International Journal of Biological Macromolecules 119 (2018) 533–539

Contents lists available at ScienceDirect

International Journal of Biological Macromolecules

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

Sialic acid involves in the interaction between ovomucin and hemagglutinin and influences the antiviral activity of ovomucin

Qi Xu [a], Yuanyuan Shan [b], Ning Wang [a], Yaping Liu [a], Maojie Zhang [a], Meihu Ma [a,*]

[a] College of Food Science and Technology, Huazhong Agricultural University, Wuhan, Hubei 430070, People's Republic of China
[b] College of Food Science and Engineering, Northwest A&F University, Yangling 712100, People's Republic of China

ARTICLE INFO

Article history:
Received 23 June 2018
Received in revised form 23 July 2018
Accepted 29 July 2018
Available online 30 July 2018

Keywords:
Ovomucin
Glycosylation
Sialic acid
Avian influenza virus
Antiviral mechanism

ABSTRACT

Ovomucin (OVM) plays an important role in inhibiting infection of various pathogens. However, this bioactivity mechanism is not much known. Here, the role of sialic acid in OVM anti-virus activity has been studied by ELISA with lectin or ligand. Structural changes of OVM after removing sialic acid were analyzed by circular dichroism and fluorescence spectroscopy. OVM could be binding to the hemagglutinin (HA) of avian influenza viruses H_5N_1 and H_1N_1, this binding was specific and required the involvement of sialic acid. When sialic acid was removed, the binding was significantly reduced 71.5% and 64.35%, respectively. Therefore, sialic acid was proved as a recognition site which avian influenza virus bound to. Meanwhile, the endogenous fluorescence and surface hydrophobicity of OVM removing sialic acid were increased and the secondary structure tended to shift to random coil. This indicated that OVM molecules were in an unfolded state and spatial conformation disorder raising weakly. Remarkably, free sialic acid strongly promoted OVM binding to HA and thereby enhanced the interaction. It may contribute to the inhibition of host cell infection, agglutinate viruses. This study can be extended to the deepening of passive immunization field.

© 2018 Published by Elsevier B.V.

1. Introduction

Ovomucin (OVM) has a unique antiviral activity, the mechanism of which is not entirely known. OVM is a highly glycosylated protein containing sialic acid (SA), which belongs to the mucin family [1]. Mucins are a major component of mucus, which are widely distributed in the body's internal surface and mucosal tissues, such as the respiratory tract and intestines. They provide an important innate immune barrier to potential toxins, particles, and pathogens [2]. It can prevent pathogens from being in contact with susceptible cells. It is thought that the adhesion of mucins to pathogens is an important mechanism with a potentially significant effect.

OVM has antiviral properties, that is similar in structure and composition to the influenza virus receptor and early findings suggest that OVM has an inhibitory effect on swine influenza virus-induced hemagglutination [3]. The interaction of HA on the surface of the virus with OVM leads to the release of glycopeptide complexes. Hemagglutination inhibition assays and enzyme-linked immunosorbent assays have revealed the high affinity of OVM for bovine rotavirus, chicken new castle disease virus and human influenza virus [4–6]. It was found that *N*-acetylneuraminic acid (NeuAc, a specific subtype of sialic acid in OVM) in the β-subunit could greatly facilitate the interaction between OVM and chicken new castle disease virus. The alteration of the conformation of OVM by the alkylation of disulfide bonds leads to loss of binding to OVM antibody [4]. However, evaluations of antiviral activity in these studies focused primarily on the inhibition of viral-induced hemagglutination and did not include other more accurate and intuitive antiviral methods.

Structurally, OVM is a highly glycosylated protein whose monosaccharides are predominantly in the forms of oligosaccharides and glycosides consisting of fewer than 10 monosaccharides [7,8], including N-glycosidic bonds and O-glycosidic bonds [9]. N-glycans are linked to the aspartic acid (Asp) residues of the polypeptide sequence Asn-X-Ser/Thr, where X represents any amino acid except proline, and O-glycans are predominantly linked to the serine (Ser) and threonine (Thr) residues [10,11]. These oligosaccharides mainly include mannose (Man), galactose (Gal), *N*-acetylgalactosamine (GalNac), *N*-acetylglucosamine (GlcNac), SA, fuctose (Fuc) [12] and sulfuric acid esters [13].

Sialic acid of OVM can promote interaction with chicken new castle disease virus. Meanwhile, various viruses such as influenza virus, coronavirus and rotavirus utilize glycoproteins containing sialic acid on the surface of host cells as recognition receptors [14–16]. Sialic acid can be recognized by the epitope on the globular head of the influenza virus HA, thereby inducing interaction with the corresponding HA receptor binding sites and interfering with or blocking the adsorption of the virus to the cells [17]. Sialic acid residues are generally located at the

* Corresponding author.
E-mail address: mameihuhn@163.com (M. Ma).

https://doi.org/10.1016/j.ijbiomac.2018.07.186
0141-8130/© 2018 Published by Elsevier B.V.

terminus of the N-linked oligosaccharide chain and the O-linked oligosaccharide chain with α2,3-, α2,6- and α2,8-linkages [18]. Different influenza viruses are capable of specifically recognizing different linked types of sialic acids. Human influenza viruses are more likely to bind to the α2,6-linkage sialic acid receptor, and avian influenza viruses preferentially recognize α2,3-linkage sialic acids [19,20].

OVM is a glycoprotein containing a large amount of sialic acid. Its antiviral activity has not been studied deeply, and its anti-infective mechanism is barely understood. The role that sialic acid plays and whether it is recognized as the same receptor of the influenza virus and binds to HA remain to be revealed. Therefore, this study aims to verify the interaction between OVM and HA and demonstrate the function of sialic acid in this interaction to explain the possible mechanism of OVM for satisfactory antiviral activity and increase the knowledge of the role of OVM in passive immunity.

2. Material and methods

2.1. Isolation and purification of OVM

OVM was crude extracted according to a previously reported method [21] with modifications. In brief, 200 mL of fresh whole egg white was stirred at 4 °C for 30 min and subsequently diluted with 600 mL of 100 mM NaCl. The pH was adjusted to 6.0 with 1 M HCl, and the solution was incubated overnight at 4 °C. The egg white solution was centrifuged at 10,000*g* for 10 min at 4 °C, and the precipitate was resuspended with 500 mM NaCl for 4 h and then centrifuged under the same conditions.

After the precipitate, which was crude OVM, was washed several times with distilled water, the above procedure was repeated. The extract was freeze-dried and stored at −20 °C. OVM crude extract was purified by gel filtration chromatography (Sephacryl S-300 HR, 26 mm × 60 cm) using the AKTA purification system (GE, USA). The target elution peak was dialyzed by distilled water, and the product was purified OVM.

2.2. Removal of terminal sialic acid of OVM

Purified OVM (2 mg) was mixed with 1980 μL of pH 5.6 sodium acetate buffer and 20 μL of NA enzyme and incubated in a 37 °C water bath for 18 h.

Desialylated OVM (dSA-OVM) was dialyzed against 14 kD dialysis bags for 24 h with pH 8.6 borate buffer. The solution was removed, stirred for 1 h and centrifuged for 10 min at 6000 r/min; the supernatant was saved for subsequent experiments.

The dSA-OVM supernatant was centrifuged for 10 min in a 10 kD ultrafiltration tube at 5000*g* and concentrated to 1.5 mL. A standard curve was used to quantify the concentration of OVM.

2.3. Determination of the OVM terminal sialic acid bond

The effect of enzymatic hydrolysis and the chemical bond of sialic acid in oligosaccharide chains was evaluated by ELISA using lectins *Sambucus nigra* (SNA) and *Maackia amurensis* (MAA). A 1:25 dilution of SNA was added to the ELISA plate at 100 μL/well, and the plate was incubated overnight at 4 °C. To the control group was added PBS buffer without SNA. The plates were washed 3 times for 5 min each with PBST and then again with PBST dissolved in 5% skim milk at 300 μL/well and incubated at 37 °C for 1 h for blocking. After the plates were washed, dSA-OVM and OVM diluted to 20 μg/mL were added to the experimental and control wells, respectively, at 100 μL/well, and the plates were incubated at 37 °C for 1 h. After the plates were washed, OVM antibody diluted 1:80000 was added at 100 μL/well, and the plates were incubated at 37 °C for 1.5 h. After the plates were washed, HRP-labeled goat anti-mouse IgG diluted 1:8000 was added at 100 μL/well, and the plates were incubated at 37 °C for 80 min, washed and stained.

2.4. OVM binding HA

The function of sialic acid was assessed by the change in the binding of HA to OVM with the removal of sialic acid. The HA proteins of the influenza viruses H_5N_1 (HA5) and H_1N_1 (HA1) were added to the ELISA plate at a dilution of 1:10 at 100 μL/well, and the plate was incubated overnight at 4 °C. To the control group was added PBS buffer without HA. Other operations were the same as in Section 2.3.

2.5. OVM oligosaccharide chain inhibition assays

The common components of oligosaccharide chains (Gal, Fuc, Man and sialic acid) were separately mixed with OVM for competitive binding analysis with HA. Subsequently, the effect of free sialic acid was analyzed by different additional sequences. The additional sequences were the addition of sialic acid followed by the addition of OVM, the addition of OVM followed by the addition of sialic acid, and the addition of pre-incubation mixture. The ELISA procedure was the same as previously described.

2.6. Circular dichroism (CD) changes in OVM

The protein solution was diluted 10-fold, the final protein concentration was 0.3 mg/mL, the optical path of the quartz cell was 0.1 cm, the sensitivity was 2 mdeg/m, the wavelength scanning range was 190–240 nm, the speed was 10 nm/s, and the resolution was 0.1 nm measured at room temperature.

2.7. The intrinsic fluorescence scanning of OVM

The optical path of the quartz cell was 1.0 cm. Using tyrosine (Tyr) as an intrinsic probe, the excitation wavelength was 274 nm, and the emission spectrum was scanned at 290–400 nm. Using tryptophan (Trp), the excitation wavelength was 295 nm, and the emission spectrum was scanned at 300–450 nm. The excitation and emission monochromators each had a bandwidth of 5 nm. OVM was incubated at room temperature for 1 h, and its concentration was diluted to 100 μg/mL. Blank samples were measured under the same conditions.

2.8. OVM surface hydrophobicity

Using l-anilinonaphthalene-8-sulphonate (ANS) as a fluorescence probe, the surface hydrophobicity of OVM was measured by the fluorescence method. OVM was diluted to 0.005–0.2 mg/mL with pH 8.6 borate buffer. Fluorescence spectra of ANS were scanned by adding 8 μL of diluted sample to 100 μL of 8 mmol/L ANS solution. The excitation wavelength was 365 nm, and the scan range was 300–600 nm. The fluorescence intensity at 365 nm excitation and 484 nm emission was used to making a standard curve for protein concentration. The control was blank ANS solution with addition of the corresponding sample buffer.

2.9. Statistical analyses

All values were expressed as mean ± s.e.m. ANOVA with Bonferroni's multiple-comparison test when more than two groups were compared. All the assays were run in triplicate and were representative of at least 3 independent experiments. A P value < 0.05 was considered statistically significant and the asterisks in all figures are defined, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

Download English Version:

https://daneshyari.com/en/article/8326772

Download Persian Version:

https://daneshyari.com/article/8326772

Daneshyari.com