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Interaction of the ginsenosides with κ-casein and their effects on amyloid fibril formation by the protein: Multi-spectroscopic approaches

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ABSTRACT

The interaction of the ginsenosides (GS) including ginsenoside Rg_1, Rb_1 and Re with κ-casein and the effects of GS inhibiting amyloid fibril formation by κ-casein have been investigated in vitro by fluorescence and ultraviolet spectra. Results showed that Rg_1 and Rb_1 had dose-dependent inhibitory effects on reduced and carboxymethylated κ-casein (RCMκ-CN) fibril formation, while Re resulted in an increase in the rate of fibril formation. The enhancement in RLS intensity was attributed to the formation of new complex between GS and RCMκ-CN, and the corresponding thermodynamic parameters (ΔH, ΔS and ΔG) were assayed. The steady-state ultraviolet–visible absorption spectra had also been tested to observe if the ground-state complex formed, and it showed the same result as RLS spectra. The binding constants and the number of binding sites between GS and RCMκ-CN at different temperatures had been evaluated from relevant fluorescence data. According to the Förster non-radiation energy transfer theory, the binding distance between RCMκ-CN and GS was calculated. The fluorescence lifetime of RCMκ-CN was longer in the presence of GS than in absence of GS, which was evident that the hydrophobic interaction plays a major role in the binding of GS to RCMκ-CN. From the results of synchronous fluorescence, it could be deduced that the polarity around RCMκ-CN Trp97 residue decreased and the hydrophobicity increased after addition of Rg_1 or Rb_1. Based on all the above results, it is explained that Rg_1 and Rb_1 inhibited amyloid fibril formation by κ-casein because the molecular spatial conformation and physical property of κ-casein changed causing by the complex formation between GS and κ-casein.

1. Introduction

Native proteins can misfold via partially structured intermediates to either disordered amorphous aggregates or ordered amyloid fibrils [1]. Protein misfolding resulting in amyloid fibril formation can lead to multiple incurable diseases, such as Alzheimer's disease (AD), Parkinson's disease (PD) and Huntington's disease (HD) [2–5]. The deposition of amyloid fibril can potentially cause disruption of organ function and organ failure [6–8]. It is well accepted that the formation of amyloid fibrils is recognized to be a major contributing factor in a group of pathologic states known as amyloidosis [9,10]. Amyloidosis may mimic the appearance of a number of pathologies, both benign and malignant, which was characterized by deposition of insoluble protein fibrils in tissues and organs [11]. Herein, understanding the aggregation process and knowing how to prevent amyloid fibril formation are of critical importance for developing strategies against those diseases.

As is known to all, breast cancer (BC) is the most common malignancy as one of the leading causes of cancer-related mortality among female worldwide. Although the pathological mechanism of BC remains inconclusive, it has recently been reported that amyloidosis could involve the breast and may mimic BC [12]. Furthermore, it was also demonstrated that amyloidosis of the breast, associated with invasive BC [12], presenting clinically as a amyloid tumor, either in isolation or in association with a malignancy, can occur as organ specific nodular amyloidosis or as part of systemic amyloidosis, which can distribute in a periductal, perivascular, or intralobar pattern; can create a foreign body-like reaction with infiltration of multinucleated giant cells; and can have an affinity for calcium [13,14]. Meanwhile, it has been found that amyloidosis of the breast coincided with BC followed by vascular, interstitial, and periductal amyloid deposits and that corpora amylacea (CA), a disorder associated with the accumulation of amyloid deposits, in mammary secretory tissue engorged luminal spaces and clogged small ducts, leading to milk stasis and involution [6,15]. As the major milk protein, κ-casein readily forms amyloid fibrils under physiological conditions, it has been postulated that κ-casein may be one contributing factor of BC [15–17]. Based on the prior statements, we hypothesized

Abbreviations: RCMκ-CN, reduced and carboxymethylated κ-casein; GS, ginsenosides; Rg_1, ginsenoside Rg_1; Rb_1, ginsenoside Rb_1; Re, ginsenoside Re; AD, Alzheimer's disease; PD, Parkinson's disease; HD, Huntington's disease; ThT, thioflavin T; RLS, resonance light scattering; FRET, fluorescence resonance energy transfer; BC, breast cancer; CA, corpora amylacea; Tyr, tyrosine; Trp, tryptophan; Glc, glucopyranose; A.U., arbitrary units.

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that target inhibition of amyloid fibrillization by κ-casein may provide a novel therapeutic strategy to inhibit BC.

To date, considerable effort has been dedicated to discovering efficacious molecules to combat protein misfolding in order to prevent these diseases. Although there is still no approved therapeutic agent directed toward amyloid fibrils or fibril precursors currently, multiple inhibitors of amyloid formation have been discovered or designed, which can be divided into three general categories: small molecules, short peptides and antibodies [18–24]. And most prominent is the development of small-molecule drug candidates, which delay the onset of fibril formation or reverse the formation of toxic amyloid fibrils and pre-fibrillar entities with a different morphology [20,21,23,24].

GS, a class of steroid glycosides and triterpene saponins, are the dominant active compounds derived from *Panax ginseng* C. A. Meyer, which have various pharmacological activities such as immunomodulatory effects [25], anti-cancer [26], anti-aging [27] and anti-tumor activities [28]. It has been known that the major components of GS are Rg_1, Rb_1 and Re, which show diverse biological activities, including anticancer properties [29–31]. Preliminary experiments have shown that total GS hindered the aggregation process of RCMκ-CN, which is presumed to be one contributing factor of BC. However, whether Rg_1, Rb_1, and Re can inhibit RCMκ-CN amyloid fibril formation and their mechanisms remain unclear.

To address these issues, we have investigated the molecular interaction of GS on RCMκ-CN by several spectroscopic methods including thioflavin T (ThT) fluorescence assay, fluorescence spectroscopy, UV–vis absorption and resonance light scattering (RLS). In addition, the conformational changes of RCMκ-CN are discussed on the basis of synchronous fluorescence spectra. The interaction information of GS with RCMκ-CN regarding binding parameters, thermodynamic parameters, binding mode and affinity, and conformation investigation are reported in present study. Based on experiment data, it is anticipated that the research could shed some new light on the interaction for GS binding to RCMκ-CN.

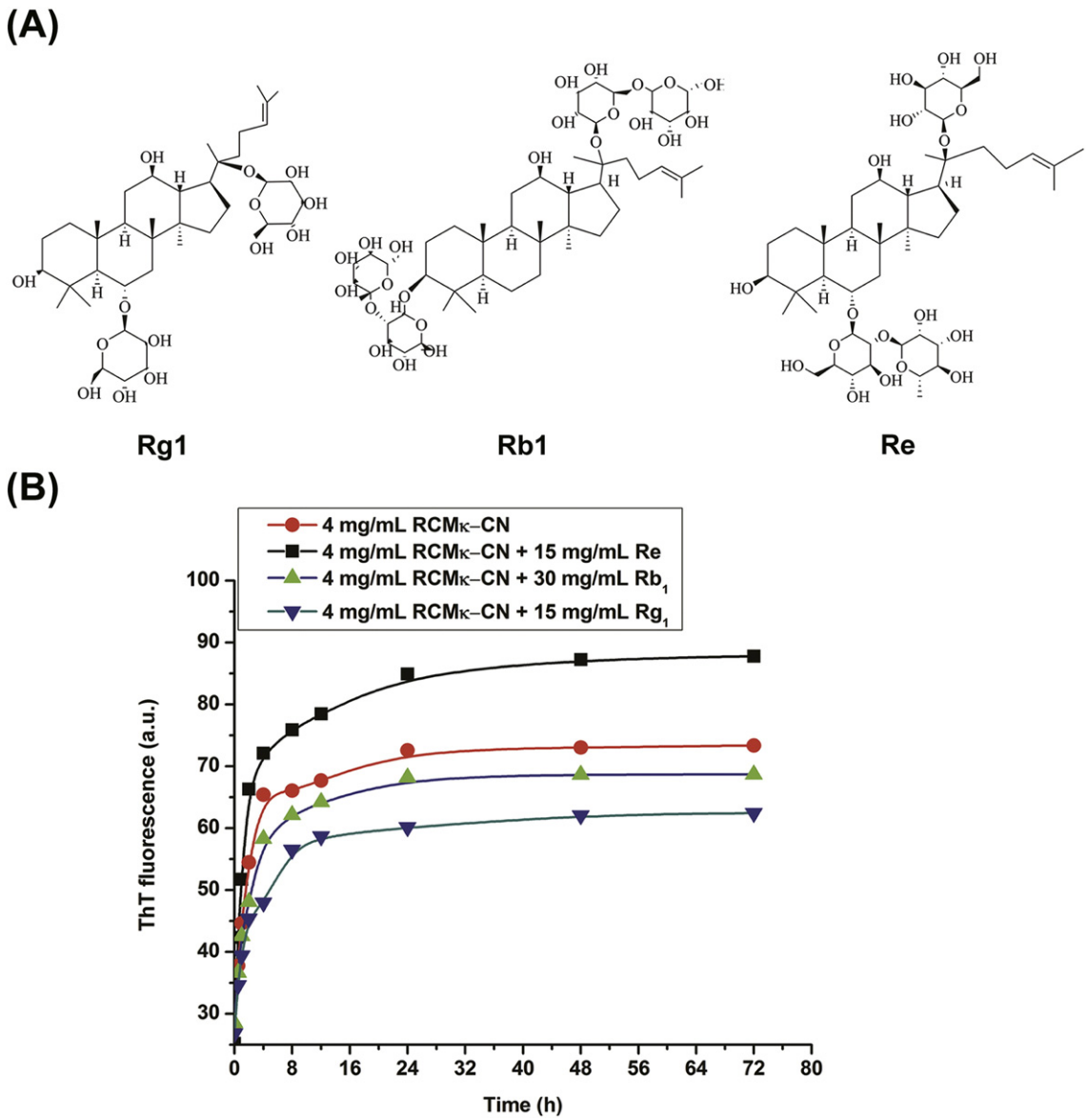

Fig. 1. Effect of GS on amyloid fibril formation by RCMκ-CN in vitro. (A) The structure of Rg_1, Rb_1 and Re. (B) ThT fluorescence assay of RCMκ-CN (4 mg/mL) incubated at 310 K in the presence and in the absence of GS. Values shown are the mean readings from three individual experiments, and the standard errors for each data point are within 4% (not visible). The rate constants for fibril growth (elongation) were as follows: Re (15 mg/mL, 10.8 h^{-1}), RCMκ-CN (4 mg/mL, 8.9 h^{-1}), Rb_1 (30 mg/mL, 6.9 h^{-1}), and Rg_1 (15 mg/mL, 4.8 h^{-1}).