

The Interaction between EGCG3''Me in Tea and Alpha-glucosidase

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This paper aims to make up for the research gap on the interaction between epigallocatechin3-O-(3''-O-methyl) gallate (EGCG3''Me) and alpha-glucosidase (α -glucosidase). Therefore, the interaction between the EGCG3''Me in tea and α -glucosidase was analysed with fluorescence spectrometry to ascertain the binding constants, the number of binding sites and thermodynamic parameters. The results indicate that the binding between EGCG3''Me and α -glucosidase quenched the internal fluorescent intensity of the latter; the binding constants decreased with the rise of temperature, revealing that the binding ability was alleviated and the fluorescence quenching obeyed the static quenching mechanism; ΔH was negative and ΔS was positive in the binding reaction, signifying that the main interaction forces include the hydrophobic force and hydrogen bonds; the ΔG of the two molecules was negative, an evidence of the spontaneity of the reaction. Combined with the 3D fluorescence spectra changes of α -glucosidase, these results mean that the binding of the molecules was realized through the tryptophan and tyrosine residues in the α -glucosidase. The research proves that the EGCG3''Me in tea is potentially a natural inhibitor of α -glucosidase, and sheds new light on the prevention and treatment of type 2 diabetes.

1. Introduction

Alpha-glucosidase (α -glucosidase), an enzyme naturally produced in many organisms, is capable of catalysing and hydrolysing the α -glucosyl residue from the nonreducing end of substrates containing α -glycosidic bond. Hence, this enzyme can reduce glucose in starch and other polysaccharides through the α -1,4-glycosidic bond (Chen et al., 2009; Kou et al., 2006). The molecular mass of α -glucosidase varies greatly depending on the source, ranging from 40,000 to 150,000.

The α -glucosidase (EC 3.2.1.20) is one of the enzymes located on the brush border of small intestinal villus cell. The membrane-bound intestinal α -glucosidases hydrolyse oligosaccharides, trisaccharides, and disaccharides to glucose and other monosaccharides in the small intestine. The hydrolysis process pushes up the postprandial glucose level, and indirectly contributes to the occurrence of type 2 diabetes (Holman et al., 2014; Ceriello, 2005). To prevent the disease, it is necessary to find an effective inhibitor of α -glucosidase, aiming to slow down the production of glucose and the increment of postprandial glucose level.

It has been reported that tea polyphenols can effectively inhibit the activity of α -glucosidase, and slow down the rapid elevation of postprandial glucose (Geng et al., 2007; Quan et al., 2006). Yilmazer-Musa et al. (2012) disclosed that the epigallocatechin gallate (EGCG) monomer in green tea and white tea have an obvious inhibitory effect on α -glucosidase. Jishu Quan et al. (2008) discovered that the extracts of the green tea strongly suppress the activity of α -glucosidase. Liu et al. (2016) explored the inhibitory effect of EGCG on activity of α -glucosidase in Caco-2 cells.

Recently, epigallocatechin3-O-(3''-O-methyl) gallate (EGCG3''Me) has attracted much attention. In the existing studies, EGCG3''Me was isolated and synthesized through the screening of tea germplasm, and proved to have excellent antiallergic and anti-tumour effects (Luo et al., 2008; Wu et al, 2010; Zhao, 2012). For instance, Qunqin Fei et al. (2014) studied the inhibitory effect of EGCG3''Me on α -glucosidase. However, there has been no report on the interaction between EGCG3''Me and α -glucosidase. To make up for the gap, this paper takes EGCG3''Me monomer as the inhibitor to analyse the effect of EGCG3''Me on α -glucosidase by

fluorescence spectrometry, aiming to disclose the interaction mechanism between the two and lay a theoretical basis for the development of α -glucosidase inhibitors.

2. Materials and Methods

2.1 Reagents

The α -glucosidases (*Bacillus thermophilus*) were purchased from Sigma USA, the EGCG3"Me was procured from Sichuan Victory Biological Technology Co., Ltd., and the other chemical reagents were analytically pure.

2.2 Instruments

The following instruments were adopted for our research: XS 205DU analytic balance (Mettler Toledo), constant temperature water bath (DAIHAN Scientific), and F-7000 fluorescence spectrophotometer (Hitachi).

2.3 Methods

(1) Fluorescence spectra analysis

Various concentrations (0.01, 0.02, 0.04, 0.08 and 0.10mg/mL) of EGCG3"Me were separately added into 0.5U/mL α -glucosidase solution, and the volume of the mixture was normalized to 3mL with phosphate-buffered saline (PBS). The 1.0cm quartz cells were used, the slit width of excitation and emission gratings was 5.0nm, and the excited wavelength (λ_{ex}) was 280nm. The fluorescence emission spectra of α -glucosidase and the mixture of α -glucosidase and EGCG3"Me were scanned under the constant temperature within the wavelength range of 290nm~450nm.

(2) 3D fluorescence spectra analysis

The scanning ranges for both excited and emitted wavelengths were from 200 to 600nm. The slit width was 5nm. The author obtained the 3D fluorescence spectra of pure α -glucosidase, pure EGCG3"Me, and the mixture of α -glucosidase and EGCG3"Me.

3. Results and Analysis

3.1 The inhibitory effect of EGCG3"Me on α -glucosidase

The fluorescence quenching effect:

The internal fluorescence of α -glucosidase mainly came from the tryptophan and tyrosine residues. At the excited wavelength of 280nm, the α -glucosidase emitted its internal fluorescence at the wavelength of 358nm, while EGCG3"Me did not emit any fluorescence signal at this wavelength.

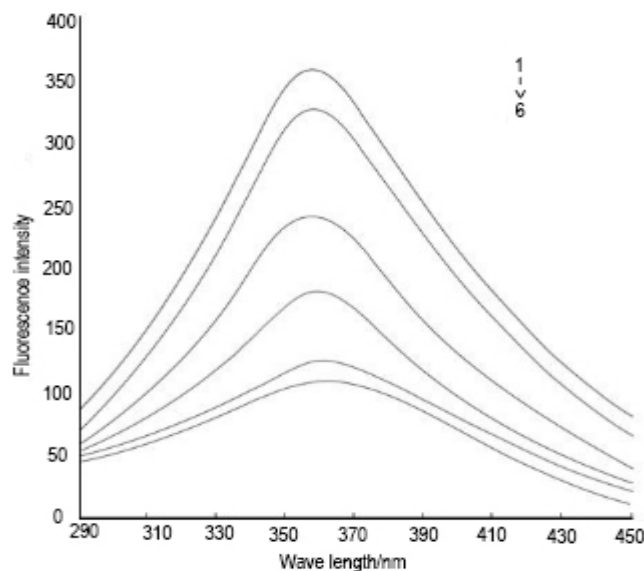


Figure 1: The fluorescence quenching effect of EGCG3"Me on α -glucosidase.

As shown in Figure 1, when the concentration of α -glucosidase remained constant, the shape of α -glucosidase peak did not change despite the increase of EGCG3"Me concentration; meanwhile, the fluorescence intensity of α -glucosidase decreased significantly, a signal of regular fluorescence quenching.

Furthermore, the faint redshift (from 358 to 362nm) at the maximum emission wavelength (360nm) reveals the occurrence of the interaction between EGCG3^{Me} and α -glucosidase, the formation of the compound, and the conformational change of enzyme protein. In this case, the microenvironment of the chromophoric groups was more polarized, and the hydrophobicity was altered, leading to the decline in enzyme activity (Wang et al., 2013; Liu et al., 2014; Lan et al., 2015).

3.2 The binding constants and thermodynamic parameters

During the interaction between EGCG3^{Me} and α -glucosidase, the relationship between the fluorescence quenching intensity and α -glucosidase concentration was calculated by the Stern-Volmer equation (Lakowicz, 2006):

$$F_0/F = 1 + K_q \tau_0 [Q] = 1 + K_{sv} [Q] \quad (1)$$

where F_0 and F are the fluorescence intensity of α -glucosidase before and after the addition of the quencher, respectively; K_q is the rate constant of the bimolecular quenching process; τ_0 (10⁻⁸s) is the lifetime of the fluorophore without the quencher; $[Q]$ is the concentration of the quencher; K_{sv} is the quenching constant. The Stern-Volmer quenching curve was obtained using $[Q]$ as the x-axis and the ratio of F_0 to F as the y-axis.

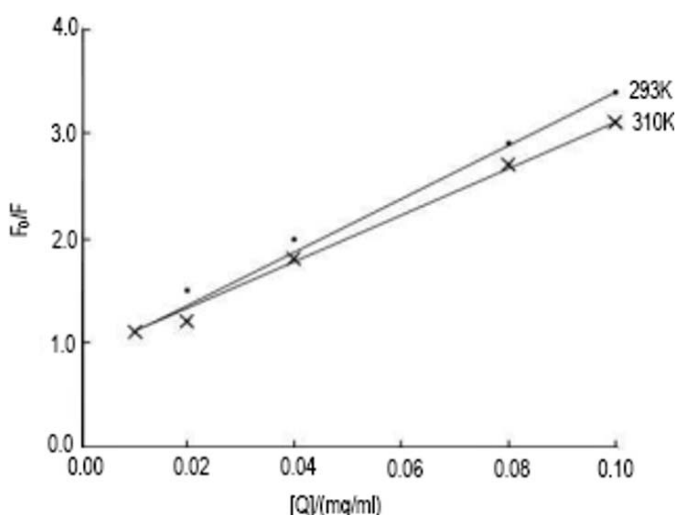


Figure 2: The Stern-Volmer curve of EGCG3^{Me} on α -glucosidase at different temperatures.

Then, the rate constant K_q and quenching constant K_{sv} of the interaction between EGCG3^{Me} and α -glucosidase were calculated according to the quenching curve.

Table 1: The rate constant K_q and quenching constant K_{sv} of EGCG3^{Me} on α -glucosidase at different temperatures

Samples	Temperature T/K	K_q /[L/(mol·S)]	K_{sv} /(L/mol)
EGCG3 ^{Me}	293	5.29×10^{12}	5.29×10^4
	310	2.12×10^{12}	2.12×10^4

After that, the Stern-Volmer equation was revised. Then, the relationship between the fluorescence quenching intensity and the quencher concentration can be expressed as:

$$\lg[(F_0 - F)/F] = \lg K_a + n \lg [Q] \quad (2)$$

where F_0 and F are the fluorescence intensity of α -glucosidase before and after the addition of the sample, respectively; K_a are the binding constants; n is the number of the binding sites; $[Q]$ is the concentration of the sample (mg/mL). The curve was obtained with $\lg [Q]$ as the x-axis and $\lg [(F_0 - F)/F]$ as the y-axis. The binding constants (K_a) and the number of binding sites (n) of the interaction were calculated by the intercept and slope of a line (Table 2).

Table 2: The binding constants and the number of binding sites of the interaction between EGCG3" Me and α -glucosidase at different temperatures.

Samples	Temperature T/K	K_a (L/mol)	n	ΔG (kJ/mol)	ΔH (kJ/mol)	ΔS [J/(mol·K)]
EGCG3"Me	293	2.09×10^5	1.23	-29.85	-13.33	56.37
	310	1.55×10^5	1.03	-30.80		

The binding forces between small molecules and proteins include hydrogen bonds, van der Waals force, electrostatic attraction, and hydrophobic force (Wu et al, 2013). The binding type of the two types of molecules was identified based on the enthalpy change (ΔH) and entropy change (ΔS) before and after the reaction. The values of ΔH and ΔS can be calculated by the Van't Hoff equation.

$$\ln K = -\Delta H / RT + \Delta S / R \quad (3)$$

$$\ln K_2 - \ln K_1 = (1/T_1 - 1/T_2) \Delta H / R \quad (4)$$

$$\Delta G = \Delta H - T \Delta S \quad (5)$$

Where K is the binding constant in the reaction system at a certain temperature; R is the gas constant. Table 2 lists the values of ΔH , ΔS and ΔG calculated by the equation.

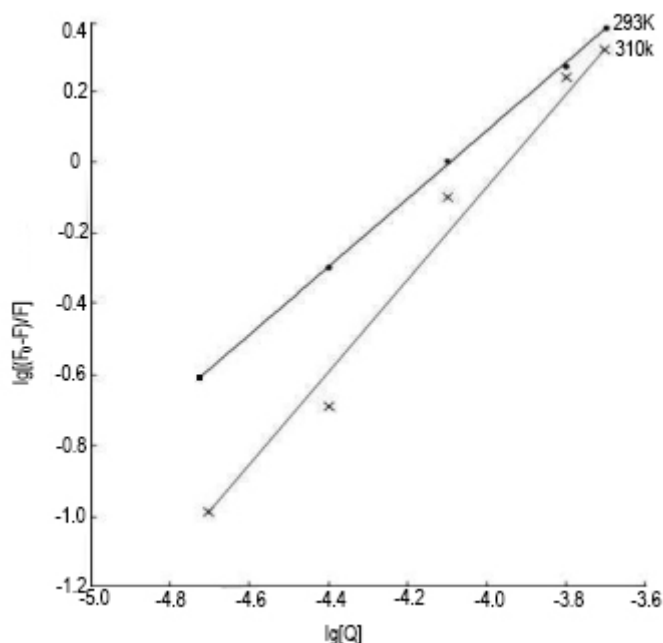


Figure 3: Double logarithmic plot of the fluorescence quenching effect of EGCG3" Me on α -glucosidase at different temperatures.

The above data demonstrate the strong binding forces between EGCG3" Me and α -glucosidase. It can be seen that the binding constants decreased with the rise in temperature, revealing a decline in binding forces and an alleviation of the quenching effect. This means the fluorescence quenching obeys static quenching mechanism (Lakowicz, 2006). The value of 'n' was around 1, indicating that the two molecules had one binding site. In addition, ΔH was negative and ΔS was positive in the binding reaction of EGCG3" Me and α -glucosidase. Hence, the main interaction forces include the hydrophobic force and hydrogen bonds. Furthermore, the ΔG of the two molecules was negative, an evidence of the spontaneity of the reaction (Ross D P and Subramanian S, 1981; Saini S et al, 2006).

3.3 The 3D fluorescence quenching effect of EGCG3" Me on α -glucosidase

According to Figure 4, the α -glucosidase exhibited two strong fluorescence peaks at the excited wavelengths of 230nm and 280 nm, and the emitted wavelength of 340 nm. The 230nm/340nm fluorescence peak is

related to the skeleton structure of enzyme protein peptide chain, reflecting the secondary structure of the protein. Meantime, the 280nm/340nm fluorescence peak is attributed to the tyrosine and tryptophan residues in the enzyme protein (Singha et al., 2013). In the detection range, EGCG3"Me did not emit any fluorescence signal. The two fluorescence peaks of α -glucosidase were heavily quenched after the addition of EGCG3"Me, a signal of the binding of EGCG3"Me and α -glucosidase. Owing to the binding action, the α -glucosidase protein peptide chains were extended, the microenvironment of intramolecular chromophores residues was altered, and, most importantly, the enzyme activity was inhibited.

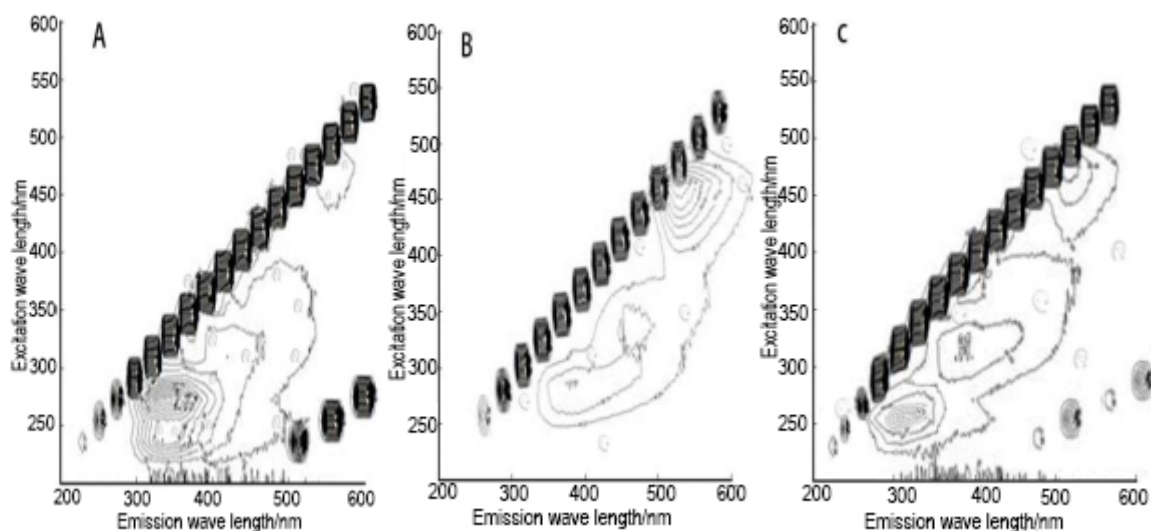


Figure 4: 3D fluorescence spectra of pure α -glucosidase, pure EGCG3"me and the interaction product of α -glucosidase and EGCG3"Me.

4. Conclusion

This paper explores the interaction mechanism between EGCG3"Me and α -glucosidase. The fluorescence spectrometry results indicate that EGCG3"Me and α -glucosidase formed a relatively stable compound, which induced the fluorescence quenching of α -glucosidase. The interaction of these molecules relied on both hydrophobic force and hydrogen bonds. There was one binding site, and the reaction was spontaneous. In the meantime, the binding of the molecules induced the change of 3D fluorescence spectra, which, in turn, altered the microenvironment of intramolecular fluorescent chromophores. The resulting extension of α -glucosidase protein peptide chains further changed the conformation of the enzyme protein and inhibited its enzyme activity. The research proves that the EGCG3"Me in tea is potentially a natural inhibitor of α -glucosidase, and sheds new light on the prevention and treatment of type 2 diabetes.

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