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CHEMISTRY OF GARLIC [ALLIUM SATIVUM] AND ITS MEDICAL USES

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Abstract:

*Garlic [*Allium sativum *L.] is considered one of the twenty most important vegetables, with various uses throughout the world, either as a raw vegetable for culinary purposes, or as an ingredient of traditional and modern medicine. Furthermore, it has also been proposed as one of the richest sources of total phenolic compounds, among the usually consumed vegetables, and has been highly ranked regarding its contribution of phenolic compounds to human diet.*

Keywords: vegetables, modern medicine, phenolic compounds.

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INTRODUCTION:

Chemistry of garlic

Nonvolatile sulfur-containing precursors in intact garlic.

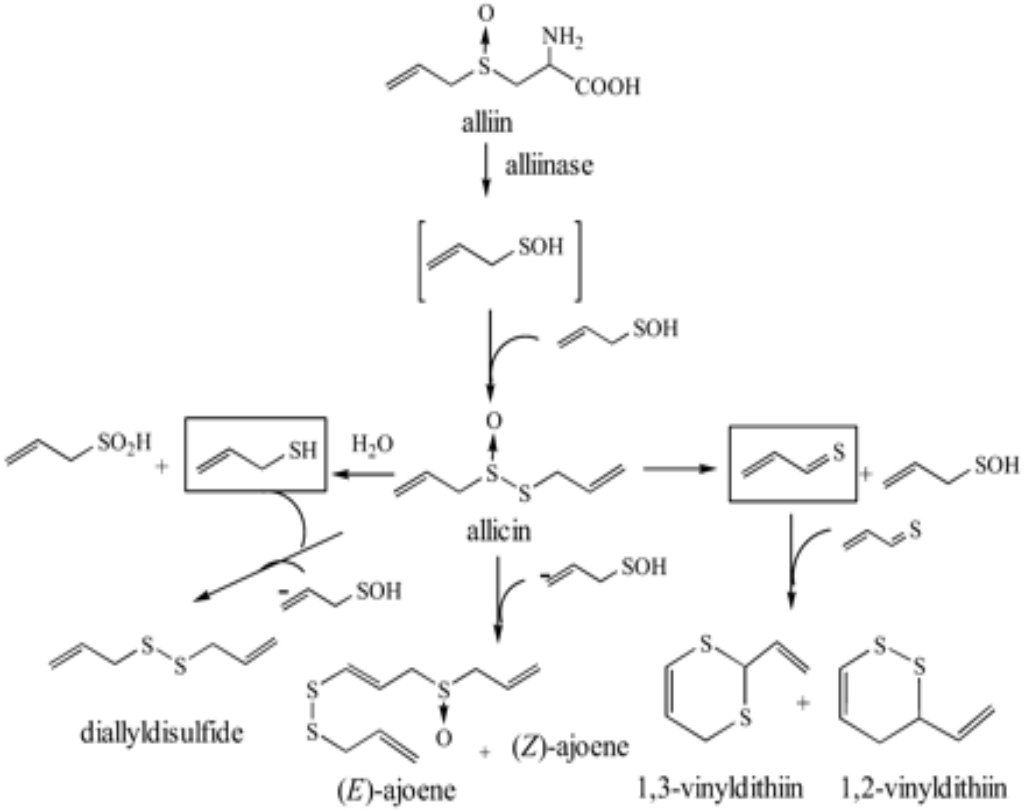
The major sulfur-containing compounds in intact garlic are γ-glutamyl-*S*-allyl-L-cysteines and *S*-allyl-L-cysteine sulfoxides [alliin]. Both are abundant as sulfur compounds, and alliin is the primary odorless, sulfur-containing amino acid, a precursor of allicin [12], methiin, [+]-*S*-[*trans*-1-propenyl]-L-cysteine sulfoxide, and cycloalliin [13]. These sulfoxides, except cyloalliin, are converted into thiosulfinates [such as allicin] through enzyme reactions when raw garlic is cut or crushed. Thus, no thiosulfinates are found in intact garlic.

γ-Glutamyl-*S*-allyl-L-cysteines are converted into *S*-allyl-cysteines [SAC] through an enzymatic transformation with γ-glutamyltranspeptidase when garlic is extracted with an aqueous solution [14]. SAC, a major transformed product from γ-glutamyl-*S*-allyl-L-cysteine, is a sulfur amino acid detected in the blood that is verified as both biologically active and bioavailable. Determining the contents of these key precursor compounds is important for evaluating raw garlic.

Organosulfur compounds in the process of garlic-product preparation.

Thiosulfinate formation.

The disruption of garlic bulbs causes the formation of thiosulfinates such as allicin through the enzymatic reaction of sulfur-substituted cysteine sulfoxides, compartmentalized in the cytoplasma with alliinase in the vacuole, via sulfur-substituted sulfenic acids as a highly reactive intermediate [**Fig. 1**]. The finding that allicin killed microrganisms in a Petri-dish [15] was a sensational discovery. However, hopes for a medicinal or antiseptic use of allicin based upon this Petri-dish study soon faded because of its extreme instability and toxicity. Other thiosulfinates, including allylmethyl-, methylallyl-, and *trans*-1-propenyl-thiosulfinate, were found in the garlic homogenates, and, like allicin, they are all unstable [16,17]. When allicin itself was kept at 20°C for 20 h, it decomposed to diallyl disulfide [DADS] [66%], diallyl sulfide [DAS] [14%], diallyl trisulfide [9%], and sulfur dioxide [18]. Allicin easily reacts with amino acids and proteins, creating an -SH group. Freeman found that allicin binds to protein and fatty acids in the plasma membrane, is thus trapped before absorption, and cannot circulate in the blood [19]. In fact, no allicin was detected in the blood after the ingesting raw garlic or pure allicin [5,20].



Alliinase is the key enzyme that facilitates the transformation of cysteine sulfoxides to thiosulfinates. The purified enzyme possesses a pH optimum of 6.5 with *S*-methyl-L-cysteine as substrate [21]. In addition, pyridoxal phosphate stimulates alliinase activity as a cofactor [22]. A pH dependency of alliinase activity is indicated when allicin and other thiosulfinates are released during incubation of garlic powder in buffer solutions adjusted from pH 2 to 10. Thiosulfinates are not formed below pH 3.6, which is the usual pH range in the stomach [23]. Furthermore, thiosulfinates are never generated through the neutralization of a mixture previously incubated below pH 3. Thus, alliinase is completely and irreversibly inhibited under the acidic conditions found in the stomach. Freeman et al. [24] also reported that no processed garlic preparations contain allicin, and furthermore, allicin is not generated in simulated gastric solution. Therefore, allicin-producing potential, which is defined as the allicin released from garlic preparations in water, should not be a meaningful chemical evaluation for garlic products. Findings clearly indicate that allicin itself does not contribute to any of garlic's beneficial effects inside the body. Allicin is thought to be a transient compound that is rapidly decomposed into other sulfur-containing compounds and is not a genuine active compound of garlic.

Organosulfur volatiles.

Processed garlic contains a wider variety of organosulfur volatiles than the intact garlic clove. Typical volatiles that have been identified in crushed garlic and garlic essential oil include DAS, DADS, diallyl trisulfide, methylallyl disulfide, methylallyl trisulfide, 2-vinyl-4H-1, 3-dithiin, 3-vinyl-4H-1, 2-dithiin, and [E,Z]-ajoenes. Over 20 sulfides have been identified in steam-distilled garlic oil and oil-soluble extract of garlic, and many of them, especially sulfides having an allyl group, are responsible for the characteristic smell and taste after ingesting garlic. The major sulfides in garlic oil include DAS [57%], allylmethyl [37%], and dimethyl [6%] mono- to hexasulfides, in some cases, together with a small amount of allyl 1-propenyl and methyl 1-propenyl di-, tri-, and tetrasulfides]. Diallyl trisulfide is the most abundant in fresh garlic oil, but commercially available garlic-oil products have an increased amount of DADS [24,25]. The level is speculated to be dependent upon the disproportionation of diallyl trisulfide in the oil. The component of these sulfides varies according to extraction temperature or time [26].

Vinyldithiins were first demonstrated to be thermal-degradation products derived from allicin during gas chromatographic analysis of allicin. These structures were elucidated to be 2-vinyl-4H-1, 3-dithiin and 3-vinyl-4H-1, 2-dithiin on the basis of spectroscopic analysis. The formation mechanism has been confirmed to be a type of Diels-Alder dimerization of thioacrolein derived from the β-elimination of allicin. A remarkable production of vinyldithiins from allicin is observed when less-polar solvents such as hexane are used. Vinyldithiins, especially 2-vinyl-4H-1, 3-dithiin, are rich in the oil macerate of raw garlic [27].

Apitz-Castro et al. [28] first isolated ajoene from the ether fraction of garlic extract as a potent antithrombotic agent. Block and Ahmad determined ajoene structure was E and Z isomers of 4, 5, 9-trithiadodeca-1, 6, 11-triene-9-oxide. They also proposed that it is formed by *S*-thioallylation of allicin, followed by Cope-type elimination and readdition of 2-propenesulfenic acid. Iberl et al. elaborated the influence of different media on allicin transformation including the E:Z ratio of ajoene. Another ajoene-type organosulfur compound, E-4,5,9-tritriadeca-1,7-diene-9-oxide, was isolated from oil-macerated garlic extract.

Water-soluble organosulfur compounds.

Alcoholic and aqueous garlic extracts contain primarily *S*-allyl-L-cysteines derived from γ-glutamyl-*S*-allyl-L-cysteines [**Fig.1**]. *S*-Allyl-L-cysteine and *Trans*-*S*-1-propenyl-L-cysteine, together with a small amount of *S*-methyl-L-cysteine, are found in garlic extract such as AGE. These cysteine derivatives are colorless crystals and are odorless and stable in the solid state or aqueous solution under neural or slight acidic conditions. SAC provides protection against oxidation, free radicals, cancer, and cardiovascular diseases. In addition, *S*-allylmercapto-L-cysteine, which demonstrates an in vivo hepato-protective effect an in vitro cancer-preventive effect in human prostate carcinoma cells, as well as antioxidant activity in vitro, is a characteristic compound present in AGE.

Cholesterol reduction in clinical studies

Meta-analysis has been done on studies of cholesterol reduction and concludes that dehydrated garlic powder is ineffective in lowering blood-cholesterol levels; there is no reasonable explanation for this inconsistency with research results that demonstrate the cholesterol-lowering effects of garlic. However, it is wrong to use allicin as the standardization marker for potential or yield, because allicin's lack of bioavailability means that it is not a genuinely active compound of garlic. The media and lay publications that report such negative studies and meta-analyses have a strong impact on the public; they create

confusion and skepticism, thereby reducing the intake of garlic supplements that can have health-promoting effects, especially among populations at high risk for disease.

However, the above meta-analysis excluded the results of several clinical studies of the effects of AGE on cholesterol. AGE has consistent effects on risk factors for cardiovascular disease, including cholesterol and others, in some of these studies; blood SAC level was measured in the subjects as a compliance marker. The blood SAC level in the group taking supplements was significantly higher than that of the placebo group, it is clear that SAC is bioavailable because it was absorbed into the blood and is therefore active in the human body. The bioavailability of a chemical compound such as SAC makes it possible to obtain consistent measured effects for the standardization of garlic products.

Anti-oxidation

Reactive oxygen species [ROS], or free radicals, have been implicated in mediating various pathological processes such as cancer, ischemia, inflammatory diseases, diabetes, and atherosclerosis. Garlic has been reported to be effective against diseases of which ROS are considered a main cause. The studies suggest that garlic may work by reducing ROS or interacting with them to minimize the negative impact on the body. However, the degree of anti-oxidative efficacy of various garlic preparations differs according to variations in chemical structures and standardization procedures.

Since antioxidative activity is caused by the relative electron status of the materials, in vivo reaction in the whole body should be taken into account when considering the active compounds of garlic. LDL oxidation has been recognized as playing an important role in the initiation and progression of atherosclerosis. Popov et al. observed the antioxidant effect of the aqueous extract from a dehydrated garlic–powder preparation by using photochemiluminescence on the $Cu[^{2+}]$-initiated oxidation of LDL. The formation of conjugated diene, which accompanies the lipid peroxidation process, was detected photometrically. Allicin-free AGE and its constituent SAC have a similar preventative effect against $Cu[^{2+}]$-initiated oxidation of LDL taken from the human subjects who consume AGE. Ide et al. investigated and found clear supportive data that AGE and SAC significantly prevent membrane damage, loss of cell viability, and lipid peroxidation in bovine pulmonary artery endothelial cells [PAECs] exposed to oxidized LDL. Wei et al. and Yamasaki et al., using PAECs, also observed that AGE suppresses hydrogen peroxide $[H_2O_2]$ and superoxide anion $[O_2^-]$ generation, and thus protects vascular endothelial cells from oxidant injury. It also significantly increases the activities of superoxide dismutase [SOD], catalase, and glutathione peroxidase in PAECs. AGE pretreatment significantly reduced the loss of cell viability induced by H_2O_2. AGE and SAC inhibited both lactate-dehydrogenase release and lipid peroxidation induced by H_2O_2. These data indicate that the antioxidative capabilities of AGE and SAC may be useful in preventing of atherosclerosis. Furthermore, Geng et al. showed that AGE increases intracellular glutathione levels, glutathione disulfide reductase, and SOD activity in PAECs, whereas the level of glutathione disulfide decreased. These results suggest that the antioxidant effect of AGE may be due to its modulation of the glutathione redox cycle and SOD activity in vascular endothelial cells.

ROS are involved in signal transduction pathways leading to nuclear factor kappa B [NF-κB] activation that has been implicated in the regulation of gene transcription. Geng et al. determined the effects of SAC on NF-κB cultivation in human T lymphocytes [Jurkat cells] induced by tumor necrosis factor alpha and H_2O_2. SAC consistently inhibited NF-κB activation induced by both tumor necrosis factor alpha and H_2O_2 in nuclear extracts. The results suggest that SAC might act through antioxidant mechanisms to block NF-κB activation in Jurkat cells. These studies are meaningful because SAC is bioavailable and can be delivered to such cells in vivo. If SAC could not reach the target cells in vivo after consumption of garlic, it would not act like an active compound. Therefore, analysis of the bioavailability of such compounds is important, especially for in vitro studies and designing isolated systems.

Horie et al. demonstrated that AGE prevents the formation of thiobarbituric acid–reactive substances and fluorescent substances during lipid peroxidation of rat liver microsomes. AGE protects the membranes from lipid peroxidation and serve to maintain membrane fluidity. Imai et al. [3] compared the antioxidant properties of 3 garlic preparations and organosulfur compounds in garlic. AGE inhibited the emission of low-level chemiluminescence and the early formation of thiobarbituric acid–reactive substances in a liver microsomal fraction initiated by t-butyl hydroperoxide. However, the water extracts of raw and heat-treated garlic enhanced the emission of low-level chemiluminescence. Among a variety of organosulfur compounds, SAC and *S*-allylmercaptocysteine [SAMC], major organosulfur compounds found in AGE, showed radical scavenging activity in both chemiluminescence and

1,1-diphenyl-2-picrylhydrazyl assays, indicating that these compounds may play an important role in the antioxidative activity of AGE. Numagami et al.examined effects of AGE and its thioallyl components on rat brain ischemia using a middle cerebral artery occlusion model and a transient global ischemia model. SAC significantly prevented the elevation of water content in ischemic brains and reduced infarct volume. On the other hand, neither allyl sulfide nor allyl disulfide was effective.

The direction of in vitro research must be considered and designed based upon the information from both in vivo and pharmacokinetic analysis of candidates for the active compounds of herbs and botanicals.

Drug-garlic interaction and influence on metabolizing enzymes

Herbal and botanical preparations used as complementary medicines with drugs are heavily scrutinized due to their capability to influence P450 enzymes in the liver, which are responsible for metabolizing exogeneous chemical compounds. Several studies demonstrated the stimulating effect of garlic on P450 enzymes, indicating it has an influence on medications and their levels in the blood. Many herbal supplements are now being closely studied for their potential interaction with medication, especially ones that have an influence on P450 isozymes. Many herbal supplements are consumed by people also taking medications, and these medications may interact with the supplements through the metabolizing systems in the body. The issue is therefore of great interest to the medical, academic, and public communities. Further research in this area must be undertaken and reflected through the development of herbal extract preparations that are less interactive with traditional synthesized drugs. Piscatelli reported that cytochrome enzyme P450 isozymes were significantly influenced by the intake of a dehydrated garlic–powder supplement, and blood concentration of the AIDS medication Saquinavir [Forlovase, Roche Laboratories] was drastically reduced due to the stimulation of P450 isozymes responsible for metabolizing the drug. Because the study was small and the research protocol was criticized, the National Center for Complementary and Alternative Medicine supports both basic-mechanism and clinical studies to confirm and compare the effects of the two different garlic preparations, that is, dehydrated garlic powder and AGE, on saquinavir metabolism in humans.

Dehydrated garlic–powder products contain oil-soluble sulfur compounds derived from allicin, and AGE mainly contains water-soluble sulfur compounds such as SAC. Because previous reports indicate that oil-soluble, but not water-soluble, sulfur compounds stimulate P450s, it may be interesting to learn whether these products have different results on saquinavir metabolism.

Hu et al. observed the effects of DAS on oxidative metabolism and hepatotoxicity induced by acetaminophen in rats. Treatment with DAS significantly protected rats from acetaminophen-related mortality and elevation of serum lactate dehydrogenase. DAS was also found to induce cytochrome P450 2B1 in rat livers but to inhibit and inactivate P450 2E1 . It is also reported that DAS induced liver microsomal pentoxyresorufin dealkylase activity, a representative activity of P450 2B1. Correspondingly, the levels of P450 2B1/2 protein and P450 2B1/2 mRNA were markedly increased by DAS treatment. In contrast, the level of P450 2E1 mRNA in the liver was not changed. Nakagawa et al. demonstrated the hepato-protective effects of SAC and SAMC using mice with acute hepatitis induced by hepatotoxins. SAC and SAMC reduced the rise of serum enzyme levels and liver necrosis caused by acetaminophen. By studying the mechanism of SAMC's hepatoprotective effect, Sumioka et al. observed that SAMC pretreatment significantly suppressed declines in hepatic-reduced glutathione levels that were induced by administering acetaminophen. SAMC pretreatment also suppressed the increase in hepatic lipid peroxidation and the decrease in levels of hepatic-reduced coenzyme CoQ9H2 that were induced by administering acetaminophen. Dion et al. found that water extracts of AGE significantly reduced the in vitro formation of N-nitrosomorpholine, a mutagen and liver carcinogen. Water-soluble sulfur compounds reduce cancer risk or prevent carcinogenesis without modifying the P450 system. SAC and its nonallyl analog, *S*-propyl cysteine, effectively blocked the formation of N-nitrosomorpholine. Because water-soluble sulfur compounds like SAC or SAMC protect the liver through P450-independent pathways, this suggests that another hepatoprotective mechanism of SAMC may be attributable to its antioxidant activity.

CONCLUSION:

Many clinical, preclinical, and in vitro studies have shown that allicin-free garlic products, such as AGE, have clear and significant biological effects in cardiovascular, immunological, cancer, hepatoprotective, and other areas. Various chemical constituents have also been identified in this allicin-free preparation, such as nonsulfur compounds, saponins, Maillard-reaction compounds, protein fractions, and others. Each compound is closely

related to and responsible for the various biological effects, and it is unnecessary to retain allicin or its degraded odorous oil-soluble sulfur compounds in the garlic product

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