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Research paper

A-type dimeric epigallocatechin-3-gallate (EGCG) is a more potent inhibitor against the formation of insulin amyloid fibril than EGCG monomer

Rong-zu Nie [a], Wei Zhu [a], Jin-ming Peng [a], Zhen-zhen Ge [a], Chun-mei Li [a, b, *]

[a] College of Food Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China
[b] Key Laboratory of Environment Correlative Food Science, Huazhong Agricultural University, Ministry of Education, Wuhan, 430070, China

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ABSTRACT

Because fibrillary protein aggregates is regarded to be closely associated with many diseases such as Alzheimer's disease, diabetes, and Parkinson's disease, growing interest and researches have been focused on finding potential fibrillation inhibitors. In the present study, the inhibitory effects of epigallocatechin-3-gallate (EGCG) and A-type dimeric epigallocatechin-3-gallate (A-type EGCG dimer) on the formation of insulin fibrillation were compared by multi-dimensional approaches including thioflavin-T (ThT) fluorescence assay, 1-anilinonaphthalene-8-sulfonic (ANS) fluorescence assay, dynamic light scattering (DLS), transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy and circular dichroism (CD) spectroscopy. Our results confirmed that A-type EGCG dimer is a more potent inhibitor against the formation of bovine insulin amyloid fibril than EGCG. In addition, A-type EGCG dimer could not only inhibit insulin amyloid fibril formation, but also change the aggregation pathway and induce bovine insulin into amorphous aggregates. The results of the present study may provide a new guide on finding novel anti-amyloidogenic agents.

1. Introduction

Increasing evidence suggests that a large variety of proteins and polypeptides can form fibrillar protein aggregates under certain conditions, which is regarded to be a common hallmark of many diseases such as Alzheimer's disease, diabetes, and Parkinson's disease [1]. Although the fibril formation ability of proteins and polypeptides seems to be sequence and native structure independent, the final amyloid fibrils share some common features, including a β-sheet rich structure, fibril morphology, surface hydrophobic property, and birefringence upon staining with Congo red [2,3]. Therefore, many proteins and peptides can serve as model proteins for studying amyloid aggregation and screening amyloid aggregation inhibitors. Among the most used model proteins, insulin is an ideal model not only because it is easy to form amyloid fibrils and easily available at a reasonable price, but also because the kinetic of fibril formation of insulin is a typical nucleation-dependent polymerization, making the aggregation mechanism study representative [4].

Because no effective treatments for amyloid fibrils related diseases are available at present [5], searching for agents that can effectively and directly prevent the formation of amyloid fibrils or disaggregate the preformed amyloid fibrils is a promising therapeutic strategy [6].

Previous studies demonstrated that many natural polyphenol compounds such as quercetin, gallic acid (GA), EGCG, myricetin and curcumin showed strong inhibitory effects on the aggregation and formation of amyloid fibrillation [4,7,8]. Among them, EGCG (Fig. 1a) attracted prominent attention, not only because it is the major catechin found in the widely consumed green tea, but also because it can interact with a lot of amyloid forming proteins thus exerting many health benefits such as diabetes and neurodegenerative diseases prevention [9]. It was reported that EGCG could remodel alpha-synuclein and amyloid-beta fibrils and reduce cellular toxicity by stabilization conformation of native protein [10]. It could depolymerize the preformed hen egg white lysozyme amyloid and islet amyloid polypeptide fibrils to amorphous

* Corresponding author. College of Food Science and Technology, Huazhong Agricultural University, Wuhan, 430070, China.
E-mail address: lichmyl@126.com (C.-m. Li).

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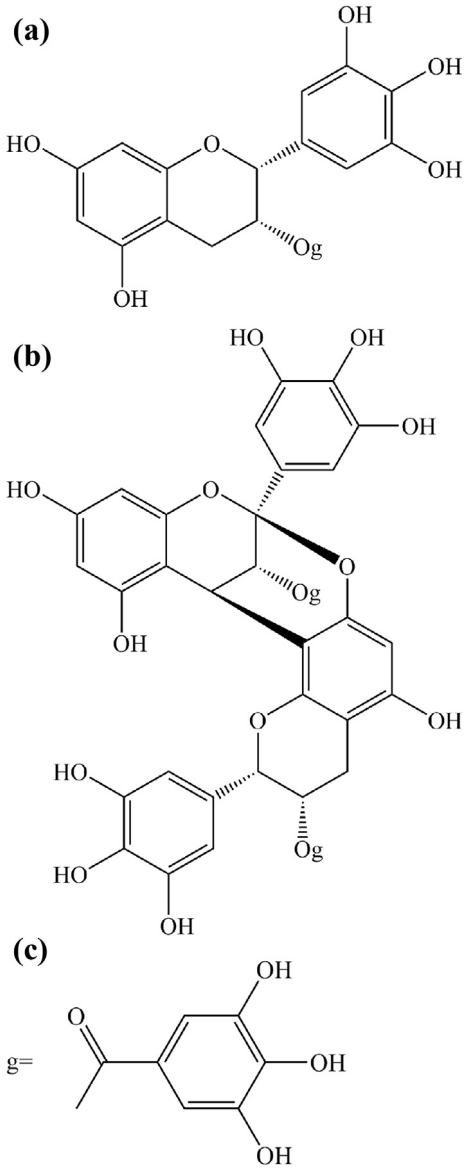

Fig. 1. Chemical structures of EGCG (a) and A-type EGCG dimer (b) and gallate moiety(c).

aggregates in a dose-dependent manner [11,12]. It was also capable of binding with native bovine insulin to prevent the transition of α-helix to β-sheet and the formation of insulin amyloid fibril [13]. Moreover, it could interfere with the aromatic hydrophobic core of Aβ thus exerting anti-amyloid effects [14].

In our previous study, we obtained a new proanthocyanidins dimer, namely A-type EGCG dimer (Fig. 1b), from persimmon fruits. Interestingly, we found that the ability of this dimer on unfolding the structure of snake venom PLA_2 was significantly stronger than that of EGCG [15]. At the molar ratio of phenolic compound to PLA_2 of 5:1, EGCG unfolded about 23% of the β−sheet structure of PLA_2, but A-type EGCG dimer unfolded the β−sheet structure of PLA_2 completely. This finding drove us to propose a hypothesis that A-type EGCG dimer might be a potent amyloid fibrils inhibitor.

In the present study, the inhibitory effects of A-type EGCG dimer and EGCG on the formation of insulin fibrillation were compared by thioflavin-T (ThT) fluorescence assay, 1-anilinonaphthalene-8-sulfonic (ANS) fluorescence assay, dynamic light scattering (DLS), transmission electron microscopy (TEM), Fourier transform infrared (FTIR) spectroscopy and circular dichroism (CD) spectroscopy. And the possible mechanisms were also discussed. We believed that our results could shed new light on finding potent anti-aggregative agents against amyloid diseases.

2. Materials and methods

2.1. Materials

EGCG, Thioflavin-T (ThT) and Bovine pancreas insulin (MW: 5733.49) were purchased from Yuanye biological technology Ltd. (Shanghai, China). Bovine pancreas insulin was used without further purification. Acetic acid, KBr and methanol were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). 1-anilinonaphthalene-8-sulfonic (ANS) was obtained from Aladdin-reagent (Shanghai, China). All other reagents and chemicals were of analytical grade.

2.2. Sample preparation

A-type EGCG dimer was prepared using persimmon (Diospyros kaki L.) as starting material by the method we have previously reported and further purified by preparative high-performance liquid chromatography (HPLC) [16]. The purity of A-type EGCG dimer was determined to be 97.86% by RP-HPLC.

2.3. Preparation of bovine insulin amyloid fibril

Bovine insulin was dissolved in 20% acetic acid (pH 2.0) containing 100 mM NaCl to a final concentration of 1 mg/ml. Incubation was carried out in Eppendorf tubes to avoid solvent evaporation. The sample was incubated at 60 °C without agitation during the course of aggregation.

2.4. Thioflavin-T (ThT) fluorescence assay

The stock solution of ThT was performed by dissolving ThT in 95% ethanol to a final concentration of 1 mM. It was stored at 4 °C in dark. 30 μL of bovine insulin (1 mg/ml) solutions with or without polyphenol were diluted with 9 ml of 20 mM phosphate buffer (pH 7.4), then 90 μL of ThT stock solution was added. The mixture was incubated for about 1min and mixed thoroughly before measuring. ThT fluorescence intensity was obtained using a fluorescence spectrophotometer (Hitachi, Japan) with the excitation at 440 nm and recording the emission at 485 nm. The fluorescence of the solvent blank was subtracted. The IC_{50} value was obtained by regression analysis from the dose-response curve plotted with percentage inhibition rate against log of sample concentration.

2.5. Dynamic light scattering (DLS)

The distribution of particle size in each sample was assayed using a light-scattering spectrometer (Malvern, U.K.) equipped with a 4 mW He−Ne laser with a wavelength of 633 nm. Insulin solutions were directly placed into dust-free disposable cuvettes with a 1 cm optical path. Lighting scattering was monitored at 25 °C with the angle of 173°. Each scan contained 11 runs and the duration of each run was 10 s. Scans were repeated four times for every sample. The particle size distribution of samples was obtained using the non-negative least squares method (NNLS).

2.6. Transmission electron microscopy (TEM)

Insulin samples with or without polyphenol were diluted with