NUTRITION RESEARCH 36 (2016) 648–657

Available online at www.sciencedirect.com

ScienceDirect

www.nrjournal.com

Original Research

Low-molecular-weight fractions of Alcalase hydrolyzed egg ovomucin extract exert anti-inflammatory activity in human dermal fibroblasts through the inhibition of tumor necrosis factor–mediated nuclear factor κB pathway

Xiaohong Sun[a,1], *Subhadeep Chakrabarti*[a,b,1], *Jun Fang*[c,d], *Yulong Yin*[c,d], *Jianping Wu*[a,b,*]

[a] *Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada*
[b] *Cardiovascular Research Centre, University of Alberta, Edmonton, AB, Canada*
[c] *College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha 410128, China*
[d] *Key Laboratory of Agro-ecological Processes in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Changsha, 410125, Hunan, China*

ARTICLE INFO

Article history:
Received 15 November 2015
Revised 14 March 2016
Accepted 22 March 2016

Keywords:
Ovomucin hydrolysate
Inflammation
Dermal fibroblast
Desalting
NF-κB
Ultrafiltration

ABSTRACT

Ovomucin is a mucin-like protein from egg white with a variety of biological functions. We hypothesized that ovomucin-derived peptides might exert anti-inflammatory activity. The specific objectives were to test the anti-inflammatory activities of different ovomucin hydrolysates and its various fractions in human dermal fibroblasts, and to understand the possible molecular mechanisms. Three ovomucin hydrolysates were prepared and desalted; only the desalted Alcalase hydrolysate showed anti-inflammatory activity. Desalting of ovomucin hydrolysate enriched the proportion of low-molecular-weight (MW) peptides. Indeed, ultrafiltration of this hydrolysate displayed comparable anti-inflammatory activity in dermal fibroblasts, indicating the responsible role of low-MW bioactive peptides in exerting the beneficial biological function. The anti-inflammatory activity of low-MW peptides was regulated through the inhibition of tumor necrosis factor–mediated nuclear factor κ–light-chain-enhancer of activated B cells activity. Our study demonstrated that both peptide composition and MW distribution play important roles in anti-inflammatory activity. The low-MW fractions prepared from ovomucin Alcalase hydrolysate may have potential applications for maintenance of dermal health and treatment of skin diseases.

Abbreviations: BAY 11-7085, (E)-3-(4-t-butylphenylsulfonyl)-2-propenenitrile; CBB, Coomassie Brilliant Blue; HDFs, human dermal fibroblasts; IκBα, inhibitor κBα; ICAM-1, intercellular cell adhesion molecule-1; MW, molecular weight; NF-κB, nuclear factor κ–light-chain-enhancer of activated B cells; PAGE, polyacrylamide gel electrophoresis; SDS, sodium dodecyl sulfate; TNF, tumor necrosis factor; TFA, trifluoroacetic acid; UPLC, ultra-performance liquid chromatograph.

* *Corresponding author at:* 4-10 Ag/For Centre, Department of Agricultural, Food & Nutritional Science, University of Alberta, Edmonton, AB, Canada T6G 2P5. Tel.: +1 780 492 6885; fax: +1 780 492 4265.
E-mail address: jwu3@ualberta.ca (J. Wu).

[1] Both authors contributed equally.

http://dx.doi.org/10.1016/j.nutres.2016.03.006
0271-5317/

1. Introduction

Egg is a widely used, affordable, and high-quality source of protein. Egg protein–derived compounds have been extensively studied for their potential uses either as functional foods or as nutraceutical preparations [1]. Ovomucin is a mucin-like glycoprotein from egg white, accounting for 2% to 4% of total egg protein [2]. Each ovomucin molecule is composed of a carbohydrate-poor α-subunit and a carbohydrate-rich β-subunit, containing 11% to 15% and 50% to 57% (wt/wt) carbohydrate, respectively [3,4]. It is well documented that ovomucin and its hydrolysates exert a number of beneficial bioactivities such as antitumor and cholesterol-lowering activities [5]. A structurally similar molecule, MUC1 mucin, a transmembrane glycoprotein expressed on the surface of mucosal epithelial cells, has been demonstrated to have anti-inflammatory property by suppressing the Toll-like receptor 5 signaling pathway [6]. In addition, ovomucin is rich in sialic acid, which can further contribute to anti-inflammatory effects [7]. Thus, ovomucin might have the potential to modulate inflammation, although its specific anti-inflammatory functions remain largely unknown.

Proteolytic digestion has been used to enhance both solubility and bioactivities of ovomucin [8]. For example, glycopeptides generated upon pronase digestion of ovomucin can exert antiadhesive activity against *Escherichia coli* O157:H7 as well as antitumor activity in a double-grafted tumor system [9,10]. Ovomucin hydrolysates generated by microbial proteases such as Protamex, Flavourzyme, and Alcalase also possess antioxidant activity as determined by free radical scavenging assays [11].

However, not all protein hydrolysates are created equal. Indeed, depending on the enzymes used, the posthydrolysis treatments undertaken and the intrinsic properties (molecular weight [MW], solubility, hydrophobic vs hydrophilic nature) of the substrate protein, hydrolysates from the same protein source can differ widely in their observed biological functions [12]. For example, a recent publication by Schadow et al [13] has demonstrated the stark differences in biological effects of several commercially available collagen hydrolysates on articular cartilage function, whereas other studies suggested that lower-MW peptides were better suited for topical use and those rich in proline and/or hydroxyproline had higher bioavailability upon oral ingestion [14,15]. Thus, there is a need to identify those enzymatic hydrolysates that might exert beneficial actions such as anti-inflammatory effects. Development of bioactive preparations from proteins like ovomucin can then lead to their use in the management of inflammatory conditions such as those affecting the skin, the largest organ in the body.

Inflammatory damage to the skin occurs from external injurious stimuli (excessive exposure to sun rays, trauma, radiation, infections) as well as from internal processes such as aging and the dermatologic manifestations of systemic illnesses [16]. Wound healing is also dependent on a low level of dermal inflammation, yet excessive and uncontrolled inflammatory changes may lead to widespread scarring and weaknesses of the healed skin [17]. Anti-inflammatory drugs and laser therapy are widely used for the treatment of skin diseases and aging, but are associated with their own complications and adverse effects, especially upon long-term usage [18,19]. As such, there is increasing interest in using naturally derived compounds for maintenance and enhancement of skin health and reversal of skin aging effects. Natural compounds such as those derived from food sources are generally perceived as safer alternatives to pharmacologic agents and also likely to be better tolerated due to their preexisting use as dietary components [20]. Indeed, several enzymatic hydrolysates from food protein sources have shown promise in ameliorating pathological mechanisms associated with skin aging and dermal diseases [21,22].

Egg white as a natural agent has been used in many parts of the world as an ingredient of folk cosmetics such as facial mask for improving skin health [23]. Given the implied anti-inflammatory potential in skin health and possible anti-inflammatory activity of ovomucin, we hypothesized that ovomucin-derived peptides might exert anti-inflammatory activity. The specific objectives were to test the anti-inflammatory activities of different ovomucin hydrolysates and its various fractions (enzymatically prepared and/or subjected to desalting or ultrafiltration) using human dermal fibroblasts (HDFs), a widely used model system for examining skin health [24,25], and to understand the possible molecular mechanisms of the anti-inflammatory activity.

2. Methods and materials

2.1. *Materials and reagents*

Fresh eggs from White Leghorn laid within 24 hours were obtained from the Poultry Research Centre of the University of Alberta (Edmonton, AB, Canada) and used same day for ovomucin extraction. Sodium dodecyl sulfate (SDS) and trifluoroacetic acid (TFA) were obtained from Sigma-Aldrich (St Louis, MO, USA). Coomassie Brilliant Blue (CBB) R-250 was purchased from Bio-Rad laboratories Inc (Hercules, CA, USA). Acetonitrile was bought from Fisher Scientific Inc (Ottawa, ON, Canada). Milli-Q water was prepared by the milli-Q water supply system (Millipore Corporation, Billerica, MA, USA).

2.2. *Isolation of ovomucin*

The ovomucin was prepared by the method of Wang and Wu [4] with modifications. Briefly, fresh egg white was diluted 3 times with milli-Q water and stirred for about 120 minutes. Then, the above slurry was adjusted to pH 5.0, settled in cold room (4°C) for 24 hours, and centrifuged at 15 344*g* for 10 minutes at 4°C (Beckman Coulter, Inc, Fullerton, CA, USA) to collect the precipitate. The precipitate was lyophilized and stored at −20°C until further use.

As an aside, the 2-step method for ovomucin preparation was not applied because the 2-step method is complicated and time-consuming, involves the use of large amounts of salts, and is difficult for scale-up preparations compared with the 1-step method used in this work. One-step method for ovomucin extraction is a potential plant approach and can easily address the issue of salt residue in the final product. Thus, 1-step method is a practical way to enrich ovomucin for preparation of bioactive ingredients or functional foods.