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Ovomucin – a glycoprotein with promising potential

Dileep A. Omana, Jiapei Wang and Jianping Wu*

Department of Agricultural, Food and Nutritional Science (AFNS), University of Alberta, 4–10 Ag/For Building, Edmonton, Alberta, Canada T6G 2P5
(Tel.: +1 780 492 6885; fax: +1 780 492 4265; e-mail: jwu3@ualberta.ca)

Ovomucin, accounting for ~3.5% of total egg white protein, is responsible for the thick gel characteristics of liquid egg white. Besides its excellent foaming and emulsion capacities, it possesses anti-viral, anti-bacterial, anti-tumor and other bio-activities. This paper reviews compositional, structural, physicochemical, functional and biological properties of ovomucin, as well as development of methods of extraction. As one of the least defined proteins in egg white, further study is required to characterize the structure and to explore its full potential in new applications as functional foods and nutraceuticals.

Composition and structure

Ovomucin appears to have a long linear molecule with more coiled regions at its extremities, forming a randomly coiled structure. Hence ovomucin is believed to have a highly polymerized macromolecular structure as that of mammalian mucins (Gallagher & Corfield, 1978), which firmly support the involvement of disulfide bridges in ovomucin polymerization. Ultracentrifugation separates thick egg white into precipitate and liquid portion. Insoluble and soluble ovomucin can be prepared from the precipitate and liquid portions, respectively (Kato, Nakamura, & Sato, 1970). Electrophoretic studies of ovomucin from hen egg albumen revealed three components; two carbohydrate poor (α1- and α2-ovomucin) and one carbohydrate rich (β-ovomucin) components (Itoh, Miyazaki, Sugawara, & Adachi, 1987). They separated these components by gel filtration in presence of SDS and 2-mercaptoethanol. Similar electrophoretic pattern for crude ovomucin in reduced condition has also been observed by Guerin and Brule (1992) and Toussant and Latshaw (1999). In another study by Hiidenhovi, Makinen, Huopalahti, and Ryhanen (2002) while determining the purity of the prepared ovomucin by gel filtration they found that ovomucin eluted at 3 peaks; peak 1 was β-ovomucin and peak 2 and 3 were α2- and

* Corresponding author.

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α1-ovomucin respectively. Molecular weight of different forms of ovomucin based on earlier studies has been detailed in Table 1. Light scattering measurements showed that the molecular weight of insoluble ovomucin as 23,000 kDa in the presence of guanidine hydrochloride (Tomimatsu & Donovan, 1972), whereas the molecular weight of soluble ovomucin was found to be 8300 kDa (Hayakawa & Sato, 1976).

Ovomucin is a highly glycosylated glycoprotein, which consists of a carbohydrate poor subunit (α-ovomucin) with 11–15% carbohydrate and a carbohydrate-rich subunit (β-ovomucin) with 50–57% carbohydrate (Itoh et al., 1987; Kato & Sato, 1971; Robinson & Monsey, 1971). Kato, Fujinaga, and Yagishita (1973) showed that there are at least three types of carbohydrates side chains in ovomucin; a chain composed of galactose, galactosamine, sialic acid and sulfate in a molar ratio of about 1:1:1:1, a second chain composed of galactose and glucosamine in a molar ratio of about 1:1 and a third chain composed of mannose and glucosamine in a molar ratio of about 1:1. On an average, ovomucin consists of 33% of carbohydrate content (Mine, 1995). Various carbohydrates in ovomucin are mannose, galactose, N-acetyl-D-galactosamine, N-acetyl-D-glucosamine, N-acetylneuraminic acid and fructose (Donovan, Davis, & White, 1970), and sulfated saccharides (Robinson & Monsey, 1971). Previous studies on the structure and composition of carbohydrate content of hen ovomucin have been carried out (Strecker, Wieruszeski, Cuvillier, Michalski, & Moutreuil, 1992; Strecker, Wieruszeski, Martel, & Moutreuil, 1987; Strecker, Wieruszeski, Martel, & Moutreuil, 1989). Different types of carbohydrates like mannose (Man), galactose (Gal), N-acetyl-D-galactosamine (GalNAc), N-acetyl-D-glucosamine (GlcNAc), N-acetylneuraminic acid (NeuAc) was found in ovomucin. Further the structure of sulfated saccharides present in ovomucin has also been elucidated (Strecker et al., 1987).

Ovomucin is highly insoluble at neutral pH or in the absence of denaturing agents. It can be made soluble mechanically by homogenization and sonication in mild alkaline conditions or chemically in presence of dissociation agents like urea, guanidine hydrochloride, SDS and by reducing agents like mercaptoethanol or dithiothreitol (Itoh et al., 1987; Robinson & Monsey, 1971). However, these methods usually cause cleavage of disulfide bonds and release of carbohydrate chains. Hayakawa and Sato (1976) reported a molecular weight of 8300 kDa in mild alkaline conditions, which is reduced to 1100 kDa after sonication and to 230 kDa after sonication followed by treatment with reducing agents.

The β-component is predominant of hydroxyl amino acids like threonine and serine while that of α-component are acidic amino acids like glutamic acid and aspartic acid (Itoh et al., 1987). Amino acid analysis of the two ovomucin fractions prepared by lysozyme—sepharose 4B chromatographic column revealed higher proportion of serine and threonine in the first fraction and aspartic acid in the second fraction (Kato et al., 1977). This shows that these fractions are similar to β-ovomucin and α-ovomucin of Itoh et al. (1987), respectively. Robinson and Monsey (1971) also noticed a higher content of glutamic acid and aspartic acid in reduced α-ovomucin, while serine and threonine were predominant in the β-ovomucin. However, no noticeable difference was found in the amino acid composition of ovomucin prepared from thick and thin egg white using gel permeation method (Adachi, Azuma, Janado, & Onodera, 1973). Amino acid sequence of α-ovomucin from hen thick egg white showed 2087 amino acid residues with relative molecular mass of 230.9 kDa (Watanabe et al., 2004). The amino acid composition of ovomucin prepared by the 2-step method (Omana & Wu, 2009a) is comparable with that of previous reports (Donovan et al., 1970; Kato et al., 1970).

Details on the proximate composition of prepared ovomucin are scarce except its protein content. Robinson and Monsey (1971) reported a protein content of 60.6% (w/w) for the prepared and dried ovomucin complex containing 2.1% (w/w) ash. Ovomucin prepared by Donovan et al. (1970) contained 12.6% nitrogen, 9.24% moisture and 2.11% ash, after freeze drying. Ovomucin prepared by the 2-step method developed by Omana and Wu (2009a) revealed a protein content of 69.7%.

### Table 1. Molecular weight of different forms of ovomucin by earlier studies.

| Different forms of Ovomucin | Molecular weight (kDa) | References |
|----------------------------|------------------------|------------|
| Native ovomucin            | 8000                   | Lanni et al., 1949 |
| Ovomucin monomer unit      | 163                    | Donovan et al., 1970 |
| Insoluble ovomucin         | 23,000                 | Tomimatsu & Donovan, 1972 |
| Soluble ovomucin           | 8300                   | Hayakawa & Sato, 1976 |
| Sonicated ovomucin         | 1100                   | Hayakawa & Sato, 1976 |
| Reduced ovomucin           | 230                    | Hayakawa & Sato, 1976 |
| α-ovomucin                 | 210                    | Robinson & Monsey, 1971 |
| β-ovomucin                 | 200                    | Kato et al., 1991 |
| α1-ovomucin                | 220                    | Tsuge et al., 1997a, 1997b |
| α2-ovomucin                | 150                    | Itoh et al., 1987 |
| α2-ovomucin                | 220                    | Itoh et al., 1987 |
| β1-ovomucin                | 210                    | Hidenhovi et al., 1999 |
| β1-ovomucin                | 523–743                | Robinson & Monsey, 1972 |
| β2-ovomucin                | 400                    | Hayakawa & Sato, 1976 |
| β2-ovomucin                | 700                    | Itoh et al., 1987 |
| β2-ovomucin                | 700                    | Kato et al., 1991 |

### Methods of preparation

Ovomucin was first prepared by Eichholz (1898), while working with glucoside constitution of proteins. His method involved dilution of egg white with distilled water (1:4) and separating the precipitate by centrifugation. The precipitate was then repeatedly washed with distilled water, boiled in rectified spirit, washed in ether and dried to get ovomucin. Different methods of preparation of

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**Note:** The content is a portion of a larger text, and the table is provided as an example of a structured data representation within the text. The full text is not included here for brevity. The table provides molecular weight values for various forms of ovomucin as determined by different methods and studies. The text also discusses the carbohydrate composition, amino acid analysis, and methods of preparation of ovomucin.
ovomucin have been detailed in Table 2. As a conventional method, ovomucin is extracted and purified from egg white by isoelectric precipitation method (Brooks & Hale, 1961; Donovan et al., 1970; Kato et al., 1970; Robinson & Monsey, 1971). Briefly, egg albumin is diluted with water, followed by adjusting the pH to isoelectric precipitation of ovomucin. The gelatinous precipitate obtained is repeatedly washed with water and 2% KCl, in order to get rid of the major co-precipitating proteins like ovalbumin and lysozyme (Brooks & Hale, 1959; Kato et al., 1970). This method was promising to adopt in industrial scale, however the precipitated ovomucin contained other egg white proteins even after repeated washings. Beveridge and Nakai (1975) prepared ovomucin as per the method of Robinson and Monsey (1971) except the replacement of 2% KCl with 2% NaCl. Recently Omana and Wu (2009a) developed a novel method for preparation of more pure ovomucin. The procedure includes precipitation of egg white using 100 mM NaCl solution at pH 6.0, followed by treating the precipitate by 500 mM NaCl solution. The precipitate was then separated by centrifugation. This method seems economically feasible to scale up for the preparation of ovomucin of purity of over 90%.

Isolation of proteins from egg white by gel filtration is not uncommon. Attempt to purify ovomucin by gel permeation chromatography was first carried out by Young and Gardner (1970), using Sepharose 4B column with a size exclusion limit of 3 x 10^6. Ovomucin was found in the first fraction eluted in the void volume due to its high molecular size. Several other researchers (Awade, Moreau, Molle, Brule, & Maubois, 1994; Hiidenhovi, Aro, & Kankare, 1999; Itoh et al., 1987) also attempted to prepare ovomucin by gel filtration. This method of extraction of ovomucin was found to be analytical rather than preparative. However an attempt has been made in preparative scale as well. Obtained crude ovomucin after isoelectric precipitation was dissolved in phosphate buffered saline, which was further passed on to preparative gel filtration chromatography to obtain 97% pure ovomucin (Hiidenhovi, Huopalahti, & Ryhänen, 2003).

Donovan et al. (1970) prepared ovomucin by modifying the method of Mac-Donnell, Lineweaver, and Feeney (1951). Briefly, after lysozyme crystallization, the supernatant was adjusted to pH 4.5 and dialyzed. The precipitated ovomucin was separated by centrifugation and washing with water. Sleigh, Meltrose, and Smith (1973) prepared ovomucin by ultracentrifugation method. After initial blending of thick white with 2.2 M KH2PO4, the mixture was centrifuged at 105,000g (5 °C) for 75 min. The gelatinous residue was then blended in 0.5 M KH2PO4 with repeated centrifugation (105,000g) for two times, followed by dialysis. Despite the low yield obtained by this method, it was attractive because of rapid isolation. Soluble ovomucin can be prepared by ultracentrifugation (59,000g) of homogenized egg white, followed by washing the gel fraction by 2% KCl until washings was free from protein (Kato, Imoto, & Yagishita, 1975).

### Table 2. Various methods (including modifications) used for ovomucin isolation.

| Different methods of ovomucin isolation | References |
|----------------------------------------|------------|
| First isolation of ovomucin | Eichholz (1898) |
| Precipitation at pH 4.5 after lysozyme crystallization | Mac-Donnell et al. (1951), Donovan et al. (1970) |
| Isoelectric precipitation (at pH 6.0) methods | Brooks and Hale (1961), Kato et al. (1970), Donovan et al. (1970), Robinson and Monsey, (1971), Beveridge and Nakai (1975), Guerin and Brule (1992) |
| Gel permeation chromatography method | Itoh et al. (1987), Awade et al. (1994), Awade and Efstathiou (1999), Hiidenhovi et al. (1999), Sleigh et al. (1973) |
| Ultracentrifugation (105,000 × g) method | Kato et al. (1975) |
| Preparation of soluble ovomucin by ultracentrifugation (59,000 × g) method | Omana and Wu (2009a) |
| 2-step method using NaCl solutions | |
Table 3. Yield of ovomucin obtained by various researchers from different egg white sources.

| Source of ovomucin          | Yield (mg/100 g of egg white) | References (in chronological order) |
|-----------------------------|-------------------------------|-------------------------------------|
| Whole egg white             | 90–117                        | Brooks and Hale (1961)              |
| Thick egg white             | 132                           | Baliga et al. (1971)                |
| Thin egg white              | 26                            | Baliga et al. (1971)                |
| Thick egg white             | 200                           | Robinson and Monsey (1971)          |
| Thick egg white             | 260                           | Adachi et al. (1973)                |
| Thin egg white              | 180                           | Adachi et al. (1973)                |
| Whole egg white             | 530                           | Toussant and Latshaw (1999)         |
| Whole egg white             | 280                           | Hidiendhovi et al. (2002)           |
| Thick egg white             | 340                           | Hidiendhovi et al. (2002)           |
| Liquid egg white            | 500                           | Hidiendhovi et al. (2002)           |
| Filtration by-product       | 520                           | Hidiendhovi et al. (2002)           |
| Thick egg white             | 246                           | Hidershoj et al. (2008)             |
| Thin egg white              | 127                           | Hidershoj et al. (2008)             |
| Whole egg white             | 400                           | Omana and Wu (2009a)                |
| Whole egg white             | 410                           | Omana and Wu (2009b)                |

Role of ovomucin in egg white thinning

The thick gel like egg white undergoes thinning to a low viscous state, during storage of egg, generally called as egg white thinning. The chemical changes during the natural thinning of egg white are not fully understood; however several mechanisms have been proposed (Feeney & Allison, 1969; Li-Chan & Nakai, 1989). The major explanations are (1) depolymerization of ovomucin by reduction of disulide bonds; (2) dissociation of lysozyme–ovomucin complex (3) lysozyme complexes with ovomucin in such a way as to change the physical state of the ovomucin molecules and destroy the gel structure and (4) alkaline hydrolysis of the disulide bonds of ovomucin (Burley & Vadehra, 1989; Donovan, Davis, & Wiele, 1972; Robinson, 1972). This section mainly deals with the proposals based on the role of ovomucin in egg white thinning.
Gel-like properties of thick egg albumin is mainly due to ovomucin, in the case of avian eggs. Hence the most accepted reason for egg white thinning is the degradation of ovomucin complex (Kato, Nakamura, & Sato, 1971; Robinson & Monsey, 1972). This finding was further confirmed later (Kato & Sato, 1972). They observed a gradual solubilization of β-ovomucin in the thick egg white, while α-ovomucin remains unchanged during egg white thickening. This view was supported by Prins (1988), stating that thinning is associated with disaggregation of ovomucin’s α and β-subunits. He noticed a decrease in β-subunits (highly glycosylated) in the thick albumen gel during storage, causing decrease in the carbohydrate proportion of the water-insoluble ovomucin.

As mentioned earlier, ovomucin is a complex of two distinct glycoprotein components viz., α- and β-ovomucins (Hayakawa & Sato, 1976; Kato & Sato, 1971). Its β-ovomucin is reported to have stronger interaction with lysozyme than α-ovomucin. The nature of interaction is primarily electrostatic, involving the negative charges of the terminal sialic acid residues in ovomucin and the positive charges of the lysyl ε-amino groups in lysozyme (Kato et al., 1975; Kato, Yoshida, Matsudomi, & Kobayashi, 1976). It has been calculated that 1.97 g of lysozyme are required to cross-link 2 g of ovomucin at pH 7.4 (Robinson, 1972). Ovomucin usually present in egg white as a complex with lysozyme (Cotterill & Winter, 1955) and other egg white proteins (Kato et al., 1976). Hawthorne (1950) suggested that egg white thinning might result from the slow insolubilization of ovomucin caused by its combination with lysozyme. Maximum interaction of ovomucin with lysozyme was observed at a pH of 7, and the interaction reduced at alkaline pH during storage (Cotterill & Winter, 1955). Brooks and Hale (1961) have also the same suggestion that the changes in network of ovomucin chains associated/cross linked with lysozyme may be the reason for egg white thickening.

It is well known that egg proteins contain a great deal of sulphhydryl groups (Greenstein, 1939). A role of sulphhydryl groups was also suggested in egg white thinning. Smith and Back (1962) indicated the possibility of involvement of sulphhydryl (SH) groups of ovalbumin during egg white thinning, while Donovan et al. (1972) suggested that alkaline hydrolysis of the disulphide bonds of ovomucin was responsible for the thinning. Although later study showed that ovalbumin undergoes a transition on heating or on storage resulting in a more stable protein (Smith & Back, 1965), the role of sulphhydryl group in ovalbumin on egg white thinning is unclear. Further study also suggested that disulphide cleavage of ovomucin did not occur in egg white thinning (Kato, Ogata, Matsudomi, & Kobayashi, 1981). They found that α-ovomucin polymerized by disulphide bonds remained insoluble for long time storage of 60 days, suggesting the occurrence of non-covalent disaggregation of ovomucin might be the reason for egg white thinning.

## Bioactivity

At present, proteins from food are always valued not only by their nutritional value and functional properties, but also by their biological activities. There has been a growing interest within food industry towards health-promoting substances. The anti-bacterial, anti-viral, anti-tumor and other bioactivities of ovomucin and its glycopeptides were already been demonstrated. Important bioactivities showed by ovomucin and its derived components are given in Table 4.

### Anti-viral and anti-bacterial activities

Anti-haemagglutination activity of ovomucin against influenza virus was reported in early 1940s (Gottschalk & Lind, 1949). They found that the interaction between virus enzyme and the virus haemagglutinin inhibitory component of ovomucin resulted in the liberation of a carbohydrate—peptide complex. Later investigations on the biological function

| Table 4. Important bioactivities of ovomucin/ovomucin derived components. |
|---------------------------------------------------------------|
| **Ovomucin/Ovomucin derived component** | **Bioactivity** | **References** |
| Ovomucin | Anti-haemagglutination activity against influenza virus | Gottschalk and Lind (1949) |
| Ovomucin | Proliferation of mouse spleen lymphocytes | Otani and Maenishi (1994) |
| Ovomucin | Higher affinity towards bovine rotavirus, hen Newcastle disease virus (NDV), and human influenza virus | Tsuge et al. (1996a); Tsuge et al. (1996b); Tsuge et al. (1997a) |
| Ovomucin | Inhibitory activity against colonization of Helicobacter pylori | Kodama and Kima (1999) |
| Ovomucin | Hypcholesterolemic action | Nagaoka et al. (2002) |
| β-ovomucin | Cytotoxic effect on cultured tumor cells | Oharni et al. (1993) |
| β-ovomucin | Suppress the growth of subcutaneously xenografted sarcoma-180 cells in mice and cure the tumor | Yokota et al. (1999b) |
| Glycosylated fragment of α-ovomucin | Anti-tumor activity | Oguro et al. (2000) |
| Sulfated glycopeptides of ovomucin | Activate cultured macrophage-like cells | Tanizaki et al. (1997) |
| Ovomucin glycopeptides | Anti-tumor activity | Watanabe et al. (1998) |
| Ovomucin glycopeptides | Binding to Escherichia coli O157:H7 | Kobayashi et al. (2004) |
| Ovomucin hydrolysate | Strong inactivation action on food poisoning bacteria | Ryoko et al. (2004) |
| Sialic acid | Anti-infection activity against virus or bacteria | Kelm and Schauer (1997); Schauer (2004) |
of ovomucin using haemagglutination inhibition test and ELISA revealed the higher affinity of ovomucin towards bovine rotavirus, hen Newcastle disease virus (NDV), and human influenza virus (Tsuge, Shimoyamada, & Watanabe, 1996a; Tsuge, Shimoyamada, & Watanabe, 1996b; Tsuge et al., 1997a). They also found that the N-acetylneuraminic acid (NeuNac) residue in the β-subunit greatly contributed to the binding of ovomucin to NDV, and the reduction and alkylation of disulfide bonds in ovomucin markedly altered its conformation and resulted in no ability to bind to anti-ovomucin antibodies (Tsuge, Shimoyamada, & Watanabe, 1997b).

In addition, ovomucin showed anti-bacterial activity. Ovomucin was found to have inhibitory activity against colonization of Helicobacter pylori (Kodama & Kimura, 1999). Enzymatically hydrolyzed products (2.0 kDa-70.0 kDa average molecular weight) obtained by reacting ovomucin with one or more kinds of proteases selected from serine protease, papain, metalloproteinase, trypsin and pepsin exhibit strong inactivation action on food poisoning bacteria (one or more kinds of bacteria selected from enterotoxigenic Escherichia coli, pathogenic E. coli, cell invasive E. coli, vero toxin-producing E. coli, pathogenic Vibrio bacterium, Bacillus dysentericus, Pseudomonas aeruginosa and Pseudomonas cepacia) (Ryoko et al., 2004).

As mentioned earlier, ovomucin consists of α- and β-components. The former is N-glycosidic glycoprotein with or without a little sialic acid and the latter is O-glycosidic glycoprotein containing large amounts of sialic acid. Most studies suggested that the anti-infection activity of ovomucin resulted from sialic acid. Kobayashi et al. (2004) prepared ovomucin glycopeptides with pronase digestion and found that ovomucin glycopeptides could bind to Escherichia coli O157:H7. Sialydase treatment of ovomucin glycopeptides prevented its ability to bind Escherichia coli O157:H7, demonstrating that sialic acid played an important role in the binding.

Sialic acids represent a family of more than 50 structurally distinct sugars with α-keto acids on a nine carbon backbone (Hao, Balagurumoorthy, Sarilla, & Sundaramoorthy, 2005). The most common form of sialic acid contains NeuNac, N-glycolylneuraminic acid (NeuNGc) and 2-keto-3-deoxy-D-glycero-D-galacto-nonulosonic acid (KDN). Many studies reported that sialic acid showed anti-infection activity against virus or bacteria such as Influenza A and C viruses, cholera, tetanus, diphtheria toxin, corona viruses, polyoma viruses, adenoma viruses, rota viruses, mouse hepatitis virus, HIV viruses, E. coli, Streptococci, H. pylori (Kelm & Schauer, 1997; Schauer, 2004). Sialic acid could be directly involved in a variety of recognition processes (Kelm & Schauer, 1997). Many viruses, bacteria and protozoa attach to host cells via sialic acid on the surface of cell, in which sialic acid are the most frequent ligands. The anti-infection activity of sialic acid might be caused by occupying the recognition sites of pathogenic microorganisms. The mechanism of anti-infection activity from ovomucin glycopeptides might act the same way, which should be further studied.

Anti-tumor activity

Ohami, Ohishi, Yokota, Mori, and Watanabe (1993) reported the cytotoxic effect of β-ovomucin from egg white on cultured tumor cells such as SEKI cell (human melanoma cell) and 3LL (Lewis lung cancer cell) by scanning electron microscopy. Further study on dose and time dependent cytotoxic effect of β-subunit on sarcoma-180 (SR-180) cells has also been carried out (Yokota, Ohishi, & Watanabe, 1999a). SR-180 cells treated with β-subunit showed changes such as swelling and bleb formation of microvilli on cell membrane, irregular clumping of chromatin, irregular nuclear shape, and marked swelling of organelles in cytoplasm associated with cell degeneration in necrotic change. Yokota, Ohishi, and Watanabe (1999b) suggested that β-subunit prepared from egg white ovomucin was found to suppress the growth of subcutaneously xenografted sarcoma-180 cells in mice and cure the tumor. The β-subunit-treated tumor cells were in the states of degenerated and necrotic cells, and massive accumulations of neutrophils, macrophages and lymphocytes were found at the margin of the degenerated and necrotic tumor tissue area. These findings suggested that β-subunit brought about the regression of tumor, probably by activating the immune system. Watanabe, Tsuge, Shimoyamada, Ogama, and Ebina (1998) found that (220 and 120 kDa, highly glycosylated peptides) ovomucin separated from pronase-treated hen egg white could cure directly and entirely tumor and inhibited indirectly and slightly its growth. Desialylated experiments indicated that the sialic acid residues in the 120 kDa fragment are not necessarily essential for direct anti-tumor activity but might be indispensable for regression of distant tumors. In non-inhibitory activity, the increase of immunosuppressive acid protein in serum suggested the slight activation of the immune system. Later Oguro, Watanabe, Tani, Ohishi, and Ebina (2000) proved the anti-tumor activities of a 70 kDa highly glycosylated fragment (OVMz70F) in the α-subunit separated from pronase-treated hen egg white ovomucin. In the tumor tissues of OVMz70F-treated mice neutrophils, macrophages and lymphocytes were found to have massively accumulated and the angiogenesis (the formation of new capillary blood vessels) was inhibited.

Other bioactivities

The sulfated glycopeptides in ovomucin were found to activate cultured macrophage-like cells (Tanizaki, Tanaka, Iwata, & Kato, 1997). The macrophage-stimulating activity was estimated by the growth and morphology of the cells, H2O2 generation, and interleukin-1 (IL-1) production from the cells. The in vitro culture assay with macrophages showed that the protease digestion of ovomucin induced morphologic alteration and increased H2O2 generation and IL-1 production in lower concentration (100 µg/ml). It is also reported that ovomucin could enhance the proliferation of mouse spleen lymphocytes, stimulated by lipopolysaccharide (Otani & Maenishi, 1994). Furthermore,
ovomucin also exhibited cholesterol lowering effect. Cholesterol uptake in Caco-2 cells was inhibited by ovomucin and ovomucin-feeding could significantly lower the serum cholesterol in rats; which proved the hypocholesterolemic action of ovomucin (Nagaoka, Masaoaka, Zhang, Hasegawa, & Watanabe, 2002). In fact, not only α, β-sub-units of ovomucin but also peptides from ovomucin protein also showed different kinds of biological activities, which suggest ovomucin as a highly potential source of bioactive ingredients.

Future perspectives
Ovomucin was among the focus of previous studies aiming to establish a role of this protein in egg white thinning. Even though, various hypotheses proposed regarding the involvement of ovomucin in egg white thinning, the science behind that is still unknown. As an extremely large molecule, the structure of egg white ovomucin has not been fully investigated. Although there are several detailed studies on the sugar chain structures of ovomucin, further study is needed to fully map the sugar chain structures and their linkages to elucidate the structure–function relationship of this unique protein. Recent research indicated that ovomucin have anti-bacterial, anti-viral, anti-tumor and macrophage stimulating activities; however, further research needs to be done on its activity to bind food borne pathogens. Till date the purity of the protein was the major challenge in industry. It is expected that the new method of ovomucin preparation recently reported might further advance the structural information of the unique protein and thus would open new windows for further utilization of this important glycoprotein.

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