International Journal of Biological Macromolecules 64 (2014) 162–167

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

journal homepage: www.elsevier.com/locate/ijbiomac

Inhibitory effects of β-ionone on amyloid fibril formation of β-lactoglobulin

Baoliang Ma [a], Xiong You [b,*], Fujiao Lu [a]

[a] Department of Physics, Science of College, Nanjing Agricultural University, Nanjing 210095, PR China
[b] Department of Applied Mathematics, Science of College, Nanjing Agricultural University, Nanjing 210095, PR China

ARTICLE INFO

Article history:
Received 16 September 2013
Received in revised form 9 November 2013
Accepted 2 December 2013
Available online 8 December 2013

Keywords:
β-Lactoglobulin
Amyloid fibrils
Lag time
β-Ionone
Protein stability

ABSTRACT

β-Lactoglobulin (β-LG) is the major constituent of whey food, which has been shown to interact with a wide range of aroma compounds. In the present work, a model aroma compound, β-ionone, is used to investigate the influence of aroma compounds on the urea-induced unfolding of β-LG at pH 7.0. β-Ionone is observed to enhance the stability of β-LG at pH 7.0. Moreover, the amyloid fibrils are observed when β-LG at pH 7.0 is incubated for 12–20 days at 37 °C in the presence of 3–5 M urea. However, the formation of amyloid fibrils is inhibited when β-ionone is added into the samples and the inhibitory effects follow a concentration-dependent fashion. There is a clear correlation between C_m and lag time. The correlation demonstrates that protein stability affects the amyloid fibril formation of β-LG. The results highlight the critical role of protein stability and provide an approach to prevent the formation of amyloid fibrils in vitro.

1. Introduction

Proteins are basic and important molecules in cells. It has been shown that proteins can spontaneously fold into their native structures, which are related to their biological functions [1]. However, it has also been demonstrated that a number of proteins cannot correctly fold into their native structures in certain pathological conditions, on the contrary, aggregate together and form the amyloid fibrillar structures [2]. The formation of such amyloid fibrils usually causes several serious diseases, such as Alzheimer's, Parkinson's and Huntington's diseases [3,4]. It is found that the amyloid fibrils are composed of cross-β structures and their morphological features are not related to behaviors of the specific proteins [5]. Nowadays, several kinds of mechanisms for the formation of the amyloid fibrils have been proposed. In general, the formation of such fibrils has been hypothesized to occur stepwise with a slow phase of nucleation of precursors of the amyloid fibrils and then a relatively fast elongation phase [6]. Furthermore, it is generally believed that the amyloid formation is a common property of all proteins rather than a characteristic feature of the proteins which have been found to cause diseases [7].

Since the formation of amyloid fibrils generally can result in many neurodegenerative diseases, recent research has focused on the inhibition of fibril formation. This research area is becoming more important nowadays, because of the population aging in the world, which will lead to a large increase in the number of patients with these diseases. In previous studies, a number of inhibitors of fibril formation have been investigated. It has been shown that the amyloid formation can be inhibited by various compounds, such as pyrroloquinoline quinine (PQQ) [8], nitrophenols [9], salvianolic acid B [10], biocompatible nanogels [11], benzofurans [12], baicalein [13], curcumin [14].

β-Lactoglobulin (β-LG) is a globular protein which is composed of 162 amino acids with molecular mass of 18.3 kDa. The structure has been characterized by nuclear magnetic resonance (NMR) spectroscopy at acidic pH, where the protein exists in monomeric form, and by X-ray crystallography at neutral pH, where the protein exists in its dimeric form [15,16]. Native β-LG (Fig. 2) is composed of nine antiparallel strands, labeled β-A to β-I, forming a central calyx and one major terminal three-turn α-helix. These secondary structure elements are arranged to form at least two hydrophobic binding sites that accommodate small ligands. β-LG has been extensively studied in protein folding and aggregation. It has been shown that β-LG can readily form amyloid fibrils upon heating at acidic pH or in the presence of denaturants at neutral pH [17–20]. Previous studies showed that the growth kinetics curves of β-LG fibril are sigmoidal curves showing a clear lag phase. Furthermore, the results of electron micrographs also revealed that these fibrils of β-LG are unbranched, twisted fibrils with diameters of 8–10 nm [19].

β-Ionone (Fig. 1) is one phytochemical compound with an end ring analog of β-carotenoid. It represents a subclass of cyclic

* Corresponding author. Tel.: +86 13605184263.
E-mail address: youx@njau.edu.cn (X. You).

0141-8130/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.ijbiomac.2013.12.003

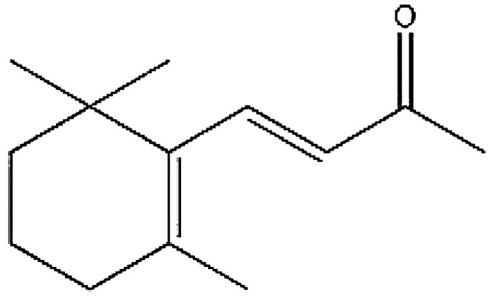

Fig. 1. The chemical structure of β-ionone.

isoprenoids. Its physiological functions include the inhibition of the growth of fungi [21] and the regulation of the synthesis of mevalonate-derived constituents [22]. Previous studies have also demonstrated that β-ionone can inhibit the growth of breast-, gastric-, colon- and HL-60-cancer cells in a dose-dependent manner [23]. In addition, β-ionone can also suppress mammary carcinogenesis, proliferative activity and induce apoptosis in the mammary gland of the Sprague-Dawley rat [24].

Interactions between aroma compounds and β-LG are highly diverse and have received much attention because of their influence on the organoleptic properties of food [25–27]. In this work, the effects of β-ionone on the urea-induced unfolding and amyloid fibrils of β-LG are investigated. These results demonstrate that β-ionone can enhance the stability and inhibit the fibril formation of β-LG at pH 7.0.

2. Materials and methods

2.1. Chemicals

Bovine β-lactoglobulin (β-LG) was obtained from Sigma (Sigma–Aldrich Co., St. Louis, MO) and used without purification. β-Ionone, Thioflavin T, sodium azide (NaN_3) and Congo Red were purchased from Sigma. All the other chemical regents used were of analytical grade and were made in China.

2.2. Urea-induced unfolding monitored by tryptophan fluorescence

A stock solution of β-LG was prepared in 10 mM sodium phosphate buffer at pH 7.0. In the urea-induced unfolding experiments, urea stock solution (10 M) and β-LG sample were mixed in different ratios to prepare β-LG-denaturant mixtures with urea concentrations ranging from 0 to 8 M. The final concentration of protein was 0.05 mg ml^{-1} in the buffer containing different concentrations of β-ionone (0, 20 μM, 40 μM, 60 μM, 80 μM), respectively. The solutions were incubated for 10 min at 37 °C and used for the following spectral measurements.

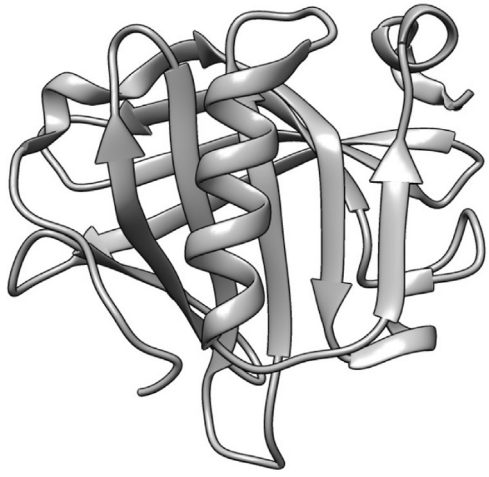

Fig. 2. Schematic representation of β-LG structure.

The tryptophon fluorescence measurements were performed on Fluorescence Spectrophotometer (Hitachi F-4600, Tokyo, Japan) with a cuvette of 1 cm light path. The excitation wavelength of fluorescence spectrum was set at 290 nm, and the emission spectra were recorded in the wavelength range of 305–420 nm. The bandwidths of excitation and emission slits were set 2.5 nm and 5 nm, respectively. Spectra were collected with a scan speed of 240 nm ml^{-1} and a response time of 1 s. Each spectrum was the average of 3 scans. Data were obtained by monitoring the fluorescence intensity at 340 nm. The analytical methods for these data of the fluorescence were described in detail in the previous publication [28].

2.3. Fibril formation

A stock solution of β-LG was prepared in 10 mM sodium phosphate buffer at pH 7.0. Dilution with 10 M urea in 10 mM sodium phosphate buffer resulted in solutions of 1.2 mg ml^{-1} β-LG with urea concentrations ranging from 0 to 7 M at pH 7.0. To study the effect of β-ionone on the fibril formation, the desired concentrations of β-ionone were added into the solutions containing 1.2 mg min^{-1} β-LG and 5 M urea with 0.01% (w/v) sodium azide (NaN_3). β-LG solutions were first well mixed via vortexing and then incubated at 37 °C during the course of experiments.

2.4. ThT assay

To monitor the degree of fibril formation, 50 μL aliquots of proteins were taken at each time point and diluted 100 times with 4 mL of ThT solution in 10 mM sodium phosphate buffer (pH 7.0, final ThT concentration 25 μM). ThT fluorescence intensity measurements were performed by exciting samples at 450 nm with slit-width of 5 nm and recording emission intensity at 482 nm with slit-width of 5 nm using a Hitachi F-4600 Florescence Spectrophotometer. All measurements were taken in triplicate. The data from ThT fluorescence measurements were fitted against sigmoidal curves described by the following equation:

$$I = I_0 + m_i t + \frac{I_f + m_f t}{1 + e^{-[(t-t_0)/\tau]}} \quad (1)$$

where I is the fluorescence intensity at time t, t is incubation time and t_0 is the time to reach 50% of maximal fluorescence, and $I_0 + m_i t$ and $I_f + m_f t$ represent the initial baselines and the final plateau line, respectively. Thus, the apparent rate constant, k_{app}, for the growth of fibrils is given by $1/\tau$ and the lag time is given by $t_0 - 2\tau$ [29].

2.5. Congo red binding assays

A stock solution of 200 μM Congo Red (CR) was prepared in 10 mM sodium phosphate solution (pH 7.0). CR absorbance of β-LG samples and the free dye control were determined by adding CR to a final concentration 20 μM and acquiring spectral measurements from 400 to 700 nm at 37 °C on an ultraviolet-visible spectrometer (Shimadzu UV-1800, Japan). The β-LG solutions in the absence and presence of β-ionone and the control solutions were allowed to interact with CR for at least 30 min in the dark before recording the absorbance spectra. All measurements were taken in triplicate.