary, squalene can be regarded as a versatile molecule which may become even more useful for applications in the future.

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