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Insights into the mechanism of how Morin suppresses amyloid fibrillation of hen egg white lysozyme

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ABSTRACT

This communication describes the inhibitory effect of Morin on the fibrillation of Hen Egg White Lysozyme (HEWL), a generic amyloid-forming model protein. This effect was dose-dependent and stronger than other small molecules we have tested previously. Spectrofluorometric and computational studies support a model suggesting that Morin inhibits amyloid fibril formation of HEWL by binding to the aggregation prone cleft region of the β-domain of HEWL, thereby stabilizing the molecule in its native-like state. Interestingly, transmission electron microscopy observations suggest that, along with increases in Morin concentration, the observed amorphous aggregates became larger and morphologically different. We propose that following occupation of the binding cleft, excess Morin adheres and coats the HEWL protein surface, thereby minimizing the interaction between the protein surface and water molecules.

1. Introduction

A number of chronic degenerative diseases, including Alzheimer's disease, Parkinson's disease, type-2 diabetes and some coronary heart diseases are associated with formation of amyloid fibrils [1]. Highly ordered and β-sheet rich abnormal conformations are typical characteristics of amyloid fibrils, which are formed following the aggregation of misfolded proteins [2,3]. Hen egg-white lysozyme (HEWL) is a structural homologue of human lysozyme, whose amyloidogenic variants correlate with the incidence of systemic amyloidosis [4]. The structure of HEWL consists of several α-helical and β-sheet regions, which are designated as α and β-domains, respectively. A core structure termed the HEWL K peptide (GILQINSRW, residues 54–62 in the β-domain of the cleft), isolated from trypsin-digested HEWL, is found to have the ability to self-assemble and is involved in fibril formation [5].

The search for new compounds that can interfere with the aggregation of amyloid-forming proteins is considered to be an important strategy in the development of potential new therapeutics [6]. Naturally occurring polyphenolic compounds are found extensively in a variety of foods and herbal remedies, and are considered as promising pharmaceuticals against in the treatment of amyloid diseases [7]. Among the polyphenol class of plant flavonoids, Morin (2′, 3, 4′, 5, 7-pentahydroxyflavone) was originally isolated from the members of the *Moraceae* family and is a major component of many fruits, herbs and wine [8,9].

We have previously reported the inhibitory activity of Myricetin against HEWL fibril formation, in which Myricetin exhibited a stronger inhibition than the well-characterized polyphenol Quercetin [10]. In contrast to our previous studies using other polyphenols, we find the generation of irregular structural aggregates formed by the binding of Morin to HEWL, which support a novel and distinctive model for how this small molecule inhibits amyloid formation.

2. Materials and methods

2.1. Proteins and reagents

Morin, thioflavin T (ThT), and 8-anilino-1-naphthalenesulfonic acid (ANS) were purchased from Sigma-Aldrich (St. Louis, MO, USA). HEWL was purchased from Solarbio (Beijing, China). Preparation of HEWL samples were performed as previously described [10].

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2.2. Spectrofluorometric studies and physical analysis of HEWL fibril formation

Stock solution containing 1 mM ThT was prepared in phosphate buffer (50 mM Na2HPO4, 50 mM NaH2PO4, pH 7.0) and stock solution of ANS (0.4 mM) was prepared by dissolving ANS in PBS (pH 7.0) and were both stored at 4 °C. ThT, ANS and Intrinsic fluorescence assay as well as transmission electron microscopy (TEM) analysis were performed as previously described [10]. The IC50 of Morin with respect to inhibition of HEWL fibril formation was determined using the equation $\mathbf{Y = 100/(1 + 10^{(X-LogIC50)})}$ in Prism 5 (GraphPad Software, Inc, Avenida de la Playa, CA).

2.3. Molecular dynamic simulations and docking studies

Molecular dynamics (MD) simulations were performed using GROMACS 4.0.7 program at the Supercomputer Center of Liaoning University on dual-core Pentium 2.8 G processor of Linux cluster [11]. The crystal structure of HEWL (PDB entry 1GXV) was downloaded from the Protein Data Bank (PDB) [12]. The 3D structure of Morin was obtained from Chem Spider database (http://www.chemspider.com/) [13]. Molecular dynamics (MD) simulations and the docking studies were performed by the method of He et al. [10].

3. Results and discussion

3.1. Spectrofluorometric studies of HEWL fibril formation and anti-fibrillogenic activity of Morin

ThT fluorescence assay was carried out to monitor the kinetics of HEWL fibril formation. After a lag of about one day, the ThT fluorescence intensity of HEWL increased rapidly, reaching a plateau on day four (Fig. 1A). In contrast, the presence of Morin prolonged the fibrillation lag-phase and showed a significant decrease in ThT fluorescence over the amyloid fibril formation period. To quantitatively compare the differences in the extent of fibril formation among the lysozyme samples with different concentrations of Morin, the time-dependent data points obtained from ThT fluo-

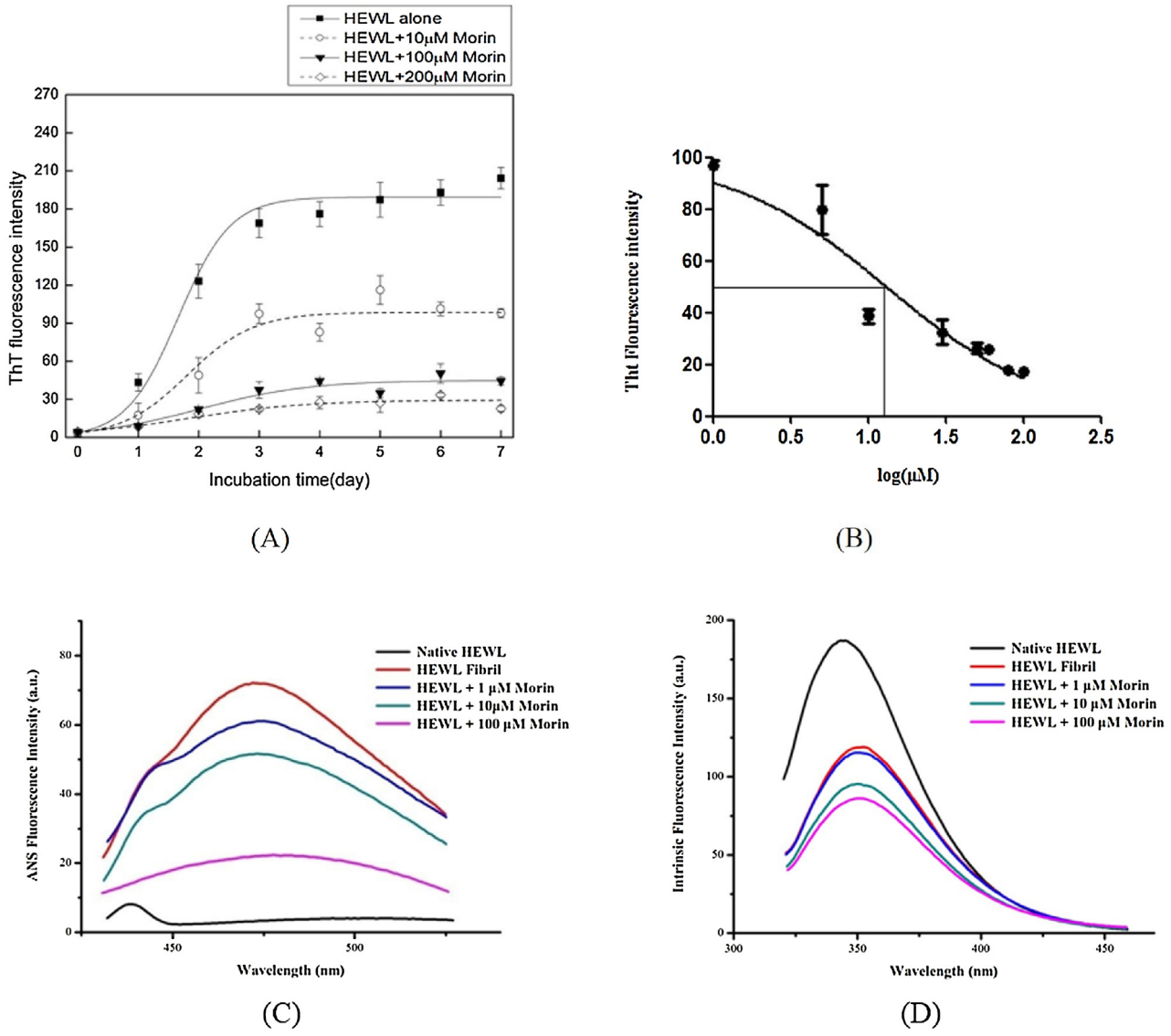

Fig. 1. Effects of different concentrations of Morin on fibril formation by HEWL amyloid. (A) Kinetics of HEWL fibril formation in the absence (filled squares) or the presence of Morin as monitored by ThT fluorescence. The curves were obtained by fitting experimental data against a sigmoidal-type equation (shown in the Result section) associated with the nucleation-dependent pathway. (B) Dose-response curve plotting the plateau value of the ThT fluorescence of the HEWL sample against Morin concentration. (C) ANS fluorescence and (D) intrinsic fluorescence emission spectra of HEWL fibrils after seven days of incubation without or with Morin.