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Characterization of Binding Interactions of (−)-Epigallocatechin-3-gallate from Green Tea and Lipase

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ABSTRACT: Understanding the interaction of (−)-epigallocatechin-3-gallate (EGCG) and lipase is important for understanding EGCG's inhibition of lipase. In this paper, the interaction of EGCG and porcine lipase was characterized by fluorescence spectroscopy, circular dichroism (CD), isothermal titration calorimetry, and molecular docking. EGCG might act as a noncompetitive pancreatic lipase inhibiter. EGCG bound to lipase with affinity of $K_a = 2.70 \times 10^4$ L mol^{-1}. Thermodynamic features suggested that the interaction process was spontaneous, with hydrogen bonds and electrostatic force perhaps primarily responsible for the interaction, with 1:1 interaction of lipase and EGCG. CD studies indicated conformation change of lipase on binding to EGCG. Furthermore, docking results supported experimental findings and revealed hydrogen-bonding interaction with Val21, Glu188, and Glu220. This noncovalent bonding between EGCG and lipase alters the molecular conformation of lipase, which decreases the enzyme catalytic activity. This study will help further understand the antiobesity mechanisms of green tea.

KEYWORDS: *epigallocatechin gallate, green tea, lipase, obesity, interaction*

INTRODUCTION

Obesity has increased at an alarming rate in recent years and is becoming a severe health threat.[1] In Asian countries, green tea is traditionally thought to help with weight loss.[2] Green tea is known to possess many health benefits, including antioxidative, anticancer, anti-inflammatory, and antibacteria effects.[3−9] Today, there is increasing interest in the effects of green tea on diabetes. The health effects of green tea are mainly attributed to its polyphenols, which have strong antioxidative activity.[10] Catechins are the predominant polyphenols in green tea and comprise epigallocatechin gallate (EGCG), epigallocatechin, epicatechin gallate, and epicatechin.[11] EGCG (Figure 1) is the most abundant and potent constituent of polyphenols in green tea.[12]

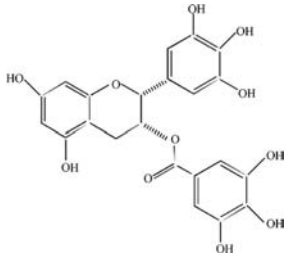

Figure 1. Chemical structure of epigallocatechin gallate (EGCG).

Numerous cell, animal, human, and clinical studies have investigated the antiobesity effects of green tea[13−16] and have scientifically supported that green tea reduces body weight by "eliminating fat". Green tea catechins, especially EGCG, appear to have antiobesity effects.[17] However, the potential mechanisms of green tea catechins in preventing obesity are complicated. They may be related to certain pathways, such as lipid and carbohydrate metabolism, modulations of energy balance, endocrine systems, redox status, food intake, and activities of different types of cells.[18] Green tea catechins can reduce carbohydrate and fat absorption by inhibiting various gastrointestinal enzymes, which is one of the mechanisms of the antiobesity effects of green tea.[19−22]

Green tea catechins have a strong ability to interact with digestive enzymes, which reduce food digestibility.[23−25] Green tea polyphenols may reduce lipid digestibility by inhibiting lipase activity. EGCG is the most active component of green tea. Therefore, understanding the interaction of EGCG and lipase is important for understanding its action against obesity.

In this study, we determined the lipase-inhibitory activity of EGCG and characterized the interactions between EGCG and porcine lipase in more detail. We used fluorescence spectroscopy, circular dichroism (CD), isothermal titration calorimetry (ITC), and molecular docking analyses to help elucidate the nature of binding between catechins and lipase.

MATERIALS AND METHODS

Materials. EGCG (with a purity of >99%) was acquired from Chengdu Biopurify Photochemicals (China), and porcine pancreatic lipase (200 units/mL) was from Sigma Chemical (St. Louis, MO, USA).

Assay of Lipase-Inhibitory Activity and Inhibitory Pattern. Lipase activity was determined using 2,4-dinitrophenyl butyrate (DNPB) as a substrate. In brief, DNPB was synthesized by using Mosmuller's method,[26] and a working solution was prepared with 10

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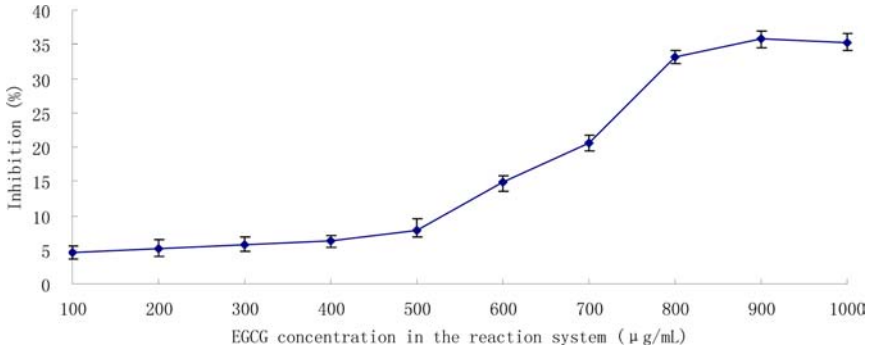

Figure 2. Inhibition of lipase by EGCG.

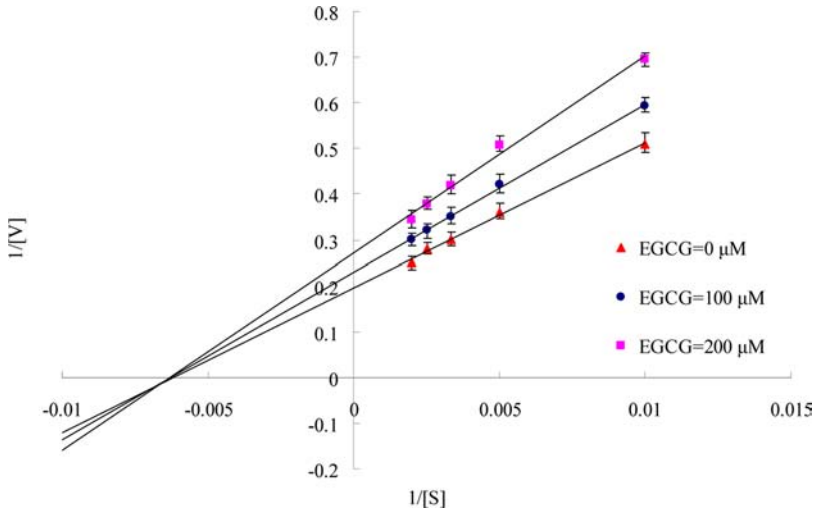

Figure 3. Lineweaver–Burk plot of the reaction of porcine lipase in the presence of EGCG.

mM phosphate buffer (pH 7.5). Lipase assay was performed in a reaction medium (0.7 mL) containing 25 mM DNPB and 0.1 mg of lipase, and assays were conducted in the presence or absence of EGCG. EGCG solution was prepared by dissolving in working solution and added to reaction mixtures just before the reaction. The reaction mixture was kept at 37 °C. The hydrolysis of DNPB to 2,4-dinitrophenol was monitored at 360 nm after incubation for 1 min. The control lipase activity was determined as described above but without EGCG. All samples were analyzed in triplicate. The inhibition of lipase activity in the presence of EGCG was calculated as inhibition % = [(activity control − activity test)/activity control] × 100.

The inhibition pattern of EGCG at different concentrations was measured with increasing concentrations of DNPB as a substrate against lipase activity. The inhibition type was determined by Lineweaver–Burk plot analysis calculated according to Michaelis–Menten kinetics.

Fluorescence Spectroscopy. EGCG solution was prepared with 10 mM phosphate buffer (pH 7.5) at 0, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0×10^{-5} M and then incubated with 1.0×10^{-5} M lipase for 1 h at 37 °C. The fluorescence emission spectra were recorded on a Hitachi-850 spectrofluorometer (Hitachi, Japan) in a 1 cm quartz cell at an excitation wavelength of 280 nm. The excitation and emission bandwidths were 5 nm. The emission spectra were recorded from 300 to 400 nm.

CD Measurements. The lipase concentration was 1.0×10^{-6} M, and spectra were recorded at EGCG concentrations of 0.0, 0.5, 1.0, and 2.0×10^{-6} M at 37 °C with a Jasco-810 spectrophotometer (JASCO, Tokyo, Japan) in cells of 1.0 mm path length. The spectra were measured from 190 to 250 nm. Five scans were accumulated for each spectrum. The secondary structure was determined by the SELCON3 method in DICHROWEB.[27]

ITC Analysis. ITC measurements involved the use of a MicroCal Auto-ITC_{200} calorimeter (GE, Stockholm, Sweden) at 37 °C. Both samples were prepared in working solution and dialyzed overnight before use. The titrate was set as 0.24 mM EGCG suspension, and the titrant was 4.8 mM lipase solution. In all, 13 injections of EGCG were added into lipase at 5 min intervals with stirring at 1000 rpm. Control experiments were the working solution set as a titrant and EGCG suspension as a titrate. Integrated heat effects were analyzed by nonlinear regression with a single-site binding model. The raw data were integrated and normalized by use of ORIGIN v 7.0 (MicroCal Inc.).

Docking Studies. Docking studies involved the use of MOE software. EGCG 3-D structures were generated from PM3 semi-empirical calculations by use of Chem3D Ultra 6.0. The porcine lipase structure was downloaded from the Brookhaven Protein Data Bank (accession no. 1ETH). EGCG was considered fully flexible and lipase was considered rigid during docking. The docked complex was optimized according to its fit within the receptor pocket, for discrete and continuum hydrophobicity, van der Waals interaction, hydrogen bonding, electrostatics, and entropy.

Statistical Analysis. The digestion assay and all of the spectral tests were performed in triplicate. The standard deviation and the averaged values are documented in the corresponding tables and figures. Linear correlation coefficient was calculated by Microsoft Excel 2007.

RESULTS AND DISCUSSION

Inhibition and Inhibitory Pattern of EGCG on Lipase. The activity of lipase was increasingly inhibited with increasing concentration of EGCG (Figure 2). The inhibition ratio was 33% with EGCG at 800 μg/mL. Hence, EGCG might act as an antinutritional factor in terms of its potential to inhibit lipase activity.

We studied the reaction kinetics of lipase to determine the inhibitory pattern of EGCG. The K_m values for lipase at different EGCG concentrations were similar (Figure 3), so EGCG inhibition of lipase was noncompetitive. Because polyphenols can interact with proteins,[28] EGCG might bind lipase and then change its activities.

Analysis of Fluorescence Spectroscopy of Lipase. Fluorescence spectroscopy is used to measure the interaction between a biomacromolecule and a small molecule ligand. Porcine lipase contains seven tryptophan (Trp) molecules, and the fluorescence of lipase is mainly dominated by the Trp emission. Therefore, the activity of Trp can be used to analyze the change in the intrinsic fluorescence intensity of lipase to study interactions between EGCG and lipase. With increasing concentrations of EGCG, the fluorescence intensity of lipase decreased (Figure 4A), so EGCG binding with lipase caused microenvironment changes of lipase.

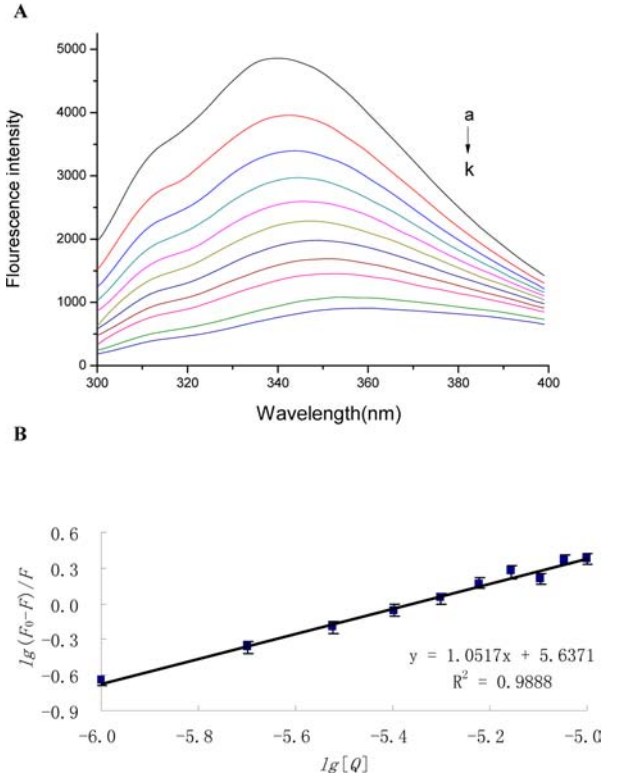

Figure 4. Quenching effect of EGCG on lipase fluorescence intensity, λ_{ex} = 280 nm: (A) lipase = 1.0×10^{-5} M, EGCG concentration increased (a–k) from 0.0 to 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, and 10.0 ($\times 10^{-5}$ M) at 37 °C; (B) plot of $\lg(F_0 - F)/F$ against lg[Q].

The relationship between fluorescence quenching intensity and the concentration of EGCG can be described by the modified Stern–Volmer equation:[29] $\lg(F_0 - F)/F = \lg K_a + n \lg[Q]$, where n is the number of binding sites per enzyme, K_a is the binding constant, and F_0 and F are the fluorescence intensities before and after the addition of EGCG, respectively. $\lg(F_0 - F)/F$ and lg[Q] showed a good linear relationship (Figure 4B), and the number of binding sites per lipase was 1.05, which suggests that EGCG interacts with lipase in a 1:1 ratio.[30] The binding constant K_a between EGCG and lipase was 3.98×10^4 L mol^{-1}.

The free energy change (ΔG) can be calculated as $\Delta G = -RT \ln K$, where K, R, and T are the binding constant, gas constant, and experimental temperature, respectively. The ΔG was calculated as −32.2 kJ mol^{-1} at 37 °C.

ITC Measurement. We used ITC to determine the thermodynamic properties of the binding interaction of EGCG and lipase. The interaction of EGCG and lipase was typically exothermic (Figure 5). The results of ITC analysis are in Table 1

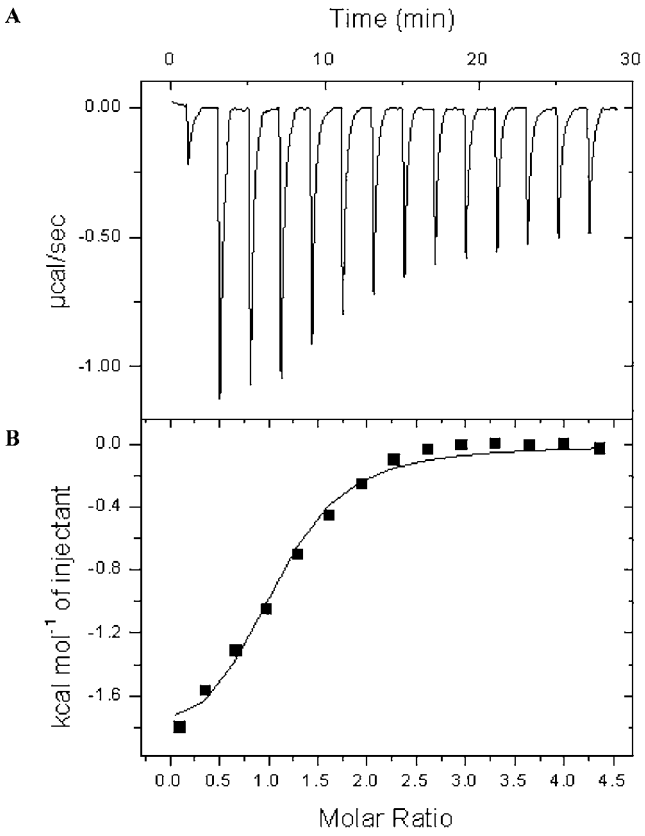

Figure 5. Results of isothermal titration calorimetry for EGCG binding to lipase: (A) raw data plot of heat flow against time for the titration of EGCG into lipase; (B) plot of the total heat released as a function of ligand concentration for the titration. The continuous line represents the best least-squares fit for the obtained data.

Table 1. Thermodynamic Parameters for Epigallocatechin Gallate (EGCG) Binding to Lipase

thermodynamic parameter	EGCG value
n	1
K_a (L mol^{-1})	2.70×10^4
ΔH (kJ mol^{-1})	−8.36
ΔG (kJ mol^{-1})	−26.25
ΔS (J mol^{-1} K^{-1})	57.72

and are similar to fluorescence spectroscopy findings. The ΔG value is negative, which indicates that the interaction was spontaneous. The enthalpies were too low for covalent bond formation (200–400 kJ mol^{-1}), which suggests that the interaction of EGCG and lipase is noncovalent.[31] ΔS is positive and ΔH is negative, so the reaction was enthalpy driven. The main source of the ΔG value (−26.25 kJ mol^{-1}) is a substantial contribution from the ΔH factor. The partial immobilization of a protein and ligand occurs in an initial step involving hydrophobic association, which results in a positive ΔS.[32] In the subsequent interacting complex, the negative ΔH contribution to the overall

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ΔG may be associated with a van der Waals interaction and hydrogen bonding.

The binding constant K_a between EGCG and lipase was >1.0 × 10^4 L mol^{-1}, representing a comparatively moderate ligand−protein interaction, which corresponds with other tea polyphenol−protein complexes with affinities from 1.0 × 10^4 to 1.0 × 10^5 L mol^{-1}.[33−36]

CD Spectra. CD spectroscopy was used to study porcine lipase conformational changes before and after EGCG binding. The CD spectra of lipase exhibited a negative band in the ultraviolet region at 208 nm (Figure 6), which is characteristic of

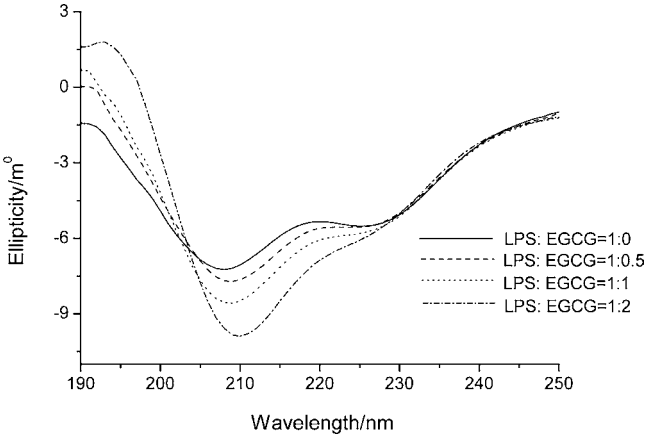

Figure 6. Circular dichroism assay.

α-helical structures in protein.[37] After EGCG binding, EGCG−lipase complexes had greater absolute θ values than did native lipase in the ultraviolet region at 208 nm, which suggests the presence of some α-helical structures in the protein.

We used SELCON3 in DICHROWEB to determine the secondary structure. On complexation of lipase with EGCG, the α-helical content of lipase changed from 21 to 46%, with a decrease in β-sheets from 24 to 14%, and the unordered structure content changed from 38 to 26% (Table 2). Thus, the binding of EGCG on lipase caused conformational changes in the enzyme.

Table 2. Circular Dichroism Analysis of Secondary Structure of Free Lipase and Its Interaction with EGCG

secondary structure element	lipase (%)	lipase/EGCG 1:0.5	lipase/EGCG 1:1	lipase/EGCG 1:2
α-helix (±1%)	21	34	38	46
β-sheet (±3%)	24	19	18	14
turn (±1%)	21	19	19	17
random coil (±2%)	38	27	28	26

Docking Studies. Molecular docking studies were used to substantiate our experimental findings. The docking results showed that EGCG was surrounded by Val21, Gln22, Ile20, Arg191, Gln188, Gln220, Cly185, Pro187, Thr186, and Gln220 in lipase (Figure 7). Hydrogen-bonding interaction appears plausible between Val21, Gln188, and Gln220. These extensive hydrogen-bonding interactions might play an important role in increased binding affinity of EGCG and lipase. The calculated ΔG for EGCG−lipase binding was −24.77 kJ mol^{-1}, which was close to the ITC-measured values of ΔG.

The catalytic sites of porcine lipase are residues Ser194, His435, and Asp320.[38] Figure 7 shows that these residues do not surround EGCG. As well, kinetics findings for lipase demonstrated that EGCG inhibition of lipase was noncompetitive. Therefore, EGCG inhibited porcine lipase activities by not binding the catalytic sites of lipase.

The conformation of an enzyme depends on noncovalent and covalent interactions among the enzyme amino acid residues. The activity of an enzyme will be changed if its specific conformation changes. The main mechanism of tea polyphenol binding proteins is considered noncovalent interaction.[23,29] We found that the interaction of EGCG and lipase was noncovalent. Noncovalent binding is often weak and nonspecific, but a combination of many noncovalent bonds may alter the conformation and function of the protein.[39] The internal noncovalent interactions of the peptide chain may be altered or even destroyed when a certain compound is added to a protein solution.[39] Inevitably, the function of the protein is affected when it binds with any chemical substance. The conformational mobility of the protein structure influences the catalytic activity of enzymes.[40]

EGCG contains hydroxyl and galloyl groups. The phenolic groups of EGCG can form hydrogen bonds with the polar groups of protein. In this study, the quenching of lipase fluorescence with EGCG produced a change in polarity of the fluorophore environment. This phenomenon may be achieved by the formation of hydrogen bonds between the OH groups of phenolic compounds and polar groups at the surface of the protein.[36] Polyphenol−protein interactions are hydrophobic in nature in the first step and then are stabilized via the formation of hydrogen bonds. However, the galloyl groups in EGCG exhibit a certain hydrophobicity.[24] Many hydrophobic amino acids are present in enzyme proteins. Such noncovalent reactions result in a conformational change. The binding effect might change the polarity of lipase and the enzyme molecular configuration, thus resulting in decreased enzyme activity. To our knowledge, one of the mechanisms of EGCG contributing to human health promotion is that EGCG can bind several enzyme proteins to inhibit their activities.[41] Our studies revealed that EGCG may bind to lipase and change its activity, which supports the above mechanism.

Furthermore, interest is growing in studying EGCG inhibiting the formation of amyloid fibrils. The accumulation of amyloid is associated with a wide range of degenerative diseases, including Alzheimer's, Parkinson's, and type II diabetes.[42−45] Recent work demonstrated that EGCG may bind to backbone sites of the monomeric species of many amyloid proteins, which was shown to inhibit both amyloid aggregation and associated toxicity.[46−49] Growing research suggests that EGCG has great potential to affect different kinds of human diseases because of its bioavailability and safety.

In summary, EGCG might act as a noncompetitive pancreatic lipase inhibiter. EGCG bound to lipase with an affinity of K_a = 2.70 × 10^4 L mol^{-1}. The thermodynamic parameters suggested that the interaction was spontaneous, with hydrogen bonds and an electrostatic force perhaps primarily responsible for the interaction, with a 1:1 interaction of lipase to EGCG. CD studies revealed that the protein conformation changed with lipase binding to EGCG. Furthermore, our docking-study findings supported our experimental results. Analysis of the inhibitory pattern of lipase by EGCG and characteristics of the interaction of EGCG with lipase indicated that lipase activity might be changed with change in lipase conformation after EGCG binding. EGCG as a lipase inhibitor may have potential for the treatment and prevention of obesity.

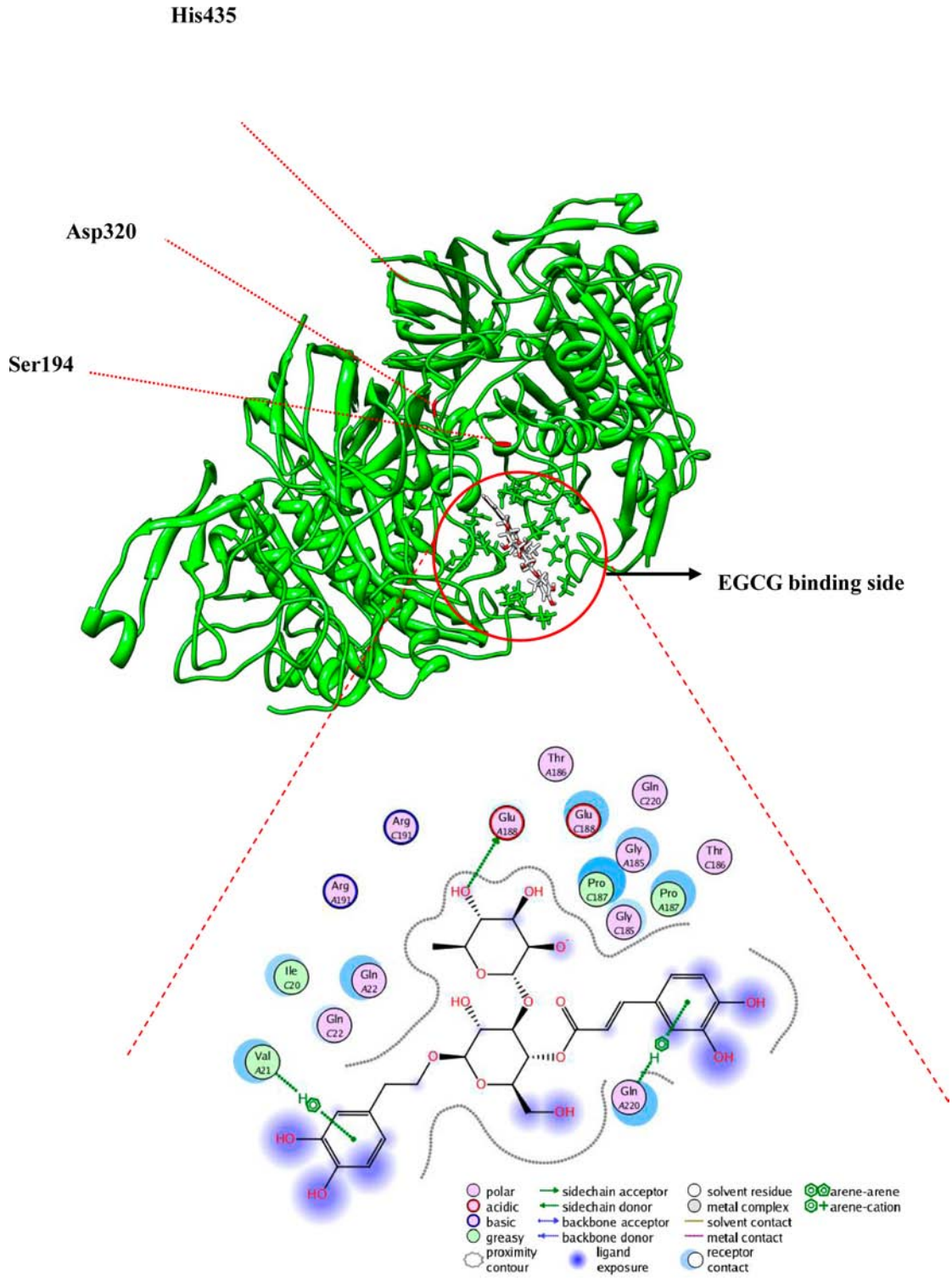

Figure 7. Best-docked conformations of EGCG−lipase complexes. EGCG is shown in white. The amino acid residues thought to interact with EGCG are shown as a 2-D representation by use of LigX in MOE.

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Notes

The authors declare no competing financial interest.

REFERENCES

(1) Cheng, T. O. Obesity crisis comprised of danger and opportunity. *J. Am. Diet. Assoc.* **2004**, *104*, 1546.

(2) Wolfram, S.; Wang, Y.; Thielecke, F. Anti-obesity effects of green tea: from bedside to bench mol. *Nutr. Food Res.* **2006**, *50*, 176−187.

(3) Kavanagh, K. T.; Hafer, L. J.; Kim, D. W.; Mann, K. K.; Sherr, D. H.; Rogers, A. E.; Sonenshein, G. E. Green tea extracts decrease carcinogen-induced mammary tumor burden in rats and rate of breast cancer cell proliferation in culture. *J. Cell Biochem.* **2001**, *82*, 387−398.

(4) Sueoka, N.; Suganuma, M.; Sueoka, E.; Okabe, S.; Matsuyama, S.; Imai, K.; Nakachi, K.; Fujiki, H. A new function of green tea: prevention of lifestyle related diseases. *Ann. N. Y. Acad. Sci.* **2001**, *928*, 274−280.

(5) Dona, M.; Dell'Aica, I.; Calabrese, F.; Benelli, R.; Morini, M.; Albini, A.; Garbisa, S. Neutrophil restraint by green tea: inhibition of inflammation, associated angiogenesis, and pulmonary fibrosis. *J. Immunol.* **2003**, *170*, 4335−4341.

(6) Haqqi, T. M.; Anthony, D. D.; Gupta, S.; Ahmad, N.; Lee, M. S.; Kumar, G. K.; Mukhtar, H. Prevention of collagen-induced arthritis in mice by a polyphenolic fraction from green tea. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 4524−4529.

(7) Sudano, R. A.; Blanco, A. R.; Giuliano, F.; Rusciano, D.; Enea, V. Epigallocatechin-gallate enhances the activity of tetracycline in staphylococci by inhibiting its efflux from bacterial cells. *Antimicrob. Agents Chemother.* **2004**, *48*, 1968−1973.

(8) Sartippour, M. R.; Shao, Z. M.; Heber, D.; Beatty, P.; Zhang, L.; Liu, C.; Ellis, L.; Liu, W.; Go, V. L.; Brooks, M. N. Green tea inhibits vascular endothelial growth factor (VEGF) induction in human breast cancer cells. *J. Nutr.* **2002**, *132*, 2307−2311.

(9) Osada, K.; Takahashi, M.; Hoshina, S.; Nakamura, M.; Nakamura, S.; Sugano, M. Tea catechins inhibit cholesterol oxidation accompanying oxidation of low density lipoprotein in vitro. *Comp. Biochem. Physiol. Part C: Toxicol. Pharmacol.* **2001**, *128*, 153−164.

(10) Ho, C. T.; Chen, Q. Y.; Shi, H.; Zhang, K.-Q.; Rosen, R. T. Antioxidant effect of polyphenol extract prepared from various Chinese teas. *Prev. Med.* **1992**, *21*, 520−525.

(11) Graham, H. N. Green tea composition, consumption, and polyphenol chemistry. *Prev. Med.* **1992**, *21*, 334−350.

(12) Kilmartin, P. A.; Hsu, C. F. Characterisation of polyphenols in green, oolong,and black teas, and in coffee, using cyclic voltammetry. *Food Chem.* **2003**, *82*, 501−512.

(13) By, Y. S.; Noriyuki, M.; Mamoru, I. Health-promoting effects of green tea. *Proc. Jpn. Acad. Ser. B: Phys. Biol. Sci.* **2012**, *88*, 88−101.

(14) Sabu, M. C.; Priya, T. T.; Ramadasan, K.; Ikuo, N. Beneficial effects of green tea: a literature review. *Chinese Med.* **2010**, *5*, 13−22.

(15) Sae-tan, S.; Grove, K. A.; Lambert, J. D. Weight control and prevention of metabolic syndrome by green tea. *Pharmacol. Res.* **2011**, *64*, 146−154.

(16) Basu, A.; Sanchez, K.; Leyva, M. J.; Wu, M.; Betts, N. M.; Aston, C. E.; Lyons, T. J. Green tea supplementation affects body weight, lipids, and lipid peroxidation in obese subjects with metabolic syndrome. *J. Am. Coll. Nutr.* **2010**, *29*, 31−40.

(17) Kao, Y. H.; Chang, H. H.; Lee, M. J.; Chen, C. L. Tea, obesity, and diabetes. *Mol. Nutr. Food Res.* **2006**, *50*, 188−210.

(18) Thielecke, F.; Boschmann, M. The potential role of green tea catechins in the prevention of the metabolic syndrome − a review. *Phytochemistry* **2009**, *70*, 11−24.

(19) Juhel, C.; Armand, M.; Pafumi, Y.; Rosier, C.; Vandermander, J.; Lairon, D. Green tea extract (ar25) inhibits lipolysis of triglycerides in gastric and duodenal medium in vitro. *J. Nutr. Biochem.* **2000**, *11*, 45−51.

(20) Shishikura, Y.; Khokhar, S.; Murray, B. S. Effects of tea polyphenols on emulsification of olive oil in a small intestine model system. *J. Agric. Food Chem.* **2006**, *54*, 1906−1913.

(21) Armand, M.; Pasquier, B.; André, M.; Borel, P.; Senft, M.; Peyrot, J.; Salducci, J.; Portugal, H.; Jaussan, V.; Lairon, D. Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. *Am. J. Clin. Nutr.* **1999**, *70*, 1096−1106.

(22) Shi, B.; He, X. Q.; Haslam, E. Gelatin-polyphenol interaction. *Am. Leather Chem. Assoc.* **1994**, *89*, 98−104.

(23) Siebert, K. J.; Troukhanova, N. V.; Lynn, P. Y. Nature of polyphenol-protein interactions. *J. Agric. Food Chem.* **1996**, *44*, 80−85.

(24) He, Q.; Lv, Y. P.; Yao, K. Effects of tea polyphenols on the activities of α-amylase, pepsin, trypsin and lipase. *Food Chem.* **2006**, *101*, 1178−1182.

(25) Koo, S. I.; Sang, K. Green tea as inhibitor of the intestinal absorption of lipids: potential mechanism for its lipid-lowering effect. *J. Nutr. Biochem* **2007**, *18*, 179−183.

(26) Mosmuller, E. W. J.; Van Heemst, J. D. H.; Van Delden, C. J.; Franssen, M. C. R.; Engbersen, J. F. J. A new spectrophotometric method for the detection of lipase activity using 2,4-dinitrophenyl butyrate as a substrate. *Biocatalysis* **1992**, *5*, 279−287.

(27) Whitmore, L.; Wallace, B. A. DICHROWEB, an online server for protein secondary structure analyses from circular dichroism spectroscopic data. *Nucleic Acids Res.* **2004**, *32*, 668−673.

(28) Charlton, A. J.; Baxter, N. J.; Khan, M. L.; Moir, A. J.; Haslam, E.; Davies, A. P.; Williamson, M. P. Polyphenols/peptide binding and precipitation. *J. Agric. Food Chem.* **2002**, *50*, 1593−1601.

(29) Wu, X. L.; Wu, H.; Liu, M. X.; Liu, Z. G.; Xu, H.; Lai, F. R. Analysis of binding interaction between (−)-epigallocatechin (EGC) and β-lactoglobulin by multi-spectroscopic method. *Spectrochim. Acta A* **2011**, *82*, 164−168.

(30) Gokara, M.; Sudhamalla, B.; Amooru, D. G.; Subramanyam, R. Molecular interaction studies of trimethoxy flavone with human serum albumin. *PLoS One* **2010**, *5*, e8834.

(31) Frazier, R. A.; Papadopoulou, A.; Green, R. J. Isothermal titration calorimetry study of epicatechin binding to serum albumin. *J. Pharm. Biomed. Anal.* **2006**, *41*, 1602−1605.

(32) Ross, P. D.; Subramanian, S. Thermodynamics of protein association reactions: forces contributing to stability. *Biochemistry* **1981**, *20*, 3096−3102.

(33) Kanakis, C. D.; Hasni, I.; Bourassa, P.; Tarantilis, P. A.; Polissiou, M. G.; Tajmir-Riahi, H. A. Milk β-lactoglobulin complexes with tea polyphenols. *Food Chem.* **2011**, *127*, 1046−1055.

(34) Tushar, K. M.; Kalyan, S. G.; Swagata, D. Interaction of (−)-epigallocatechin-3-gallate with humanserum albumin: fluorescence, fourier transform infrared,circular dichroism, and docking studies. *Proteins* **2006**, *64*, 355−362.

(35) Zerrin, Y.; Elif, A.; Yasar, K. E. Characterization of binding interactions between green tea flavanoids and milk proteins. *Food Chem.* **2010**, *121*, 450−456.

(36) Wu, X. L.; Dey, R.; Wu, H.; Liu, Z. G.; He, Q. Q.; Zeng, X. J. Studies on the interaction of EGCG with bovine β-lactoglobulin by spectroscopic methods and docking. *Int. J. Dairy Technol.* **2013**, *65*, 7−13.

(37) Venyaminov, S. Y.; Vassilenko, K. S. Determination of protein tertiary structure class from circular dichroism spectra. *Anal. Biochem.* **1994**, *222*, 176−184.

(38) Krejci, E.; Duval, N.; Chatonnet, A.; Vincens, P.; Massoulie, J. Cholinesterase-like domains in enzymes and structure proteins: functional and evolutionary relationships and identification of a catalytically essential aspartic acid. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 6647−6651.

(39) Piekarska, B.; Skowronek, M.; Rybarska, J.; Stopa, B.; Roterman, I.; Konieczny, L. Congo red-stabilized intermediates in the lambda light chain transition from native to molten state. *Biochimie* **1996**, *78*, 183−189.

(40) Andras, F. S.; Brian, C. B.; Takuji, T.; Yada, R. Y. Understanding the structure-function role of specific catalytic residues in a model food related enzyme: pepsin. *Enzyme Microb. Technol.* **2007**, *40*, 1175−1180.

(41) Suzuki, Y.; Miyoshi, N.; Isemura, M. Health-promoting effects of green tea. *Proc. Jpn. Acad. Ser. B: Phys. Biol. Sci.* **2012**, *88*, 88−101.

(42) Suzuki, Y.; Brender, J. R.; Soper, M. T.; Krishnamoorthy, J.; Zhou, Y.; Ruotolo, B. T.; Kotov, N. A.; Ramamoorthy, A.; Marsh, E. N. Resolution of oligomeric species during the aggregation of $A\beta_{1-40}$ using ^{19}F NMR. *Biochemistry* **2013**, *52*, 1903−1912.

(43) Suzuki, Y.; Brender, J. R.; Hartman, K.; Ramamoorthy, A.; Marsh, E. N. Alternative pathways of human islet amyloid polypeptide

aggregation distinguished by ^{19}F nuclear magnetic resonance-detected kinetics of monomer consumption. *Biochemistry* **2012**, *51*, 8154−8162.
(44) DeToma, A. S.; Salamekh, S.; Ramamoorthy, A.; Lim, M. H. Misfolded proteins in Alzheimer's disease and type II diabetes. *Chem. Soc. Rev.* **2012**, *21*, 608−621.
(45) Brender, J.; Salamekh, S.; Ramamoorthy, A. Membrane-disruption and early events in the aggregation of the diabetes related peptide IAPP from a molecular perspective. *Acc. Chem. Res.* **2012**, *116*, 3650−3658.
(46) Popovych, N.; Brender, J. R.; Soong, R.; Vivekanandan, S.; Hartman, K.; Basrur, V.; Macdonald, P. M.; Ramamoorthy. A site specific interaction of the polyphenol EGCG with the SEVI amyloid precursor peptide PAP(248−286). *J. Phys. Chem. B* **2012**, *116*, 3650−3658.
(47) Hyung, S. J.; DeToma, A. S.; Brender, J. R.; Lee, S.; Vivekanandan, S.; Kochi, A.; Choi, J. S.; Ramamoorthy, A.; Ruotolo, B. T.; Lim, M. H. Insights into antiamyloidogenic properties of the green tea extract (−)-epigallocatechin-3-gallate toward metal-associated amyloid-β species. *Proc. Natl. Acad. Sci. U.S.A.* **2013**, *110*, 3743−3748.
(48) Huang, R.; Vivekanandan, S.; Brender, J. R.; Abe, Y.; Naito, A.; Ramamoorthy, A. NMR characterization of monomeric and oligomeric conformations of human calcitonin and its interaction with EGCG. *J. Mol. Biol.* **2012**, *416*, 108−120.
(49) Porat, Y.; Abramowitz, A.; Gazit, E. Inhibition of amyloid fibril formation by polyphenols: structural similarity and aromatic interactions as a common inhibition mechanism. *Chem. Biol. Drug Des.* **2006**, *671*, 27−37.