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Review

Research progress on property and application of theaflavins

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Theaflavins is a group of very important material from black tea with functions as antioxidant, cancer suppressor and inhibitor of pathogenic microorganisms. A description is given on the property, chemical structure and application of theaflavin in this paper. The present research situation of theaflavins both in China and abroad is also reviewed.

Key words: Theaflavins, property, application.

INTRODUCTION

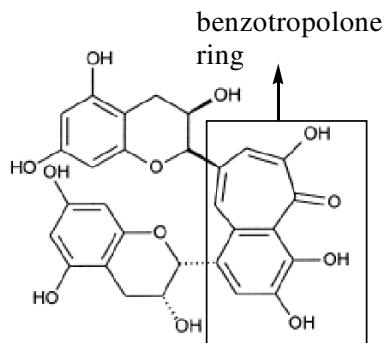
Theaflavins, which mainly consists of four compounds, is a group of polyphenol pigment formed during the fermentation of black tea. After theaflavin itself was first isolated in 1957 from black tea, this group of polyphenol pigment has been appended to several new compounds and studied broadly in the aspects of property, chemical structure, methods of purification and assay, formation mechanism, as well as the synthesis methods. In the recent years, its pharmacological function was paid much attention which includes properties as antioxidant, anti-pathogenic substance and as cancer suppressor. Furthermore, theaflavins has been used to prevent coronary heart disease and to treat diabetes in clinical trials. As the system of modeling the fermentation of black tea is constructed to product theaflavins in volume, theaflavins will be a very important kind of natural drug material with great potential in the future. This paper reviewed the present research situation of theaflavins both in China and abroad.

CHEMICAL STRUCTURE AND SYNTHESIS OF THEAFLAVINS

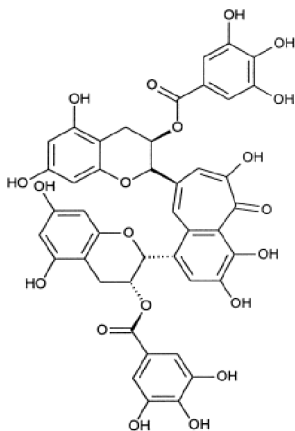
Theaflavins consist mainly of four major compounds, which are normally termed theaflavin (TF1), theaflavin-3-gallate (TF2A), theaflavin-3'-gallate (TF2B) and theaflavin-3,3'-digallate (TF3). The molecular formulas of TF1, TF2 (including TF2A and TF2B) and TF3 are C₂₉H₂₄O₁₂, C₃₉H₂₈O₁₆ and C₄₃H₃₂O₂₀, respectively. Their chemical structures are shown in Figure 1 and the conformations of TF1, TF2A, TF2B, and TF3 were modeled as shown in Figure 2 (Clark et al., 1998). With progress in the study of theaflavins, some analogs have been found. For example, epicatechin gallate (EC) was oxidized chemically using potassium ferricyanide and a new type of theaflavins was synthesized which is tentatively termed theaflavate A (Xiaochun et al., 1997). Besides, theaflavate B, isotheaflavin-3'-o-gallate and neotheaflavin-3-o-gallate were found from black tea, all of which contain a benzotropolone moiety (John et al., 1998). At present, theaflavins include 12 kinds of compounds. Although the structure of theaflavins is very complex, they have the same hydroxy-substituted benzotropolone ring which is characteristic structure of theaflavins.

Theaflavins can be synthesized through condensation of catechins, between di- and tri-hydroxylated B rings of

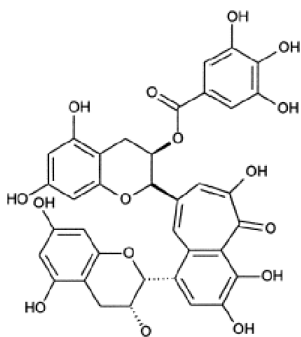
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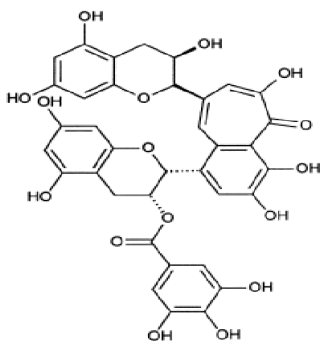
TF1



TF2A



TF2B



TF3

Figure 1. Chemical structures of TF1, TF2A, TF2B, TF3.

catechins. The reaction of condensation involves the oxidation of B ring of catechins to the quinones, followed by a Michael addition of the gallic catechin quinone to the catechin quinone, prior to carbonyl addition across the ring and subsequent decarboxylation. Generally, there are three methods for acquiring theaflavins: (1) Theaflavins could be extracted from black tea, which is the method used originally. Because the content of theaflavins in black tea is very low, the method usually costs much and cannot use industrially. The best condition for enhancing the content of theaflavins in black tea was 23°C fermentation for 80 min. (2) Modelling black tea fermentation *in vitro* has been used in synthesis of theaflavins which include both chemical oxidation mainly using potassium ferricyanide (K₃Fe(CN)₆) and sodium bicarbonate (NaHCO₃) as chemical catalyzer, and enzymatic oxidation primarily using polyphenol oxidase (PPO) as biocatalyzer (Xiaochun et al., 1997). (3) With the development of fermentation engineering and enzyme engineering, catechins were oxidized by PPO in a model fermentation system under the condition of oxygen blow (Alastair and Derek, 1983). By regulating the influence factor, the yield of theaflavins can be further enhanced. At present, the third method is the focus of synthesis of theaflavins.

PROPERTIES OF THEAFLAVINS

Antioxidant properties

Theaflavins is a kind of natural antioxidant. The phenolic hydroxy groups of theaflavins possess antioxidative activity as radical scavengers and/or metal chelaters, and the gallic acid moiety is also essential (Kyoji et al., 1994; Mayumi et al., 1994). Furthermore, the effectiveness of theaflavins was increased by esterification with gallate and was further enhanced as digallate ester. Considering the relation of the amount of metal ions in living cells to the oxidation of lipid, the ability to chelate metal ions with theaflavins is also important (Nicholas et al., 1996). Studies showed that the rate constant of theaflavins for superoxide scavenging, $K_{TF} = 1 \times 10^7 M^{-1} S^{-1}$, was higher than that of epigallocatechin gallate, $KEGCG = 4.3 \times 10^5 M^{-1} S^{-1}$ (Slobodan et al., 1997). Moreover, theaflavins could prevent the preoxidation of lipid effectively or cut off the chain reaction of oxidation of lipid. Apart from scavenging free radicals and chelateding metal ions, theaflavins could activate glutathione-S-transferase (GST), glutathione peroxidase (GPX), superoxide dismutase (SOD) and catalase (CAT) significantly which are accompanied with significant reduction in lipid peroxidation (Prosenjit and Sukta, 2003).

It was first shown that the ability of antioxidation of theaflavins was stronger than α -tocopherol (vitamin E) and propyl gallate (PG) in the rabbit erythrocyte system; but in the microsomal system, theaflavins showed less

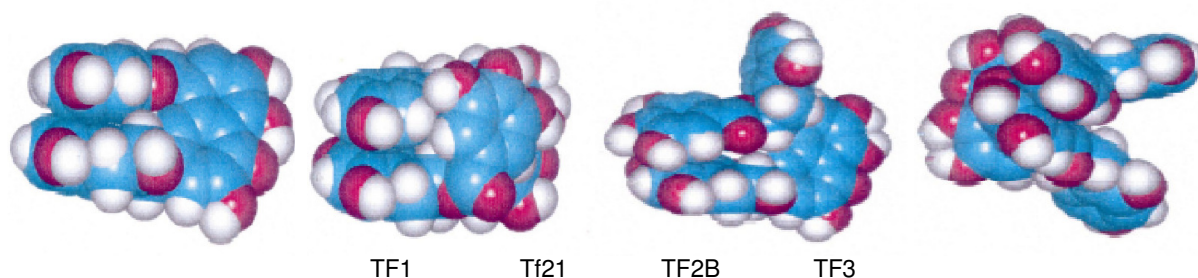


Figure 2. Proposed molecular conformations of TF1, TF2A, TF2B, TF3.

potent antioxidative activity than α -tocopherol (Mayumi et al., 1994). According to Kyoji et al. (1994), the antioxidative activity of theaflavins were more effective than glutathione (GSH), L(+)-ascorbic (AsA), dl- α -tocopherol, butylated hydroxytoluene (BHT), and butyl hydroxyanisole (BHA) in in vitro peroxidation of rat liver homogenate induced by tert-butyl hydroperoxide (BHP). Among the four major compounds of theaflavins, the hierarchy of activity as antioxidants is TF3>TF2A=TF2B>TF1 (Nicholas et al., 1996). Using an in vitro assay that measured Cu²⁺-induced oxidation of lipoproteins in human serum, theaflavins was believed to be major contributors to the antioxidant activity of black tea (Jonathan et al., 1999). The inhibitory activity of cell-mediated low density lipoprotein (LDL) oxidation was investigated and the result was TF3>TF1>epigallocatechin gallate (EGCG)> epigallocatechin (EGC)>gallic acid. The proposed mechanism of inhibitory effect was to decrease superoxide production by macrophages and to chelate ions (Hiroshi et al., 1999). But when using human LDL as the oxidation model, the antioxidant activity was TF3 > ECG EGCG TF2B TF2A > TF1 EC > EGC in decreasing order (Lai et al., 2001). This suggests that the relative antioxidant potency of various components of theaflavins depends upon their chemical structures and the assay systems. As a whole, theaflavins give a very strong antioxidant property, which has a relationship to coronary heart disease and atherosclerosis.

Cancer suppressor

Theaflavins could control tumor in multiple stages of carcinogenesis, which significantly inhibited activation of extracellular signal-regulated protein kinases and c-jun NH2-terminal kinases. TF3 appeared to have the strongest inhibitory effect (Masaaki et al., 2000). They were also believed to promote apoptosis of tumor cell and suppress the expression of proto-oncogene by modulating the transcription factors and the activity of correlating enzyme. Furthermore, tumor biomarkers including the I x B kinase (IKK) activity in activated macrophages could be inhibited and TF3 showed more activity than the other tea polyphenols (Lin, 2002).

Experiments revealed that the target of theaflavins was specific cell-signaling pathways leading to activator protein-1 (AP-1) and/or Nuclear Factor kappaB (NF-kappaB) which were responsible for regulating cellular proliferation or apoptosis (Park and Dong, 2003). All these indicate that theaflavins could inhibit cancer at the molecular level.

Protection against DNA from damage: DNA damage is a main origin for inducing cancer. Mayumi et al. (1994) firstly proved that theaflavins could inhibit DNA single-strand cleavage and mutagenicity through scavenging radicals. Not only oxidative stress-induced cytotoxicity and cellular DNA damage, but also carcinogen-related DNA damage could be inhibited by theaflavins through suppressing the elevated Cytochrome P450 1A1 (CYP1A1) in cells (Qing et al., 2002). Theaflavins showed the significant anticlastogenic effects of cyclophosphamide (CP) and dimethylbenzanthracene (DMBA) induced genetic damage (Gupta et al., 2001). Furthermore, benzopyrene (BaP)-and cyclophosphamide (CP)-induced genotoxicity in microbial and mammalian test systems could be inhibited in a dose-dependent manner by theaflavins (Yogeshwer et al., 2003). These show that protecting DNA from damage may be one of the mechanisms by which theaflavins act as cancer suppressors.

Prevention of carcinogenesis: Prevention of carcinogenesis is one of the major strategies for cancer control. Apostolides et al. (1997) observed that theaflavins inhibited the genesis of tumor through inhibiting the cytochrome P-450 enzymes. TF3 could block the nitric oxide synthase and inhibit the growth of the tumor by down-regulating the activation of nuclear factor kappa B (NF-kappa B), which may be only one of multiple transcription factor inhibited by theaflavins (Lin et al., 1999; Michiyo et al., 2002). Furthermore, UVB-induced AP-1 activity could markedly blocked by theaflavins in a concentration-dependent manner, and TF3 appeared to have the strongest effect (Masaaki et al., 2000). In addition, theaflavins could inhibit arenite-induced AP-1 transcription activity and AP-1 DNA binding activity (Chen et al., 2000). Because AP-1 is important in

the process of tumor diffusion, the inhibitory effect on AP-1 activation may further explain the anti-tumor promotion action of theaflavins. Theaflavins also showed considerable antimutagenic effects against bacterial mutagens such as sodium azide, 4-nitro-o-phenylenediamine, cumine hydro-peroxide, 2-amino-fluorene and danthron, and the antimutagenic effects were significantly higher with liver homogenate (Gupta et al., 2002). Overexposure to oxidative stress, caused by environmental pollutants, are thought to increase the risk from cancer. Theaflavins as nature antioxidant could decrease the risk from environmental pollutant. Recently, Prosenjit et al. (2003) demonstrated that theaflavins would be potential chemopreventive agents for cancer and can afford protection from irreversible DNA damage and carcinogenesis.

Tumor growth inhibition: Theaflavins prevented the growth and transfer of tumor cell and produced 81.82% ($P < 0.001$) inhibition of inflammatory swelling induced by tumor (Mari et al., 1999; Minati et al., 2002). Tumor promoters recruit inflammatory cells to the application site and cancer development may also act by aggravating inflammation in the tissue. So, the anti-inflammatory activities of theaflavins may be another mechanism for the antitumor effect. Both flavanol skeleton and galloyl moiety were necessary for the inhibitory action. 12-O-tetradecanoylphorbol-13-acetate (TPA) as tumor promoter could cause cell transformation at high frequency and markedly induced NF-kappa B activation. Experiment showed theaflavins could inhibit the TPA-induced NF-kappa B activity (Nomura et al., 2000) NF-kappa B may

Anti-pathogenic properties

TF3 has been reported to have antibacterial activity against *Trichophyton mentagrophytes*, *T. rubrum*, *Candida albicans* and *Cryptococcus neoformans* in a dose- and contact time-dependent manner (Okubo et al., 1991). Toda et al. (1991) verified the antibacterial and bactericidal activities against methicillin resistant *Staphylococcus aureus* (MRSA) and food poisoning strain of *S. aureus*. Moreover, at the concentration of 1.875 mg/ml, theaflavins inhibit *Shigella* spp. (Vijaya et al., 1995). In 1999, TF2 or TF3 was reported to kill *Bordetella pertussis* entirely at 1 mg/ml in 24 h. Theaflavins can also inhibit adsorption of virus on cells but has no effect on multiplication of the virus in the cells, including influenza A and B, rotaviruses and enteroviruses (Mikio et al., 1990; Mukoyama et al., 1991). In the study of human immunodeficiency virus (HIV), theaflavins was found to suppress the transcription of HIV in the cell, and the gallic acid moiety of theaflavins can enhance suppressive activity (Nakane et al., 1994). There is synergism among the component fractions of

TF1, TF2A, TF2B, TF3 concerning the antiretroviral activity. The combination of TF1+TF2A+TF2B+TF3 was better than the sum of the activities of these four major fractions, individually (Clark et al., 1998). Generally, theaflavins are non-cytotoxic and are effective antibacterial and antiviral agents.

APPLICATION OF THEAFLAVINS

Prevention of coronary heart disease and atherosclerosis

The pharmacological function of theaflavins is responsible for its broad application in the field of medicine. Lipid peroxidation is closely associated with a risk for atherosclerosis and heart disease, and also accelerates aging (Prosenjit and Sukta, 2003). Theaflavins have been widely used to prevent coronary heart disease and atherosclerosis through regulating the level of blood fat, preventing lipid oxidation, clearing out oxygen free radical, anti-coagulin and promoting dissolution of fibre, restricting cell hyperplasia in the smooth muscle of human aorta and inhibiting the formation of lipid cake (Hiroshi et al., 1999; Jonathan et al., 1999).

Antihyperglycaemic activity

According to Mitsui Norin Co Ltd (1993), theaflavins is used to treat hyperglycaemia in Japan. Later, many researches tried to find the mechanism of antihyperglycaemic activity of theaflavins. Using streptozotocin (STZ)-induced diabetes in mice, there is indication that theaflavins may protect β -lymphocyte escaping the toxicity of STZ (Gomes et al., 1995). Moreover, theaflavins could increase insulin activity in vitro in an epididymal fat cell assay (Anderson and Polansky, 2002). Although the mechanism of antihyperglycaemia of theaflavins is not clear, the antihyperglycaemic activity of theaflavins is doubtless.

Cancer inhibition

Recently, Weisburge et al. (2002) found that many cancers could be inhibited by theaflavins, which are caused by lifestyle element such as cigarette and tobacco. And cancer of the breast, colon, prostate and pancreas could also be inhibited by theaflavins.

Other applications

Theaflavins could strongly inhibit glucosyl transferase (GTF) to protect tooth so as to prevent decayed tooth. Weisburge et al. (2002) demonstrated that theaflavins could alleviate the hurt of smoking to body when TF2 was

added to filter tip of cigarette. Theaflavins can play a favorable role in our daily lives in the prevention of a number of diseases including hyperglycaemia, atherosclerosis, heart disease, cancers and ageing. Therefore, theaflavins would be a new medical material in the future.

PROSPECT

Tea is a kind of Chinese traditional medicine with a long history. The American Cancer Society (ACS) publication, Nutrition and Cancer Prevention also stated, "In animal studies, some teas have been shown to reduce cancer risk". Theaflavins as a important component of tea have the ability of anti-cancer as well as other properties which will determine its future in the field of medicine. The dominating problem about the progress on theaflavins is still to enhance yield and purity of product as well as to lower the cost of production. Enzymatic oxidation by PPO has developed quickly and will be the focus of synthesis in the future. Moreover, based on the promising pharmacological activities of theaflavins, the manufacture of natural and high-powered medicine using purified theaflavins or theaflavins derivatives, would be another further research focus and hotspot in future.

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