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Effects of Oolong Tea Polyphenols, EGCG, and EGCG3″Me on Pancreatic α-Amylase Activity in Vitro

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ABSTRACT: In order to investigate the inhibitory effects and possible mechanisms of Oolong tea polyphenols, (−)-epigallocatechin gallate (EGCG) and (−)-epigallocatechin 3-*O*-(3-*O*-methyl) gallate (EGCG3″Me) on pancreatic α-amylase, the inhibition, enzyme kinetics, ultraviolet (UV) absorption spectrum and fluorescence spectrum of α-amylase were investigated. The results showed that Oolong tea polyphenols, EGCG, and EGCG3″Me all exhibited inhibitory effects against α-amylase, and their half inhibitory concentration (IC_{50}) values were 0.375, 0.350, and 0.572 mg/mL, respectively. The results of Lineweaver−Burk double reciprocal plot indicated that the inhibitory types of Oolong tea polyphenols and EGCG were competitive, whereas EGCG3″Me was in a noncompetitive pattern. Oolong tea polyphenols, EGCG, and EGCG3″Me all induced red-shift of UV absorbance and quenching of fluorescence of α-amylase, suggesting possible changes in the conformation of α-amylase. The differences of inhibitory effects and inhibition types for EGCG and EGCG3″Me might be due to their structural difference (the hydroxyl group at C-3 in D ring of EGCG substituted by methoxy group, forming EGCG3″Me).

KEYWORDS: *Oolong tea polyphenols, EGCG, EGCG3″Me, α-amylase, ultraviolet absorption spectroscopy, fluorescence spectroscopy*

INTRODUCTION

With economic development, obesity is increasing at an alarming rate and has become a worldwide health problem in both developed and developing countries.[1−3] It has been reported that obesity is associated with many diseases, such as type II diabetes mellitus, obstructive sleep apnea and malignant tumors that affect the quality of life and life expectancy of obese patients.[4] Generally, the excess digestion of carbohydrates is an essential step to gain weight. Therefore, reducing or slowing the digestive availability of carbohydrate derived calories is considered to be useful in the prevention of obesity. As we know, salivary or pancreatic α-amylase (EC 3.2.1.1, α-(1 → 4)-glucan-4-glucanohydrolase), catalyzing the hydrolysis of α-(1 → 4) glycosidic linkages in starch and related compounds,[5] is one kind of the key enzymes in digestive system.[6] Furthermore, it has been reported that inhibitors of pancreatic α-amylase are useful in controlling the level of postprandial blood glucose through slowing down the digestion of starch.[5] Thus, searching for effective and nontoxic α-amylase inhibitors has important significance on the prevention and treatment of carbohydrates related diseases, such as diabetes and obesity.

Tea polyphenols, the most biologically active components of tea, including (−)-epicatechin (EC), (−)-epicatechin gallate (ECG), (−)-epigallocatechin (EGC), and (−)-epigallocatechin gallate (EGCG),[7] have shown various health benefits, such as antioxidant,[8] antitumor,[9,10] antihypercholesterolemia,[11,12] antihyperglycemic,[13] and antiobesity[14] activities. Among the tea polyphenols, EGCG (Figure 1) is the major component and is believed to be responsible for most of the beneficial effects of tea.[8,15] (−)-Epigallocatechin 3-*O*-(3-*O*-methyl) gallate (EGCG3″Me, Figure 1), the unique *O*-methylated form of EGCG that is present only in limited Oolong and green teas, has been reported to have antiallergic and antihypertensive effects, potential prebiotic-like and strong hepatoprotective activities, cold preservation of primary rat hepatocytes and anti-

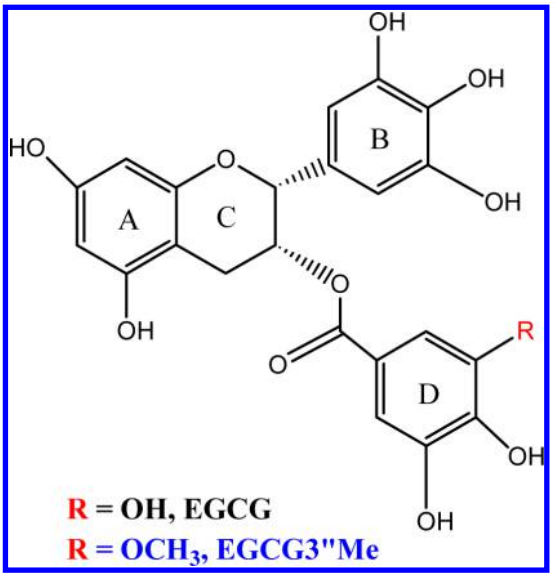

Figure 1. Chemical structures of EGCG and EGCG3″Me.

β-sheet-rich insoluble ubiquitnated protein aggregate properties.[7,16−21] In addition, Oolong tea is traditionally considered to have antiobesity and hypolipidaemic effects, and prolonged consumption of Oolong tea could maintain a lower body fat content and reduce body weight.[22−24] Some evidence have scientifically supported that the effect of Oolong tea reducing body weight is mainly attributed to its polyphenols. For example, Hsu et al.[23] have reported that polyphenol-enriched Oolong tea increased the fecal lipid excretion in humans

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without any side effects. The antiobesity effects of Oolong tea polyphenols may be produced by interacting with and inhibiting α-amylase, then reducing the carbohydrate digestibility.[25−27] However, the potential mechanisms of Oolong tea polyphenols in preventing obesity are complicated. Therefore, there is practical significance for development of α-amylase inhibitors through understanding the inhibitory effects and potential mechanisms of Oolong tea polyphenols, EGCG, and EGCG3″Me against α-amylase.

In this study, Oolong tea polyphenols, EGCG and EGCG3″Me were prepared from Oolong tea and their inhibitory effects against α-amylase were investigated. Furthermore, ultraviolet (UV) absorption spectroscopy and fluorescence emission spectroscopy were applied to analyze the nature of binding interaction of α-amylase with Oolong tea polyphenols, EGCG and EGCG3″Me.

MATERIALS AND METHODS

Materials. Oolong tea was purchased from a local market in Guangdong province of China. Toyopearl HW-40S and polyamide resins for column chromatography were purchased from Tosoh Corp. (Tokyo, Japan) and Qingdao Ocean Chemical Co., Ltd. (Qingdao, China), respectively. Standards of EGCG, ECG, EGC, and EC were purchased from Funakoshi Co., Ltd. (Tokyo, Japan), and standards of EGCG3″Me, (−)-catechin gallate (CG) and (−)-gallocatechin gallate (GCG) were prepared according to our reported method.[28] 3,5-Dinitrosalicylic acid (DNS) and porcine pancreatic α-amylase were purchased from Sigma Chemical Co., Ltd. (St. Louis, MO, U.S.A.). Folin-Ciocalteu reagent was purchased from Kayon Biological Technology Co., Ltd. (Shanghai, China). All solvents used for chromatographic purposes were grade of high performance liquid chromatography (HPLC).

Preparation of Oolong Tea Polyphenols, EGCG and EGCG3″Me. The preparation of Oolong tea polyphenols, EGCG and EGCG3″Me was performed according to the reported methods[7,28,29] with some modifications. Briefly, 100.0 g of Oolong tea was extracted with distilled water (1600 mL) at 96 °C for 40 min. The extract was centrifuged at 4500*g* for 15 min, and the resulting insoluble residue was treated again as described above. The supernatants were combined, concentrated and dissolved with deinoized water. The resulting solution was filtered through 0.45 μm microfiltration membrane and applied to a polyamide resin column (30 × 1.6 cm^2). The column was first washed with 2 times bed volume (BV) of distilled water, and then was eluted with 4 times BV of ethanol solution (80%, v/v). The elution of ethanol solution was collected, concentrated and freeze-dried by a Freeze-Dry System (Labconco, Kansas City, MO, U.S.A.), affording Oolong tea polyphenols.

Part of the Oolong tea polyphenols was dissolved with distilled water and loaded onto a Toyopearl HW-40S column (30 × 1.6 cm^2). The column was eluted with 80% ethanol solution at a flow rate of 2.5 mL/min, and the elution was autocollected (10 mL/tube). The eluted fractions were analyzed by HPLC with diode array detector (HPLC-DAD), and the fractions containing EGCG and EGCG3″Me were collected, concentrated, and freeze-dried, affording EGCG and EGCG3″Me, respectively. The structures of resulting EGCG and EGCG3″Me were characterized by HPLC, electrospray ionization time-of-flight mass spectrometry (ESI-TOF-MS) and ^{1}H nuclear magnetic resonance (NMR) spectrometry.

Determination of Contents of Oolong Tea Polyphenols, EGCG, and EGCG3″Me. The total polyphenols content in sample of Oolong tea polyphenols was determined by the Folin-Ciocalteu method according to the reported procedure.[30] The contents of EC, ECG, CG, GCG, EGCG, and EGCG3″Me were determined according to the reported method[28] by using an Agilent 1100 series HPLC-DAD system (Agilent Technologies Inc., St. Clara, CA, U.S.A.). The separation was achieved on a TSKgel ODS-100Z column (4.6 × 150 mm^2, 5 μm; Tosoh Corp., Tokyo, Japan) with a gradient mobile phase consisted of formic acid solution (A, pH 2.5) and methanol (B) at a flow rate of 1.0 mL/min. The linear gradient was as follows: mobile phase A from 82% to 40% in 15 min. The injection volume was 20 μL. The temperature of column oven was set at 40 °C. The wavelength of detector was set at 280 nm.

Assay of α-Amylase Inhibition. The determination of α-amylase inhibition was carried out according to the reported method[26,31] with slight modifications. First, α-amylase was dissolved in phosphate buffer saline (PBS, 0.02 mol/L, pH 6.8) at a concentration of 0.1 mg/mL. Various concentrations of Oolong tea polyphenols, EGCG, and EGCG3″Me solutions (0.25 mL) were mixed with α-amylase solution (0.25 mL) and incubated at 37 °C for 5 min. Then the reaction was initiated by adding 0.5 mL 1.0% (w/v) starch substrate solution to the incubation medium. After incubation at 37 °C for 3 min, the reaction was stopped by adding 0.5 mL DNS reagent (1% DNS, 0.2% phenol, 0.05% Na_2SO_3, and 1% NaOH in aqueous solution) to the reaction mixture and boiling at 100 °C for 8 min. After cooling to room temperature, the absorbance (Abs) at 540 nm was recorded by a spectrophotometer. The inhibition percentage was calculated by the following equation:

$$\text{inhibition rate } (\%) = [(\text{Abs}_1 - \text{Abs}_2)/\text{Abs}_1] \times 100$$

where Abs_1 and Abs_2 represent Abs at 540 nm without and with inhibitor, respectively. The half inhibitory concentration (IC_{50}) was calculated from inhibition curve.

Determination of Inhibition Types and V_{max} and K_m Values. The inhibition types of Oolong tea polyphenols, EGCG, and EGCG3″Me against α-amylase and values of maximum reaction velocity (V_{max}) and Michaelis constant (K_m) were determined by using the Lineweavere-Burk plots from the relevant Michaelis−Menten equations according the reported method.[32] The substrate solutions at concentrations of 6.0, 8.0, 10.0, 12.0, 14.0, and 16.0 mg/mL were reacted with α-amylase with or without inhibitor, respectively. The concentrations of α-amylase and inhibitor were 0.06 and 0.4 mg/mL, respectively, while distilled water was used as a control. The V_{max} and K_m values were obtained from the least-squares regression lines of the double reciprocal plots of the tested sample (inhibitor) concentration versus the reciprocal of reaction rate.

UV Absorption Spectroscopy. The general procedure to probe the interaction relationship between α-amylase and polyphenols by UV absorption spectroscopy was to mix the sample and enzyme solutions, incubate for 3−5 min and then determine the absorption spectrum in the range of 200−300 nm with a UV-2600 spectrophotometer (Shimadzu Corp., Kyoto, Japan). The concentration of α-amylase was 1.0 mg/mL, and the concentrations of Oolong tea polyphenols, EGCG and EGCG3″Me varied from 0.06 to 1.00 mg/mL. The control treatment was carried out without inhibitor.

Fluorescence Emission Spectroscopy. Fluorescence spectra were recorded with a Spectra Max Gemini XS spectrofluorometer (Molecular Devices, Sunnyvale, CA, U.S.A.) according to the reported method[33] with some modifications. Briefly, 3D fluorescence spectrum of α-amylase (0.1 mg/mL) was scanned under 20 °C and pH 6.9, the excitation wavelength (λ_{ex}) and emission wavelength (λ_{em}) were determined, respectively. Under the same conditions, the solutions of different concentrations of Oolong tea polyphenols, EGCG and EGCG3″Me were added into α-amylase solution and reacted for 3 min, respectively, then the relative fluorescence intensities were determined at excitation wavelength of 340 nm and emission wavelength of 280 nm.

Statistical Analysis. The results obtained were analyzed by SPSS version 16.0 (SPSS Inc.; Chicago, IL, U.S.A.). Any significant difference was determined by one-way analysis of variance (ANOVA) followed by the Tukey test for multiple comparisons at $P < 0.05$ level.

RESULTS AND DISCUSSION

The water extract of Oolong tea was first separated by polyamide column chromatography to afford Oolong tea polyphenols that the total polyphenols content was 83.92%. As shown in Figure 2B, the main components in Oolong tea

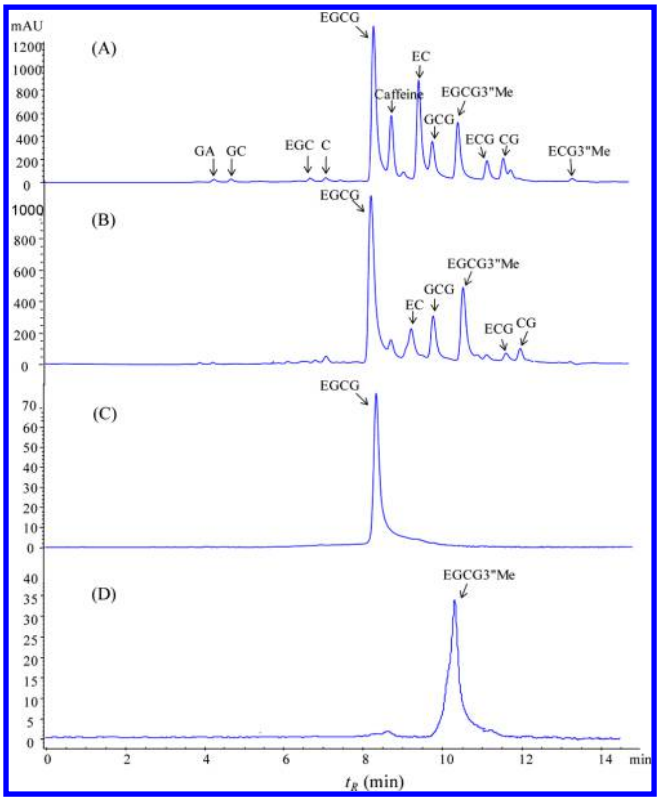

Figure 2. HPLC chromatograms of Oolong tea extract (A), Oolong tea polyphenols (B), purified EGCG (C), and purified EGCG3″Me (D).

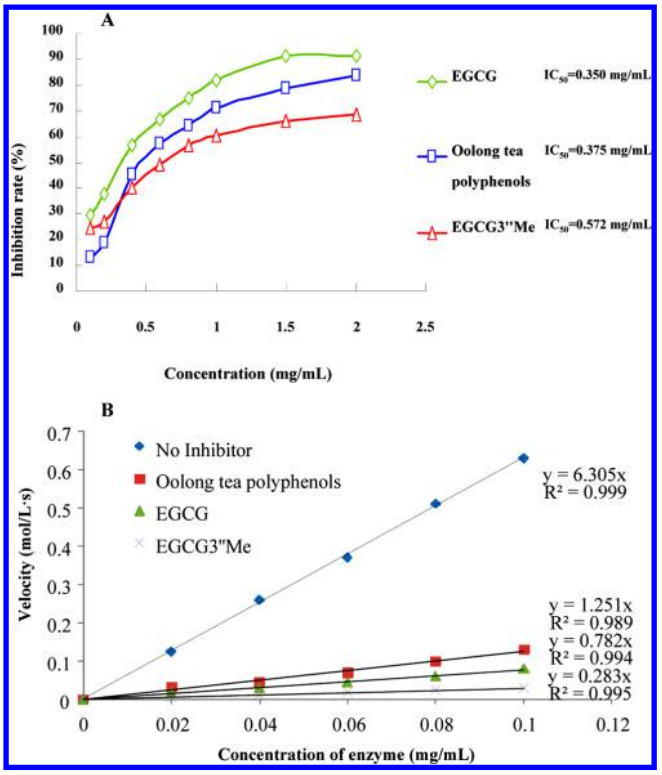

Figure 3. Inhibitory effects (A) and inhibition types (B) of Oolong tea polyphenols, EGCG, and EGCG3″Me on α-amylase.

polyphenols were EGCG (27.58 ± 1.73%), EGCG3″Me (14.30 ± 0.32%), GCG (9.28 ± 0.18%), EC (7.83 ± 0.11%), ECG (4.21 ± 0.29%), and CG (3.72 ± 0.17%). Notably, EGCG and EGCG3″Me occupied about 42% of the total polyphenols. The resulting Oolong tea polyphenols were separated by column chromatography of Toyopearl HW-40S, affording high purity of EGCG (98.00%, Figure 2C) and EGCG3″Me (96.68%, Figure 2D). By comparison with reported data,[16,34,35] their chemical structures were confirmed to be EGCG and EGCG3″Me by HPLC, ESI-TOF-MS, and ^{1}H NMR.

Inhibition of Oolong Tea Polyphenols, EGCG, and EGCG3″Me on α-Amylase. The inhibitory effects of Oolong tea polyphenols, EGCG and EGCG3″Me against α-amylase were investigated. As shown in Figure 3A, the IC_{50} values of Oolong tea polyphenols, EGCG and EGCG3″Me were 0.375, 0.350, and 0.572 mg/mL, respectively. EGCG exhibited relative lower IC_{50} value, indicating its relative higher inhibitory potency in comparison with Oolong tea polyphenols and EGCG3″Me. It is consistent with reported results that the inhibitory effects of C, EC, EGC, and EGCG on α-amylase are in the following order: EGCG > EC > EGC > C.[27,36] The results also suggested that EGCG should be one of the main inhibitory compounds against α-amylase in Oolong tea polyphenols. However, EGCG3″Me showed the highest IC_{50} value, indicating its relative weaker inhibitory effect on α-amylase. The inhibitory difference between EGCG and EGCG3″Me might be due to the difference in their structures. It has been reported that polyphenols interacted with α-amylase primarily through noncovalent interactions, such as hydrophobic interaction and hydrogen bonding between the hydroxyl groups of polyphenols and the catalytic residues of binding site in α-amylase, forming the polyphenols−enzyme complex.[27,37] Thus, the substitution of 3-OH in D ring of EGCG (Figure 1) by methoxy group might be the possible reason for relative weaker inhibitory effect of EGCG3″Me on α-amylase.[25,38] However, it has been reported that the methylation of polyphenols could greatly improve the transport through biological membranes and increase the oral bioavailability.[39,40] Thus, the in vivo inhibitory effect of EGCG3″Me is worthy of further study due to its relative higher bioavailabilty in vivo than EGCG.[41]

To explore the inhibition types of Oolong tea polyphenols, EGCG and EGCG3″Me against α-amylase, the kinetic reactions of α-amylase were investigated with different concentrations of α-amylase. As shown in Figure 3B, it was found that the lines obtained from the Lineweaver−Burk plots were all passed through the origin point (0, 0) of the coordinates, indicating that the inhibition types of Oolong tea polyphenols, EGCG, and EGCG3″Me against α-amylase were all reversible inhibition. To further investigate the inhibitory characteristics, Lineweaver−Burk double reciprocal plots were performed. As shown in Figure 4, the Lineweaver−Burk plots for Oolong tea polyphenols and EGCG showed the same intersection on *Y*-axis, indicating that the inhibition on α-amylase by Oolong tea polyphenols and EGCG were competitive. In contrast, EGCG3″Me had an intersection on *X*-axis, suggesting that EGCG3″Me inhibited α-amylase in a noncompetitive fashion. The difference of inhibition types of EGCG and EGCG3″Me, being worthy of further study, might also be due to the structural difference between EGCG and EGCG3″Me. Furthermore, kinetic inhibition parameters on α-amylase were calculated according to Lineweaver−Burk plots, and the results are shown in Table 1. In the presence of EGCG3″Me, V_{max} value for α-amylase decreased, while the value of K_m remained unchanged. In contrast, K_m value for α-

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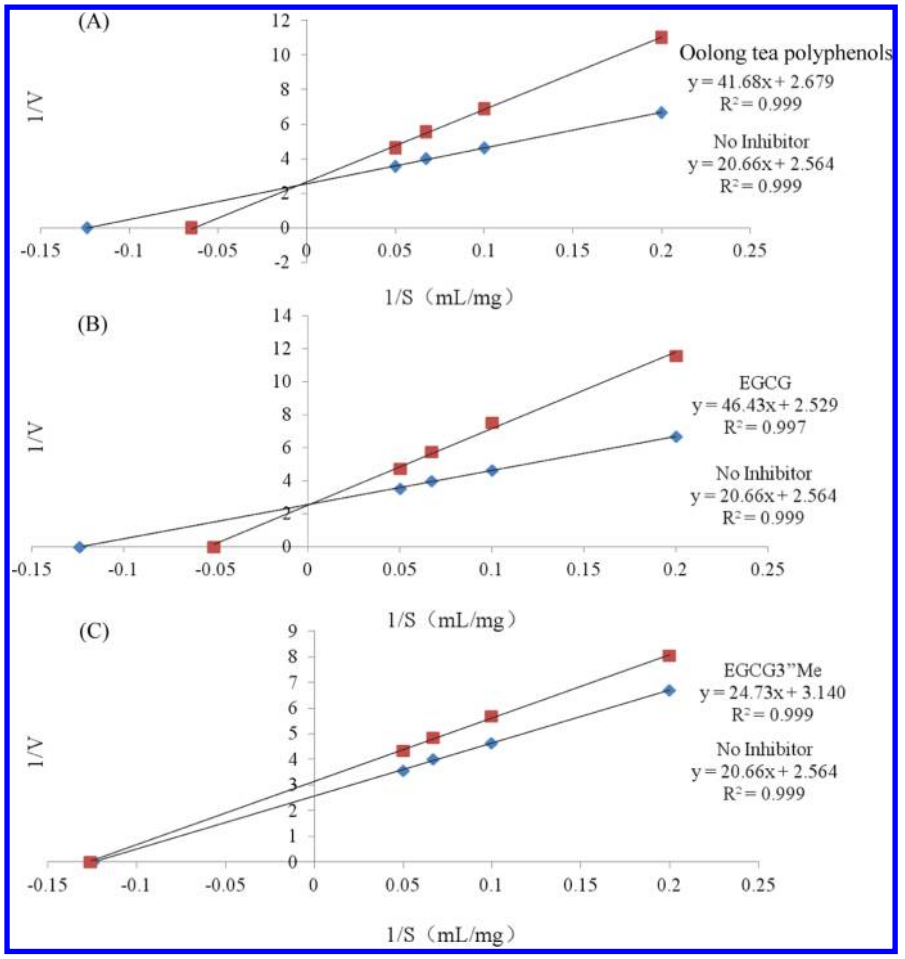

Figure 4. Lineweaver–Burk plots of the reaction of α-amylase in the presence of Oolong tea polyphenols (A), EGCG (B), and EGCG3″Me (C).

Table 1. Kinetic Results of Inhibition against α-amylase by Oolong tea Polyphenols, EGCG, and EGCG3″Me

	K_m (mg/mL)	V_{max} (mg/mL·min)	inhibition type
no inhibitior	8.06[a]	0.39[a]	
Oolong tea polyphenols	15.38[b]	0.38[a]	competitive inhibition
EGCG	18.51[c]	0.39[a]	competitive inhibition
EGCG3″Me	7.94[a]	0.32[b]	noncompetitive inhibition

amylase increased while V_{max} value remained unchanged in the presence of Oolong tea polyphenols or EGCG. Such results are consistent with their inhibitory characteristics.

Changes of α-Amylase UV Spectra Induced by Oolong Tea Polyphenols, EGCG or EGCG3″Me. α-Amylase has an absorption peak at 270 nm due to the presence of tryptophan (Trp) and tyrosine (Tyr) aromatic heterocyclic residues, thus, the change of microenvironment of the aromatic amino acid residues would cause the change of α-amylase absorption wavelength. As shown in Figure 5, changes in UV spectra of α-amylase were observed after the addition of Oolong tea polyphenols, EGCG, and EGCG3″Me. With the increase of sample concentration, a red-shift for α-amylase was found, but there was no significant difference among the samples. It has been reported that the main mechanism of polyphenols-enzyme binding was considered to be noncovalent interactions, including hydrogen bond and hydrophobic association.[42−44] Interaction of polyphenols and α-amylase was conducive to intermolecular and intramolecular agglomeration effects of enzyme, which made the residues of Trp and Tyr within the aromatic heterocyclic and hydrophobic groups surrounded by α-amylase exposed, led to the microenvironmental change of the aromatic amino acid residues in the spatial structure and caused the conformational change of protein. However, the composition and quantity of the polar groups in the enzyme protein may affect the formation and stability of hydrogen bonds between polyphenols and α-amylase.[27,37] Thus, with the increase of sample concentration, the protein (enzyme) peptide bond extension phenomenon occurred, hydrophobic interaction enhanced slightly, and then absorption peak and red-shift amplitude increased correspondingly.

Quenching of α-Amylase Fluorescence Spectra by Oolong Tea Polyphenols, EGCG, or EGCG3″Me. Fluorescence quenching measures the decrease of the quantum yield of fluorescence from a fluorophore induced by a variety of molecular interactions with a quencher molecule. It is a useful approach to investigate intermolecular interactions. In the present study, therefore, the interaction between polyphenols and α-amylase was investigated from the changes of the fluorescence emission spectra of α-amylase in the absence and presence of polyphenols. From 3D fluorescence spectrum of α-amylase (Figure 6A), it was found that α-amylase had two absorption peaks at excitation wavelength of 340 nm, whereas Oolong tea polyphenols did not show any interference (Figure

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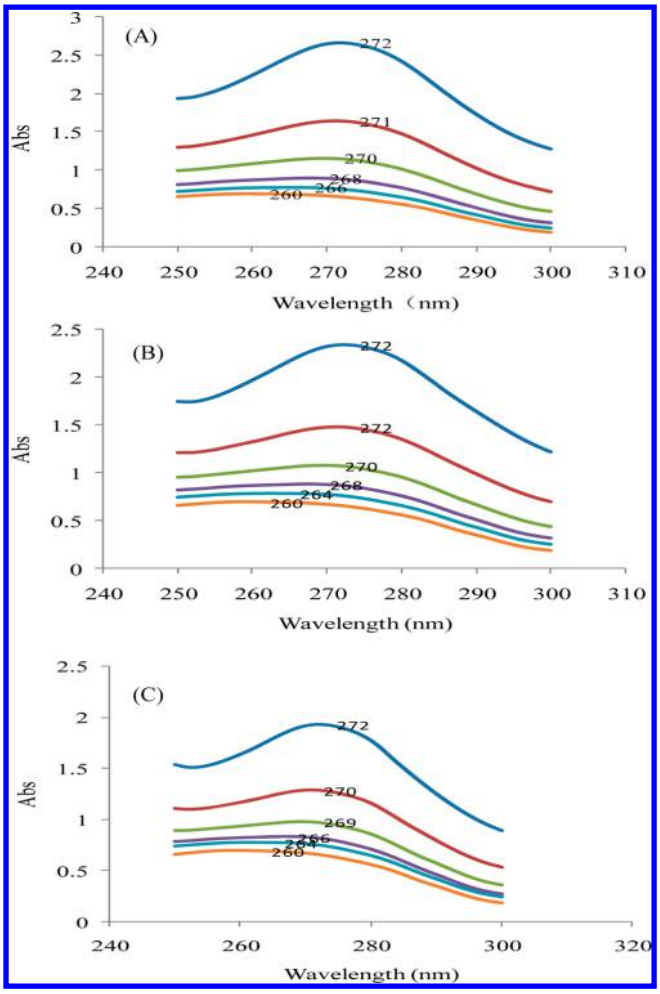

Figure 5. UV absorption spectra of α-amylase in the presence of different concentrations of Oolong tea polyphenols (A), EGCG (B), and EGCG3″Me (C). From top down, 1.00, 0.500, 0.25, 0.13, 0.06, and 0.00 mg/mL for sample concentration.

6B). With the addition of Oolong tea polyphenols, EGCG, or EGCG3″Me, the fluorescence emission intensity of α-amylase decreased (Figure 6C−H), indicating that Oolong tea polyphenols, EGCG, and EGCG3″Me could quench the intrinsic fluorescence of α-amylase. The reason might be due to the fact that polyphenols bind to α-amylase, which leads to the unfolding of α-amylase fluorescent amino acid residues and causes quenching of the inherent fluorescence property. The observed spectral changes of α-amylase reflected direct or indirect interactions of polyphenols with the hydrophobic and hydrophilic regions located in the vicinity of Trp and Tyr and induced conformational changes of α-amylase. As Brayer et al.[5] reported, inhibitors may directly contact with the side chains of Asp197, Glu233, and Asp300 of α-amylase. Computational ligand docking indicates that the inhibitory activity of EGCG on α-amylase depends on hydrogen bonds between the hydroxyl groups of EGCG and the catalytic residues of the binding site and formation of a conjugated π-system that stabilizes the interaction with active site.[37,45,46]

As shown in Figure 6C−H, Oolong tea polyphenols, EGCG, and EGCG3″Me exhibited different quenching effects on α-amylase. The absorption characteristics of Oolong tea polyphenols and EGCG with α-amylase was roughly the same, but it was significantly different from that of EGCG3″Me. The difference in quenching effects between EGCG and EGCG3″Me might be due to the difference of their structures: hydroxyl group for EGCG and methoxy group for EGCG3″Me at C-3 of D ring (Figure 1). The interaction between polyphenol and α-amylase was reported to depend on the structures of polyphenols and α-amylase.[27,37] Some researchers also explored the structure−activity relationship of inhibitors with α-amylase and possible mechanisms by molecular docking and other measurements, such as the binding of flavonols, flavones, and EGCG with human salivary α-amylase.[6,38,47] On the basis of the present results and some previous reports, thus, we conclude that Oolong tea polyphenols, EGCG, and EGCG3″Me may act as antiobesity factor, in terms of their inhibitory effects on α-amylase, due to the cooperative effects of hydrophobic association and hydrogen bond formation between polyphenols and α-amylase.

The relationship between fluorescence quenching intensity and concentration of quencher can be described by following Stern−Volmer equation.[46,48]

$$F_0/F = 1 + k_q\tau_0[Q] = 1 + K_{sv}[Q]$$

F_0 and F are the fluorescence intensities in the absence and presence of quencher, respectively. k_q is the bimolecular quenching constant, τ_0 is the native lifetime of the fluorophore, and $[Q]$ is the concentration of quencher. K_{sv}, the Stern−Volmer quenching constant, is given by $k_q\tau_0$. A linear Stern−Volmer plot is generally indicative of a single class of fluorophore in a protein, all equally accessible to the quencher. Here, the following modified Stern−Volmer equation was applied to analyze the fluorescence quenching.

$$\frac{F_0}{F_0 - F} = \frac{1}{fK[Q]} + \frac{1}{f}$$

where f is the fraction of fluorophore accessible to the quencher and K is the modified Stern−Volmer quenching constant which is very close to the binding constant. The plot of $F_0/(F_0 - F)$ versus $1/[Q]$ yields $1/f$ as the intercept and $1/fK$ as the slope. Thus, from the intercept and slope, the f and K can be obtained. The fluorescence quenching parameters are list in Table 2. On the basis of the fluorescence quenching parameters (K_{sv} and K_q), we could verified that Oolong tea polyphenols and EGCG had almost the same binding ability on α-amylase, which further proved that EGCG was one of the main inhibitory ingredients in Oolong tea polyphenols against α-amylase as mentioned above. Besides, the lower K value for EGCG3″Me indicated a lower affinity interaction between α-amylase and EGCG3″Me compared to EGCG. EGCG-α-amylase complex was reflected to have more exposure of Trp residues and unfolding of protein structure than EGCG3″Me-α-amylase. It has been reported that the binding constants for the larger polyphenol-protein complexes are larger than those of smaller polyphenol−protein adducts, which can be due to the presence of more hydroxyl groups associated with the polyphenols.[49] Due to the tightening of protein structure through intramolecular interactions such as hydrogen bonds, polyphenol−α-amylase complexes might induce Trp residues in a more hydrophobic environment. We could speculate that, therefore, hydroxyl but not the methoxy group in polyphenols played the main role in the binding process.

Fluorescence quenching can be dynamic (resulting from collisional encounters between fluorophore (α-amylase) and quencher) or static (resulting from formation of a ground state

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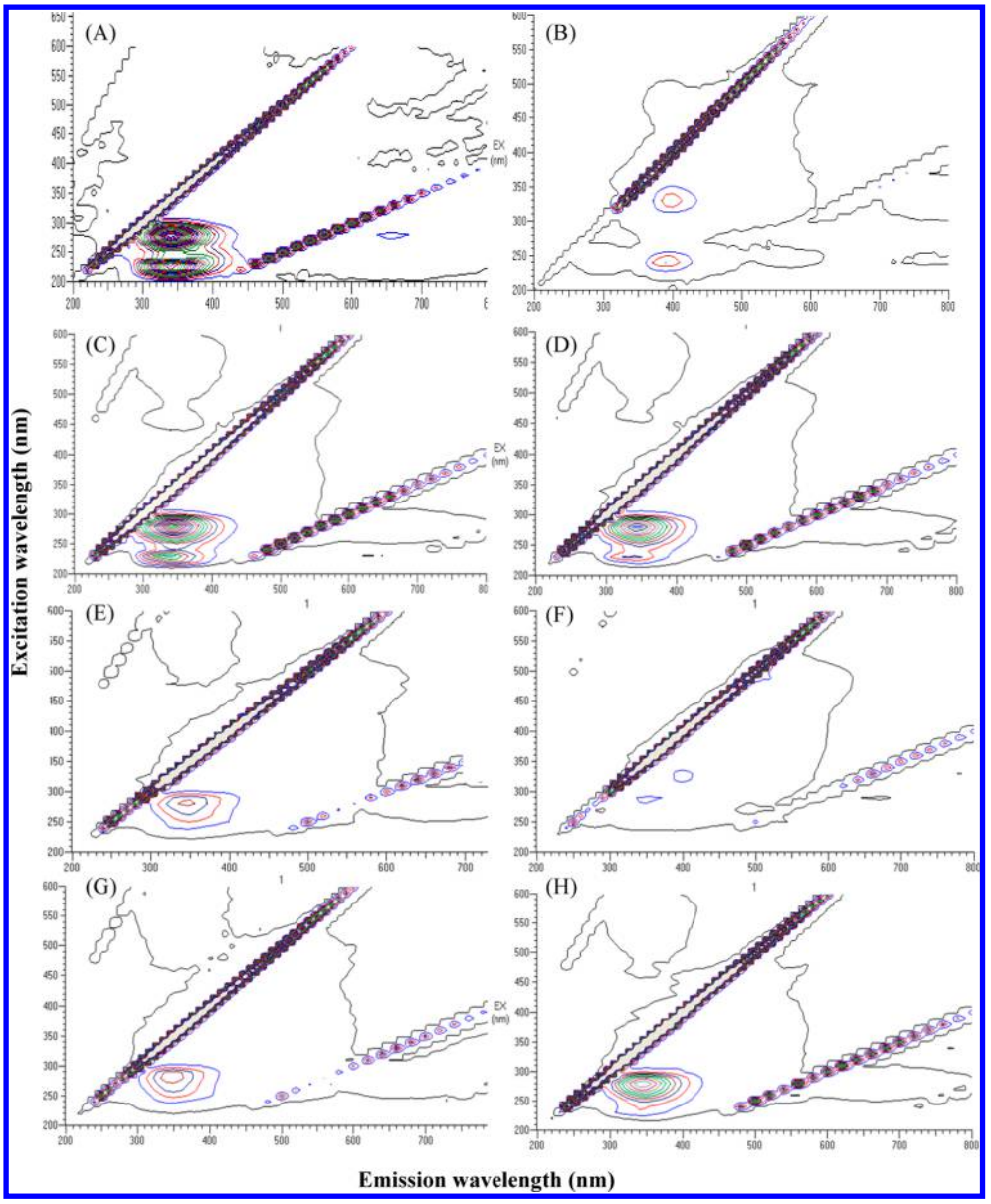

Figure 6. 3D Fluorescence spectra of α-amylase (A) and Oolong tea polyphenols (B) and fluorescence quenching effects on α-amylase by Oolong tea polyphenols (C, D, E, and F at concentrations of 10, 40, 100, and 200 μg/mL, respectively), EGCG (G, 100 μg/mL), and EGCG3″Me (H, 100 μg/mL).

Table 2. Fluorescence Quenching Parameters for the Interactions of Oolong Tea Polyphenols, EGCG, and EGCG3″Me with α-Amylase

	K_{sv} (L·g^{-1})	K_q (× 10^{12} L·g^{-1}·s^{-1})	K (L·g^{-1})
Oolong tea polyphenols	53177.0^a	5.318^a	23684.1^a
EGCG	53174.4^a	5.317^a	39272.0^b
EGCG3″Me	61542.0^b	6.154^b	33746.0^b

between fluorophore and quencher).[50] Dynamic quenching is related to diffusion, and the rising of the temperature will increase the diffusion coefficient, thus increase the bimolecular quenching constant. Conversely, if it is static quenching, the temperature rise will improve the stability of the complexes, quenching constant will diminish.[51] As shown in Figure 7, the quenching constant slope increased slightly with the increase of temperature. Hence, the fluorescence quenching of Oolong tea polyphenols, EGCG, and EGCG3″Me on α-amylase was determined to be dynamic one.

In conclusion, the inhibitory effects on α-amylase of Oolong tea polyphenols, EGCG, and EGCG3″Me, prepared from Chinese Oolong tea, were investigated in the present study. EGCG appeared to be one of the main compounds in Oolong tea polyphenols for the inhibitory effects against α-amylase. The differences of inhibitory effect and inhibition type between EGCG and EGCG3″Me might be due to substitution of the hydroxyl group in EGCG by methoxy group. In addition, it is speculated that Oolong tea polyphenols, EGCG, and EGCG3″Me exhibited their inhibitory effects against α-amylase through the cooperative effects of hydrophobic association and hydrogen bond formation between polyphenols and α-amylase. Further works on inhibitory effect in vivo and possible action mechanisms of EGCG3″Me are in progress.

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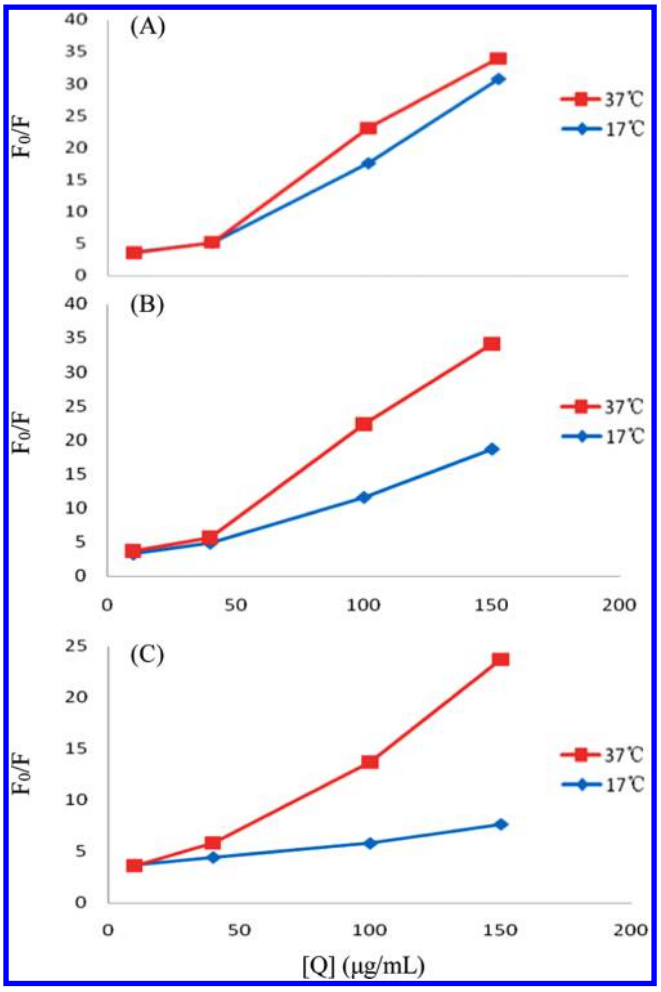

Figure 7. Stem–Volmer diagrams of Oolong tea polyphenols (A), EGCG (B), and EGCG3″Me (C).

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Notes

The authors declare no competing financial interest.

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