



Review Article

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Main Mechanisms of Action of Policosanol in Animal and Plant Cells

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ABSTRACT

Policosanol is a promising compound that can be used in various industries. In the pharmaceutical industry, policosanol and, specifically, triacontanol, octacosanol, and hexacosanol have presented biological activity against several diseases, especially those related to inflammation and hypercholesterolaemia. On the other hand, triacontanol is used as a growth promoter in a wide variety of plants and crops of economic importance, even in microalgal culture, in either its pure form or in policosanol extracts. In this review, the most relevant references to the bioactivities of policosanol in plants and animal cells are collected to compare the different mechanisms of action. Analyzing this in detail allows us to ask new research questions. The PubMed and Redalyc database were used for article search under the following keys terms: policosanol, inflammatory mechanisms, triacontanol, cellular absorption, photosynthesis and photoinhibition. Policosanol interferes with the progression of the NF- κ B and MAPK signaling pathway involved in the inflammation process. The cholesterol-lowering effect of policosanol is due to the inhibition of cholesterol synthesis in the liver, through the indirect inactivation of HMG-CoA reductase. Triacontanol improves the growth and the biochemical and physiological parameters of plants and the response to stress conditions that is related mainly to photosynthetic activity. Interestingly, octacosanol demonstrates an inhibitory activity on the effect of triacontanol in plants, a response not reported in human cells, as well as other differential aspects of the mechanisms of action in the cells of plants and animals that are interesting to analyze.

Key words: Long chain alcohols, Action mechanisms, Pharmacological activity, Growth promoter

INTRODUCTION

In general, Policosanol (POL) is a mixture of Long-chain Alcohols (LCAs) of 16 or more carbon atoms, of high molecular weight and containing an oxhydryl group. These compounds are highly insoluble substances in aqueous media at room temperature and are solid and hard, characterized by their non-polar nature and very high boiling points. Numerous beneficial properties have been reported, both to human health and as a growth promoter in plants and cyanobacteria, for POL derived from different waxy sources [1-3]. Pharmacological studies have been performed with mixtures of LCAs that form the POL mix; however, every component shows special characteristics that are beneficial for different diseases and disorders of human health [4-6]. Some of these compounds, such as Triacontanol (TRIA), Octacosanol (OCTA), and Hexacosanol (HEXA), have been evaluated in *in vitro* and *in vivo* systems [5, 6], and multiple efforts have been made to determine their mechanisms of action, metabolism, and interactions with other drugs [4, 5]. Besides, new strategies for the formulation of POL have been developed to overcome the obstacles that have arisen in evaluating this type of lipophilic compound [7] without diminishing its effectiveness, utilizing the synthesis of prodrugs [6, 8, 9].

Another important aspect is the use of POL as a growth promoter in plants and cyanobacteria, where TRIA is the only LCA that has shown this activity [1]. The mechanism of action of TRIA as a plant growth promoter and its effects in combination with other stimulants such as phytohormones and biofertilisers are under investigation [10, 11].

As a result of the above, the objective of this review is to analyze the mechanisms of action of POL, to point out the differences and similarities in their effects in both animal and plant cells that will help to propose new studies for the understanding of these mechanisms.

MATERIALS AND METHODS

This review collects the most relevant references about several studies that prove certain bioactivities of policosanol and their long chain alcohols that composed both in the pharmacological and in the agronomic area. PubMed and Redalyc database were used for article search under the following key terms: policosanol, inflammatory mechanisms, triacontanol, cellular absorption, photosynthesis and photoinhibition.

RESULTS AND DISCUSSION

Cellular uptake of pol in mammalian cells

Several studies consider POL to have promising biological functions; however, some reports have shown that individual compounds can also exert some pharmacological effects against several diseases, such as hypertension, cancer, hyperlipidaemia, Parkinson's disease, bacterial infections, gastrointestinal ulcers, and alopecia, among others [3].

Some of these compounds, such as TRIA, OCTA, and HEXA, have been evaluated to understand their mechanisms of action, and it has been proposed that their metabolic profile might be similar due to their similar structures [12]. Studies conducted with OCTA have suggested that a part of the OCTA is catabolised and converted to fatty acids or chain-shortened fatty acids as a result of β -oxidation in peroxisomes [12, 13].

Regarding absorption, previous studies demonstrated that enterocytes are capable of absorbing POL; however, scarce data about how POL enter the cells have been published, and it has been suggested that how they enter cells could be very similar to the mechanisms of entry of fatty acids, due to their similar structures and lipophilicity [13]. Fatty acids enter enterocytes by two mechanisms: passive diffusion and carrier-mediated transport [14]. Regarding drug transport, Cocucci *et al.* reported that some small therapeutic molecules, lipophilic compounds, or proteins can diffuse freely through the plasma membrane, depending on their lipophilicity [15, 16]. Passive diffusion is one of the most important mechanisms for intracellular drug entry, especially in enterocytes, notwithstanding, in the liver, kidney, and brain, carrier-mediated transport is the dominant mechanism [17, 18]. According to other studies, the intestinal absorption of very-long-chain fatty acids, and likely long-chain alcohols, require a protein-mediated pathway, such as scavenger receptor CD36 expressed on the plasma membrane of intestinal and liver cells [14].

To counteract the poor bioavailability of LCAs, some of them, TRIA and OCTA, can be conjugated with Polyethylene Glycol (PEG) to form nanoparticles, increasing their solubility by at least 14 orders of magnitude. For example, while the solubility of TRIA without PEG is 9×10^{-14} g/L, PEGylated TRIA presents a solubility of 10 g/L, and it was demonstrated that PEGylated TRIA nanoparticles enter the cells by macropinocytosis as an internalization pathway of the polymers (**Figure 1**), which improves the internalization into tumor cells, and therefore its anticancer activity is also enhanced [6, 8].

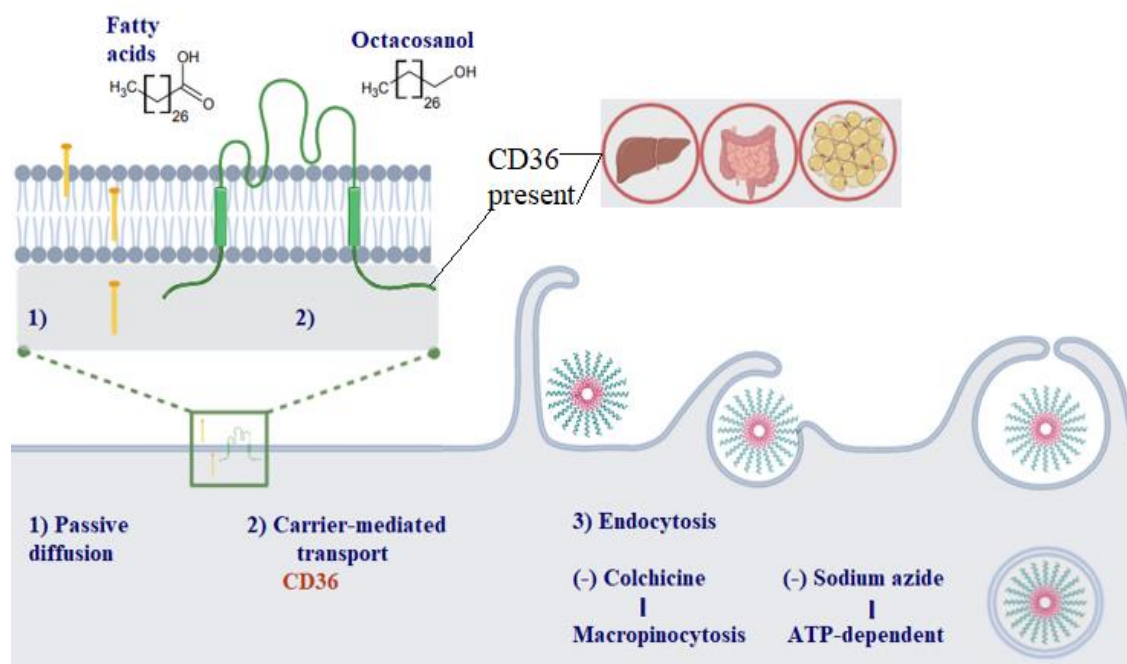


Figure 1. Graphic representation of the mechanisms used by POL to enter the cell. 1) POL could enter by passive diffusion, similarly to fatty acids, due to its structural similarity, small size, and lipophilicity; 2) POL could also use fatty acids carrier-mediated transport (CD36) present in the hepatocytes, enterocytes, and adipocytes; 3) The cell uptake of PEGylated TRIA is by ATP-dependent macropinocytosis.

The anti-inflammatory effect of pol

Inflammation is a physiological response triggered by infections or tissue injury or can be a feature of some diseases, and many studies have demonstrated that POL or its compounds show anti-inflammatory activity. Overall, the inflammatory response starts with an inducer (infection or tissue damage) recognized by sensors, which are receptors expressed in immune cells such as macrophages, mast cells, and dendritic cells. Other pro-inflammatory stimuli are triggered by cytokines such as tumor necrosis factor- α (TNF- α) and Interleukin-1 (IL-1) causing the activation of the Mitogen-activated Protein Kinase (MAPK) pathway, and transcription factors such as nuclear transcription factor- κ B (NF- κ B) and Activating Protein-1 (AP-1) in the immune cells. The MAPK family consists of three major members: Extracellular Signal-regulated Kinase (ERK), p38, and c-Jun NH₂-terminal Kinase (JNK). Every kinase triggers intracellular signaling cascades to regulate cellular processes such as the transcription of various pro-inflammatory cytokines [19], and transcriptional regulators are involved in Cyclooxygenase (COX-2), pro-inflammatory cytokines, and inducible Nitric Oxide Synthetase (iNOS) transcription [4]. The release of mediators such as cytokines, chemokines, Nitric Oxide (NO), Prostaglandin E₂ (PGE₂), and Leukotriene B₄ (LB₄) synthesised by activated immune cells causes vasodilatation, vascular permeability, and migration of blood components (plasma, leukocytes, neutrophils) to the surrounding tissue, oedema, fever and pain (**Figure 3**) [20, 21].

In different models of acute inflammation, Ravelo *et al.* demonstrated that POL purified from beeswax, named D-002, significantly decreased oedema formation and inhibited Myeloperoxidase (MPO) activity [22]. MPOs are peroxidase enzymes produced by neutrophils that generate reactive oxidants, contributing to their microbial activity [23]. These results suggest that the anti-inflammatory effect of D-002 may be due to inhibition of MPO through the reduction of neutrophil infiltration into damaged tissue. Fernández-Arche *et al.* reported that the nonglyceride fraction of pomace olive oil, composed of tetracosanol, HEXA, and OCTA, inhibits the generation of nitrite, Prostaglandin E₂ (PGE₂) and TNF- α by 88%, 38%, and 83%, respectively, in macrophages, possibly by inactivation of NF- κ B, leading to the resolution of inflammation [24]. In humans, TNF- α and Nitric Oxide Synthetase (iNOS) are upregulated by the NF- κ B pathway, and this transcriptional factor might be activated by oxidative stress, Lipopolysaccharide (LPS) and cytokines [25]. It has been reported that POL inhibits iNOS protein and controls the high production of Nitric Oxide (NO) during inflammation by inactivating NF- κ B through mechanisms such as those used by statins [24]. Therefore, this effect could be related to the hypothesis that POL inactivates or blocks the nuclear translocation of NF- κ B, although the molecular mechanisms have not yet been elucidated. Guo *et al.* demonstrated that OCTA decreases nuclear levels of p65 and c-Jun, which are components

of the transcription factors NF- κ B and AP-1. OCTA also inhibits the activity of NF- κ B and AP-1 and the phosphorylation of p38 and JNK, decreasing the expression of pro-inflammatory cytokines and leading to a reduction in the inflammatory process [4].

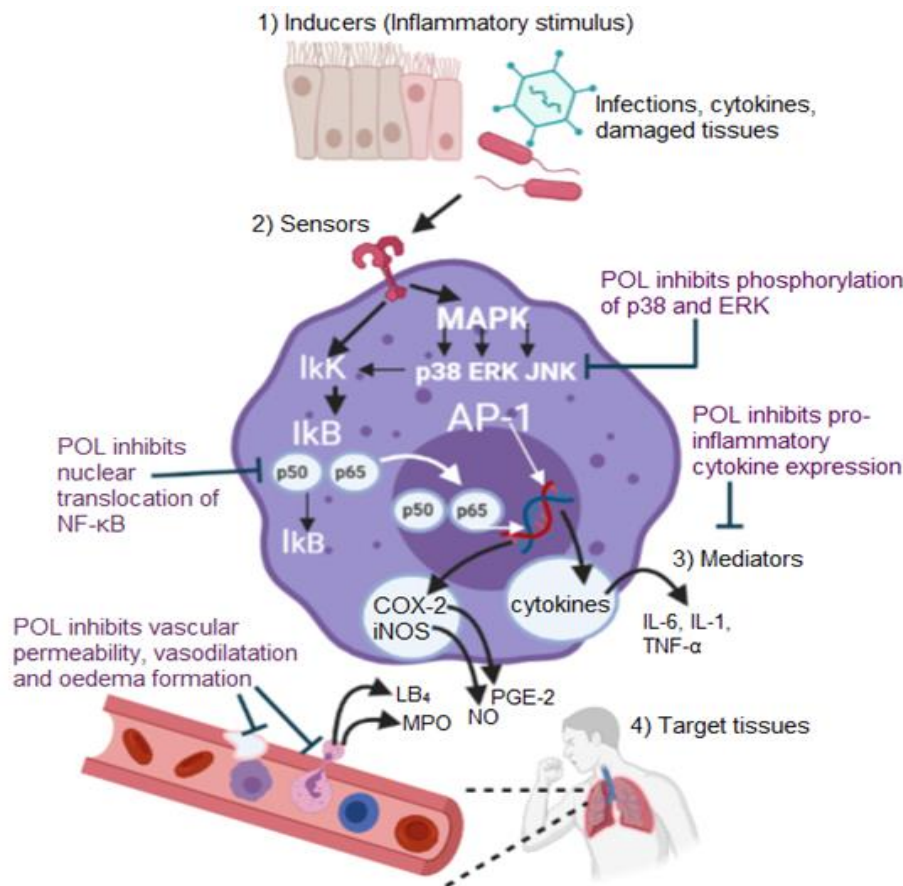


Figure 2. Molecular mechanisms of the anti-inflammatory effect of POL. NF- κ B is a family of dimeric transcription factors that includes five protein monomers (p65, RelB, cRel, p50, and p52). Activation of NF- κ B starts with the degradation of I κ B inhibitor proteins allowing nuclear translocation of free NF- κ B dimer (e.g., p50-p65). Activating Protein 1 (AP-1) is a transcription factor composed mainly of proteins belonging to the c-Jun and c-Fos families and is also related to the regulation of genes involved in inflammation.

In conclusion, POL, OCTA, and TRIA inhibit nuclear translocation of NF- κ B and the phosphorylation of MAPKs, thereby reducing the expression of the inflammatory mediators. On the other hand, POL also inhibits vascular permeability, oedema, and activity of the MPO peroxidase enzyme, decreasing the production of reactive oxidants by neutrophils (**Figure 2**).

Other biological properties of pol

Inflammation has an important association with cardiovascular disease and obesity, due to normal levels of cholesterol being required to maintain normal body function. In the cytoplasm of liver cells, endogenous cholesterol is biosynthesised through the mevalonate pathway, producing cholesterol from acetyl-CoA as a precursor. One important step in this pathway is the conversion of 3-hydroxy-3-methyl-glutaryl-CoA (HMG-CoA) to mevalonate, mediated by HMG-CoA reductase [26]. The involvement of POL in the lowering of cholesterol and serum lipids has been related to various mechanisms; among those are the down-regulation of HMG-CoA reductase activity through the phosphorylation of AMPK (adenosine 5' monophosphate-activated protein kinase) and the subsequent phosphorylation and inactivation of HMG-CoA reductase by AMPK [5]. AMPK is a Ser/Thr protein kinase and consists of a heterotrimeric complex of three subunits: the catalytic α -subunit, which can be phosphorylated at Thr172; the Thr258 and Ser485 sites; and the regulatory β and γ subunits [26, 27]. AMPK is involved in the regulation of ATP synthesis and consumption; the activation of AMPK comprises its phosphorylation by several kinases and compounds. POL increases AMPK phosphorylation at the Thr172 site in hepatoma cells leading to the inactivation of HMG-CoA reductase and a reduction in cholesterol

synthesis [28]. HMG-CoA reductase is a glycoprotein present in the endoplasmic reticulum membrane; its carboxy-terminal catalytic domain projects into the cytosol and the amino-terminal domain anchors the protein in the endoplasmic reticulum membrane [29]. In a study by Nam *et al.*, an *in vivo* model confirmed that POL decreases the HMG-CoA reductase activity but not the HMG-CoA reductase mRNA levels in hypercholesterolemic rats [30]. This effect could be related to the significant increase in AMPK phosphorylation through an indirect mechanism [30]; however, the molecular mechanism involved was not analyzed. In subsequent studies, Lee *et al.* revealed that HEXA is an allosteric activator of AMPK by binding directly to the AMPK- β subunit [5]; they also showed that HEXA phosphorylates HMG-CoA reductase on Ser872, which may result in reduced affinity for NADPH [26]. It is therefore suggested that other LCAs, such as OCTA or TRIA, could carry out this AMPK activation mechanism. Another important finding was that HEXA delays the translocation to the nucleus of Sterol-regulatory Element-binding Protein-2 (SREBP-2), which regulates HMG-CoA reductase transcription. HEXA seems to act at the post-translational level of SREBP-2, reducing its nuclear translocation and HMG-CoA reductase gene expression [5].

The presence in the liver cells of the POL mixture or the individual LCAs (HEXA, OCTA, and TRIA) or its corresponding fatty acids may cause the inhibition of cholesterol synthesis by inactivating the enzyme HMG-CoA reductase through the phosphorylation of AMPK at Thr 172 site indirectly, and activation of AMPK by direct binding to its AMPK- β subunit [28].

Applications as growth promoter

In the agricultural industry, TRIA is of particular interest because it is the only fatty alcohol used as a growth promoter in plants [1]. This is in contrast to the effects of LCAs on animal cells, where they present similar mechanisms of action. This indicates that plant cells recognize the TRIA molecule in a specific way such that it can differentiate a molecule of TRIA from others that are very similar, such as OCTA, whose difference lies only in the number of carbons – two to be specific – and that, as reported, trigger very different signals, or inclusive the inhibition of TRIA effect by OCTA [31].

TRIA was initially obtained from alfalfa (*Medicago sativa*) and subsequently from other vegetable and animal waxes. It has been studied in different species, such as tomato, sweet pepper, sugar beet, cotton, tobacco, potato, fruit crops, etc., and these studies have shown that TRIA can increase the dry and fresh weight, the height of the plant, and branch, stem and root length, as well as the yield of the fruit or seed [1, 32, 33]. Regarding physiological parameters, it has been determined that TRIA causes increases in the chlorophyll content in leaves, the photosynthetic rate, nitrogen fixation, enzymatic activity, and nutrient uptake, as well as stimulating the production of secondary metabolites and stomatal conductance [34]. Also, increases in the contents of nitrogen, phosphorus, and potassium have been reported, as well as of phenols, flavonoids, sugars, and proteins [35].

In addition to increasing the yields of crops of agricultural interest in the field, it has also been used in tissue culture of various plant species for micropropagation and the *in vitro* production of secondary metabolites of medicinal interest [1]. Other applications of TRIA include improvements in the quantity and quality of fruits, in parameters such as length, width, stiffness, Total Soluble Solids (TSS), acidity, and content of phenolic compounds [36].

However, TRIA not only presents effects such as growth promotion in plants but also in cyanobacteria, improving their biomass, photosynthesis, photorespiration, and chlorophyll, and protein content, which in turn, cause an increase in Fatty Acid Methyl Ester (FAME) yields, which is of particular interest for biodiesel production and in the removal of pollutant compounds resulting from the increase in biomass of cyanobacteria [2].

Photosynthesis is one of the main processes stimulated by the exogenous application of TRIA, increasing parameters related to the efficiency of excitation capture by the PSII reaction centers (Fv/Fm) [37], the minimum fluorescence (F0), the maximum fluorescence (Fm), the light efficiency of the electron transport of the PSII (PSII) and the photochemical quenching (qP), and decreasing the Non-photochemical Quenching (NPQ) [34, 37] and chlorophyll content, even under stress conditions, saline stress, cooling and water stress [1, 32, 35]. Kathuria *et al.* demonstrated that TRIA hinders the activity of chlorophyllase but does not enhance the biosynthesis of chlorophyll [38]. Other aspect are changes of the fluidity of the chloroplast membrane and alterations in the dynamic properties of the protoplast [39], as well as the increase in the number of chloroplasts [34] and likely a better development of the thylakoid grana.

Due to the structure of TRIA, a 30-carbon aliphatic chain with low solubility, it is not able to cross the plasma membrane, which is the first contact barrier for any plant growth promoter. Furthermore, endocytosis and exocytosis are not so intense as in animal cells where TRIA and other LCAs can penetrate the cell membrane, so

the specificity of the action of TRIA may be a result of the selectivity of plant cell membranes. It has been proposed that the fluidity of the membrane is altered in some way due to the lipophilic nature of TRIA, as was observed by Ivanov and Angelov in chloroplast protoplasts and by Shripathi *et al.*, in the microviscosity of protoplast membranes of cucumber fruits (*Cucumis sativus* L.) [39, 40].

Membranes are fluid, and this fluidity depends on their lipid composition and temperature. It is known that hydrocarbon acyl chains of membrane phospholipids and glycolipids are the main determinants of membrane fluidity, although it is also modulated by the size and charge of the polar groups of these molecules and the sterol content. The presence of short-chain or *cis*-unsaturated fatty acids reduces the transition temperature, while the saturation of fatty acids and an increase in the length of the hydrocarbon chain elevate the temperature. Studies conducted by Swamy *et al.* suggested that TRIA could act as a modulating agent when intercalated in the inner membrane and, therefore, cause changes in the lipid phase and/or stimulate the membrane-bound enzymes [41]. Shripathi *et al.* discussed the different properties of TRIA and abscisic acid (ABA), where ABA may be confined to a specific spatial facet in the membrane, in contrast to TRIA [40]. The results from the same authors confirmed that the fluctuations in membrane microviscosity could be due to the fatty acid composition, as well as to a change in lipid-protein interaction, which alters the physical status of the membrane differentially, by both ABA and TRIA.

Phytohormones or growth hormones affect the fluidity of the membrane because they have a preferential affinity at the hydrophilic/hydrophobic interface. The partition of these phytohormones and arbutin (a phenolic compound induced by drought) alters the lipid phase of the membrane, therefore influencing its properties such as permeability and microheterogeneity, which regulate cellular processes [41]. At this first point, it seems that TRIA modifies this partition of phytohormones and in that way triggers a cascade of signals that affects other metabolic processes. Swamy *et al.* demonstrated that there is a differential modulation between TRIA and jasmonic acid (JA) at the level of the cell membrane, and this differential effect could explain the antagonistic effect of TRIA concerning JA [41]. TRIA also inhibits the effect of ABA [40], which, together with JA, is a phytohormone involved in stomatal stress closure. Recently, stomatal regulation and inhibition of stomatal closure by ABA through TRIA application was reported [34].

Concerning the differential response to TRIA and OCTA, it has been reported that the first triggers the formation of a compound known as TRIM at the level of the tonoplast (the membrane that covers the vacuole), which suggests that TRIM causes a cascade of signals within cells that promote greater metabolic activity in plants. In contrast, it was shown that OCTA, which inhibits the effect of TRIA, triggers the formation of a second messenger called OCTAM [31]. TRIM, whose structure is 9- β -L(+)-adenosine (9H-purin-6-amine, 9- β -L-ribofuranosyl), is formed from adenosine, which is a constituent of Adenosine Triphosphate (ATP) and a precursor in the biosynthesis of cytokinins [42]. Therefore, it is likely that the second messenger caused by the application of TRIA (TRIM), could present a function similar to that of cytokinins, due to its structural similarity, or could perhaps provide adenosine or adenine for the formation of ADP and ATP, which are the precursors in the biosynthesis of cytokinins. However, this may only be a response that synergizes or inhibits the effect of other phytohormones due to the modulating effect of TRIA at the membrane level. Data published by Kunjammal *et al.*, demonstrated a synergistic effect on both growth and yield parameters in rice plants between TRIA (2 ppm) and cytokinin (10 ppm), compared to those of the treatments applied alone [10].

In addition to the hormonal modulation of TRIA at the plasma membrane level, the stimulation of a second messenger (TRIM), and the stimulation of the enzyme NADP oxidase, it has been reported that TRIA also stimulates the ATP content by ATPase stimulation [31]. ATP is synthesized by the activity of ATP synthetase, which is a complex that makes use of the proton potential created by the action of the electron transport chain during photosynthesis, transporting a proton down the gradient and using energy to complete the phosphorylation of ADP to ATP. The synthesis of ATP occurs mainly at the membrane level in the thylakoids of the chloroplasts, where it is part of the photosynthetic complex for energy production.

During light and heat stress, inhibition of photosynthetic activity is induced via oxidative damage to the PSII proteins, with the D1 protein binding to the PSII reaction center, which is more prone to oxidative damage by ROS produced during the photoinhibition process, therefore, in the inactivation of PSII by heat, D1 seems to be damaged by lipid peroxidation that occurs near PSII [43]. In addition, the fluidity of the thylakoid membrane seems to act as a significant mediator of the quality control of PSII under light and heat stress and could alter the ordered distribution of the proteins or protein complexes involved in the photosynthetic apparatus [43], for example the ATP synthetase. The membrane-modulating action of TRIA might affect ATP synthetase and

photosynthetic activity and in this way regulate the response of phytohormones, either synergizing or inhibiting their effects and/or participating in the biosynthesis of some compounds.

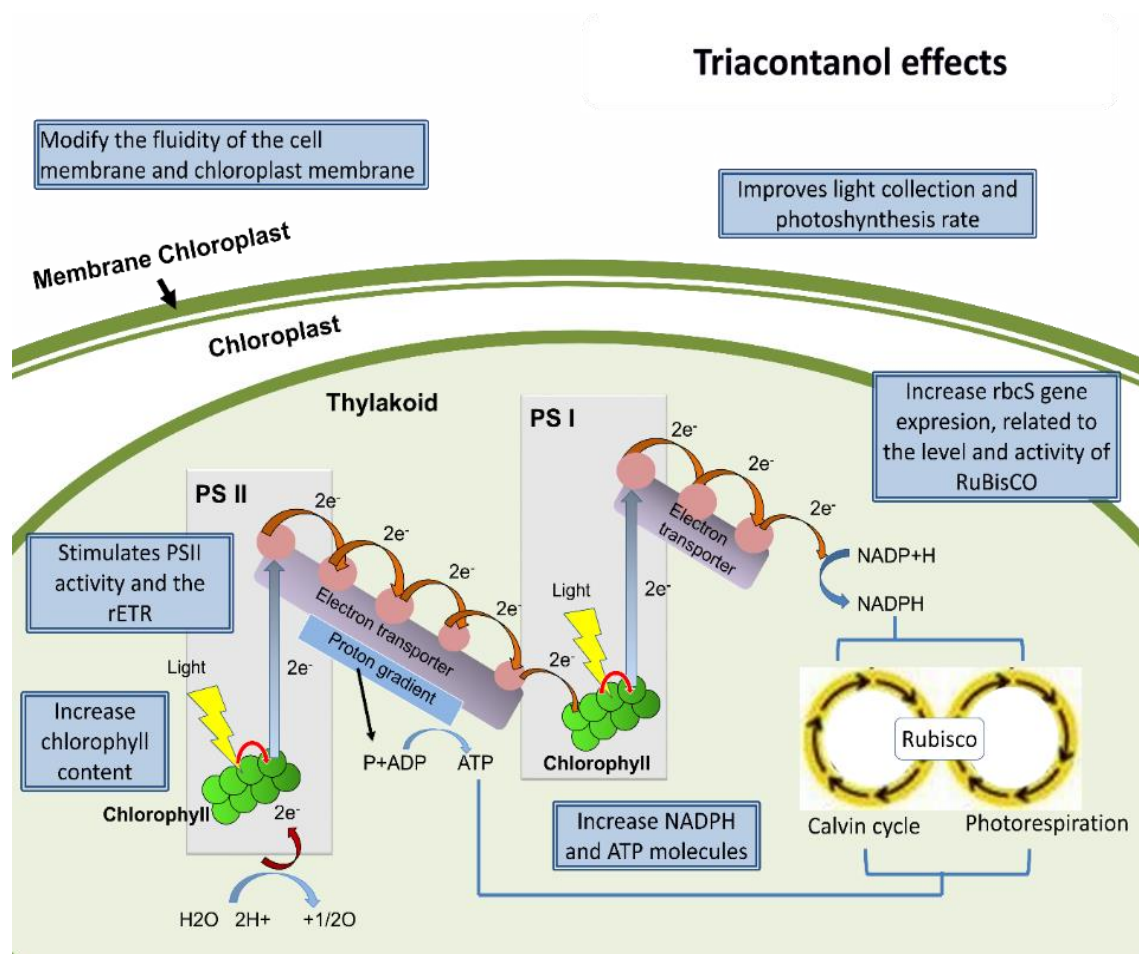


Figure 3. TRIA effects concerning photosynthetic activity. PSI: photosystem-I; PSII: photosystem-II; rETR: relative photosynthetic electron transport rate; ADP: adenosine diphosphate, ATP: adenosine triphosphate; NADP and NADPH: oxidized and reduced nicotinamide adenine dinucleotides; RuBisCO: ribulose-1,5-bisphosphate carboxylase/oxygenase.

The increase in photosynthetic activity results in a greater concentration of ATP and NADPH molecules, which are important elements in the Calvin cycle and which also intervene in its regulation. The amount of ATP in the stroma can interfere with the activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) by light [44], while that the intracellular concentration of NADPH controls the expression of genes that participate in the Calvin cycle [45]. TRIA could be favoring the fixation of CO₂ in the Calvin cycle by increasing the concentrations of ATP and NADH, resulting in increased photosynthetic activity and positively regulating the expression of the *rbcS* gene, which is related to the increase in the level and activity of RuBisCO [46]. In summary, the effect of TRIA as a plant growth promoter reported in the various studies carried out is focused on a photosynthetic activity (**Figure 3**); however, the initial mechanism by which all these effects are triggered is unclear, so it is not known whether its effect is due to the formation of a second messenger or if this is an activity is caused mainly by its modification at the membrane level.

On the other hand, it is worth mentioning that there exists in plants a protein named SnRK1 (Snf1-related kinase1), an orthologous protein of mammalian AMPK (5' AMP-activated protein kinase), whose phosphorylation, mediated by POL, inactivates HMG-CoA reductase and therefore cholesterol biosynthesis. In plants, SnRK1 acts as a metabolic sensor, integrating very diverse stress conditions, and is key in maintaining energy homeostasis for growth and survival [47]. Studies by Carianopol *et al.* suggested that SnRK1 may modulate the ABA response by phosphorylating select signaling components. There is a clear connection established between SNF1 and AMPK, due to the fact that some regulatory aspects are conserved such as phosphorylation of the T loop, the detection of adenylates and the kinase activity between them, however, in plants, the SNF1 protein could be regulated by other

regulatory mechanisms that have not been clarified [48]. Like this, the elucidation of the role of TRIA in the activation of SnRK1 may be related to its effects in improving stress tolerance. Both SnRK1 and AMPK are keys to explain the diverse effects in plant and animal cells, respectively. The activation of AMPK has several metabolic and therapeutic (anti-inflammatory and anti-tumorigenic) roles, and SnRK1 is involved in plant defense, primary and secondary metabolism, growth and development, stress hormones, and programmed cell death, all of which have been reported to be stimulated by TRIA, except cell death in plants, since there have been no studies in this regard [47-49].

CONCLUSION

The multiple effects reported for the LCAs that make up POL in both plants and animals raise the question of whether there are similarities in their mechanisms of action. Among the differences between these effects, the selectivity of plant cells for TRIA and the inhibitory effects of OCTA stands out, unlike in animal cells, whose responses to these LCAs are similar. All this is due in part to the fact that plant cells do not show as much exchange and endocytosis as animal cells.

On the other hand, the mechanisms of TRIA in plants have been little studied and have been limited to describing its effects, which range from increased yields and quality of fruit and secondary metabolites as well as stimulating various physiological and morphological parameters. The few studies on TRIA in plants have suggested a response at the membrane level and subsequently, the production of a second messenger, which some authors suggest shows a great similarity to the effect of cytokinins, phytohormones widely used for the stimulation of different physiological processes and, therefore of yields, and may have something to do with inhibiting senescence in plants; however, more studies are needed to elucidate this mechanism to compare its effects at the membrane level with other physiological responses.

Finally, the presence of the SnRK1 proteins, whose function is similar to that of AMPK in maintaining homeostasis in organisms, as well as an activation that is also similar because they are orthologous proteins, suggests a common mechanism between plant and animal cells that would explain all the effects in both cell types, limited only by the specificity, internalization and differential signaling of the cell membranes.

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