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Review

Ovomucin – a glycoprotein with promising potential

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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 bioactivities. 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.

Introduction

Ovomucin, the protein believed to be responsible for the gel like properties (Brooks & Hale, 1959) of fresh egg white, was first isolated by Eichholz (1898). This structurally important sulfated glycoprotein comprises of~3.5% of the total egg white proteins. Other major egg proteins include ovalbumin (54%), ovomucoid (11%), ovotransferrin (12%) and lysozyme (3.5%) (Kato, Tanaka, Matsudomi, & Kobayashi, 1986; Sim & Sunwoo, 2006). Considering its solubility, ovomucin complexes differs from an insoluble ovomucin complex (usually from whole thick egg white) to a soluble form (Kato, Ogino, Matsudomi, & Kobayashi, 1977). The molecular weight of soluble ovomucin complex in non-reducing condition varies between 5000 and 8000 kDa. Previous interest in this protein aimed to understand its role in egg white thinning (Robinson & Monsey, 1972); current progress indicated that there are various biological activities associated with this protein (Kodama & Kimura, 1999; Ryoko *et al*., 2004; Tsuge, Shimoyamada, & Watanabe, 1997a). However, the potential of this protein as a novel food and functional food ingredient had never been fully utilized, probably due to lack of an industry-compatible method of extraction. As a partially-defined protein, there is a need to further understand this protein to achieve its potential. Most major egg white proteins have been extensively reviewed in literature (Cegielska-Radziejewska, Lesnierowski, & Kijowski, 2008; Mine & Kovacs-Nolan, 2006; Nguyen & Smith, 1984), while ovomucin is not described to that extend except the book chapter by Hiidenhovi (2007). This review detailed all aspects of ovomucin including its composition and structure, methods of preparation, purity and yield, bioactivity and its physical and chemical characteristics. Hence the review will be helpful in better understanding of this protein and will be an insight for future research needed for exploiting its potential.

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 as 3 peaks; peak 1 was β-ovomucin and peak 2 and 3 were α2- and

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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 presenting 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).

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
α-ovomucin	200	Kato *et al*., 1991
α-ovomucin	220	Tsuge *et al*., 1997a, 1997b
α1-ovomucin	150	Itoh *et al*., 1987
α2-ovomucin	220	Itoh *et al*., 1987
α2-ovomucin	210	Hiidenhovi *et al*., 1999
β-ovomucin	523–743	Robinson & Monsey, 1972
β-ovomucin	400	Hayakawa & Sato, 1976
β-ovomucin	400	Itoh *et al*., 1987
β-ovomucin	700	Kato *et al*., 1991

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 also 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 theronine 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%.

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

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 3×10^6. 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, Melrose, and Smith (1973) prepared ovomucin by ultracentrifugation method. After initial blending of thick white with 2.2 M KH_2PO_4, the mixture was centrifuged at 105,000*g* (5 °C) for 75 min. The gelatinous residue was then blended in 0.5 M KH_2PO_4 with repeated centrifugation (105,000*g*) 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 (59,000*g*) 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	Young and Gardner (1970), Itoh *et al.* (1987), Awade *et al.* (1994), Awade and Efstathiou (1999), Hiidenhovi *et al.* (1999)
Ultracentrifugation (105,000 × g) method	Sleigh *et al.* (1973)
Preparation of soluble ovomucin by ultracentrifugation (59,000 × g) method	Kato *et al.* (1975)
2-step method using NaCl solutions	Omana and Wu (2009a)

Purity and yield of isolated ovomucin

Data pertaining to ovomucin purity is scarce. Purity of ovomucin extracted from whole egg white, thick egg white and liquid egg white was found to be 64%, 65% and 71% respectively (Hiidenhovi *et al*., 2002). Awade *et al*. (1994) could purify ovomucin to 80% using superpose 6 prep gel permeation chromatography. Hiidenhovi *et al*. (2003) prepared ovomucin using a combination of conventional IEP and preparative GFC method. Isoelectric precipitated ovomucin was dissolved in phosphate buffered saline. The dissolved ovomucin underwent preparative gel filtration chromatography to obtain 97% pure ovomucin. Further the insoluble ovomucin showed 99% purity as revealed by gel filtration chromatography. Contamination level increased (43% purity) when centrifugation step (after isoelectric precipitation) was replaced by sieving step. Recently, Omana and Wu (2009a) reported ovomucin of 94.6% purity prepared by 2-step precipitation method (100 mM NaCl followed by treatment with 500 mM NaCl). They also prepared highly pure (97%) ovomucin using 2-step precipitation using $CaCl_2$ solutions (Omana & Wu, 2009b).

Studies showed that yield of ovomucin varied from 1 to 10% of total egg white protein. The higher values may be mainly due to the co-precipitation of other egg white proteins especially ovalbumin and lysozyme. The variation may be due to different purification and analysis methods used. Yield of ovomucin obtained by various researchers from different

egg white sources is given in Table 3. Ovomucin content of fresh thick and thin egg white was found to be 132 and 26 mg/100 ml of egg white, respectively (Baliga, Kadkol, & Lahiry, 1971). Robinson and Monsey (1971) reported a yield of 200 mg of insoluble ovomucin from 100 g thick egg white. The yield of ovomucin obtained by gel filtration method was 260 and 180 mg/100 g of egg white for thick and thin white, respectively (Adachi *et al*., 1973). Hiidenhovi *et al*. (2002) reported a yield of 280 mg of ovomucin from 100 g of whole egg white. However the yield was higher for ovomucin obtained from thick egg albumen, liquid egg albumen and a liquid egg albumen filtration by-product showing 340 mg, 500 mg, and 520 mg per 100 g of albumen respectively. Yield obtained for ovomucin prepared from thick egg albumin was considerably higher (246 mg/100 g) when compared to that obtained from thin part (127 mg/100 g) (Hammershoj, Nebel, & Carstens, 2008). It was proved that exhaustive washing procedures result in very low (90–117 mg/100 g egg white) yield of ovomucin (Brooks & Hale, 1961). However, Toussant and Latshaw (1999) reported a higher yield of 530 mg in 100 g of whole egg white. Recently the ovomucin yield obtained by Omana and Wu (2009a) using their novel preparation method was 400 mg/100 g of egg white compared to 185.5 mg/100 g of egg white obtained using conventional method. They also obtained a yield of 410 mg/100 g of egg white by 2-step precipitation using $CaCl_2$ solutions (Omana & Wu, 2009b).

Functional properties

Egg white is usually used in food products because of the physicochemical properties of egg white proteins; ovomucin is one of the major responsible components. A number of studies have been made on the relationship between the structural and functional properties of ovomucin such as emulsifying and foaming properties (Baniel, Fains, & Popineau, 1997; Hammershoj & Qvist, 2001; Hammershoj *et al*., 2008; Kato, Oda, Yamanaka, Matsudomi, & Kobayashi, 1985).

Ovomucin is important for its high foamability and foam stability, which is due to its molecular size, intrinsic viscosity (Kato *et al*., 1985) and due to its association with lysozyme and globulins (Johnson & Zabik, 1981). Apart from this, the carbohydrate side chain may be contributing to its excellent functional properties, since ovomucin is highly glycosylated (Kato *et al*., 1985). The foaming power of ovomucin was much higher compared to that of bovine serum albumin. Ovomucin is usually added to egg white as a foam stabilizer during preparation of food products with desired foaming capacity (Nakamura & Sato, 1964). Solubility of proteins is important for functional properties like foaming capacity. In order to improve solubility of prepared ovomucin, hydrolysis was carried out using four proteases (Hammershoj *et al*., 2008). They found that optimum foaming capacity was obtained at a degree of hydrolysis of 15–40% and was well correlated to the drop in initial surface tension. However, hydrolysis did not have effect on foam stability. Even though some studies (Kato *et al*., 1985; Kato, Osako, Matsudomi, & Kobayashi, 1983) focused on the relationship between the structural and functional properties of ovomucin; the knowledge of its emulsifying properties is scarce. It was found that hydrophobicity and flexibility are the major reason for the enhanced functional properties of ovomucin; in addition to the viscous nature caused by protein–protein interaction. It is proposed that the surface hydrophobicity of proteins plays a governing role on the emulsifying properties (Kato *et al*., 1983). The surface hydrophobicity increased with the degree of dissociation and increased in the order soluble, sonicated and reduced ovomucin. Hence ovomucin can be utilized as an emulsion stabilizer.

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	Hiidenhovi et al. (2002)
Thick egg white	340	Hiidenhovi *et al*. (2002)
Liquid egg white	500	Hiidenhovi *et al*. (2002)
A liquid egg white filtration by-product	520	Hiidenhovi *et al*. (2002)
Thick egg white	246	Hammershoj *et al*. (2008)
Thin egg white	127	Hammershoj *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 disulfide 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 thinning. 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 thinning.

It is well known that egg proteins contain a great deal of sulphydryl groups (Greenstein, 1939). A role of sulphydryl groups was also suggested in egg white thinning. Smith and Back (1962) indicated the possibility of involvement of sulphydryl (SH) groups of ovalbumin during egg white thinning, while Donovan *et al*. (1972) suggested that alkaline hydrolysis of the disulfide 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 sulphydryl group in ovalbumin on egg white thinning is unclear. Further study also suggested that disulfide cleavage of ovomucin did not occur in egg white thinning (Kato, Ogata, Matsudomi, & Kobayashi, 1981). They found that α-ovomucin polymerized by disulfide 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 valuated 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 Kimura (1999)
Ovomucin	Hypocholesterolemic action	Nagaoka *et al*. (2002)
β-ovomucin	Cytotoxic effect on cultured tumor cells	Ohami *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 bound 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 (OVMα70F) in the α-subunit separated from pronase-treated hen egg white ovomucin. In the tumor tissues of OVMα70F-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, H_2O_2 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 H_2O_2 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, Masaoka, Zhang, Hasegawa, & Watanabe, 2002). In fact, not only α, β-subunits 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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References

Adachi, N., Azuma, J., Janado, M., & Onodera, K. (1973). Solubilization and characterization of ovomucin without chemical modification. *Agricultural and Biological Chemistry, 37*(9), 2175–2180.

Awade, A. C., & Efstathiou, T. (1999). Comparison of three liquid chromatographic methods for egg-white protein analysis. *Journal of Chromatography B, 723*, 69–74.

Awade, A. C., Moreau, S., Molle, D., Brule, G., & Maubois, J. L. (1994). Two-step chromatographic procedures for the purification of hen egg white ovomucin, lysozyme, ovotransferrin and ovalbumin and characterization of purified proteins. *Journal of Chromatography A, 677*, 279–288.

Baliga, B. R., Kadkol, S. B., & Lahiry, N. L. (1971). Thinning of thick albumen in shell eggs – changes in ovomucin. *Poultry Science, 50* (2), 466–473.

Baniel, A., Fains, A., & Popineau, Y. (1997). Foaming properties of egg albumen with a bubbling apparatus compared with whipping. *Journal of Food Science, 62*, 377–381.

Beveridge, T., & Nakai, S. (1975). Effects of sulphydryl blocking on the thinning of egg white. *Journal of Food Science, 40*, 864–868.

Brooks, J., & Hale, H. P. (1959). The mechanical properties of the thick white of the hen's egg. *Biochimica et Biophysica Acta, 32*, 237–250.

Brooks, J., & Hale, H. P. (1961). The mechanical properties of the thick white of the hen's egg. II. The relation between rigidity and composition. *Biochimica et Biophysica Acta, 46*, 289–301.

Burley, R. W., & Vadehra, D. V. (1989). The albumen: chemistry. In R. W. Burley, & D. V. Vadehra (Eds.), *The avian egg: Chemistry and biology* (pp. 65–128). New York: John Wiley and Sons.

Cegielska-Radziejewska, R., Lesnierowski, G., & Kijowski, J. (2008). Properties and application of egg-white lysozyme and its modified preparations – a review. *Polish Journal of Food and Nutrition Sciences, 58*(1), 5–10.

Cotterill, O. J., & Winter, A. R. (1955). Egg white lysozyme. 3. The effect of pH on the lysozyme – ovomucin interaction. *Poultry Science, 34*, 679–687.

Donovan, J. W., Davis, J. G., & White, L. M. (1970). Chemical and physical characterization of ovomucin, a sulfated glycoprotein complex from chicken eggs. *Biochimica et Biophysica Acta, 207*, 190–201.

Donovan, J. W., Davis, J. G., & Wiele, M. B. (1972). Viscometric studies of alkaline degradation of ovomucin. *Journal of Agricultural and Food Chemistry, 20*(2), 223–228.

Eichholz, A. (1898). The hydrolysis of proteins. *The Journal of Physiology, 23*, 163–177.

Feeney, R. E., & Allison, R. C. (1969). *Evolutionary biochemistry of proteins* (pp. 52–57). New York: John Wiley and Sons, Inc..

Gallagher, J. T., & Corfield, A. P. (1978). Mucin-type glycoproteins; new perspectives on their structure and synthesis. *Trends in Biochemical Sciences, 3*, 38–41.

Gottschalk, A., & Lind, P. (1949). Product of interaction between influenza virus enzyme and ovomucin. *Nature, 164*, 232–233.

Greenstein, J. P. (1939). Sulfhydryl groups in proteins – II. Edestin, excelsin and globin in solutions of guanidine hydrochloride, urea and their derivatives. *Journal of Biological Chemistry, 128*(1), 233–240.

Guerin, C., & Brule, G. (1992). Separation of three proteins from egg white. *Sciences Des Aliments, 12*, 705–720.

Hammershoj, M., Nebel, C. C., & Carstens, J. H. (2008). Enzymatic hydrolysis of ovomucin and effect on foaming properties. *Food Research International, 41*(5), 522–531.

Hammershoj, M., & Qvist, K. B. (2001). Importance of hen age and egg storage time for egg albumen foaming. *Lebensmittel-Wissenschaft Und-Technologie, 34*(2), 118–120.

Hao, J., Balagurumoorthy, P., Sarilla, S., & Sundaramoorthy, M. (2005). Cloning, expression, and characterization of sialic acid synthases. *Biochemical and Biophysical Research Communications, 338*, 1507–1514.

Hawthorne, J. R. (1950). The action of egg white lysozyme on ovomucoid and ovomucin. *Biochimica et Biophysica Acta, 6*, 28–35.

Hayakawa, S., & Sato, Y. (1976). Studies on the dissociation of the soluble ovomucin by sonication. *Agricultural and Biological Chemistry, 40*, 2397–2404.

Hiidenhovi, J. (2007). Ovomucin. In R. Huopalahti, R. Lopez-Fandino, M. Anton, & R. Schade (Eds.), *Bioactive egg compounds* (pp. 61–68). Germany: Springer Verlag.

Hiidenhovi, J., Aro, H. S., & Kankare, V. (1999). Separation of ovomucin subunits by gel filtration: enhanced resolution of subunits by using dual-column system. *Journal of Agricultural and Food Chemistry, 47*, 1004–1008.

Hiidenhovi, J., Huopalahti, R., & Ryhänen, E. L. (2003). Ovomucin purification: some preliminary studies to modify the isoelectric precipitation method. In Y. Nys (Ed.), *Quality of eggs and egg products* (pp. 388–394). Saint-Brieuc, France: ISPAIA.

Hiidenhovi, J., Makinen, J., Huopalahti, R., & Ryhanen, E. L. (2002). Comparison of different egg albumen fractions as sources of

ovomucin. *Journal of Agricultural and Food Chemistry, 50,* 2840–2845.
Itoh, T., Miyazaki, J., Sugawara, H., & Adachi, A. (1987). Studies on the characterization of ovomucin and chalaza of the hen's egg. *Journal of Food Science, 52,* 1518–1521.
Johnson, T. M., & Zabik, M. E. (1981). Egg albumen proteins interactions in an angel food cake system. *Journal of Food Science, 46,* 1231–1236.
Kato, A., Fujinaga, K., & Yagishita, K. (1973). Nature of the carbohydrate side chains and their linkage to the protein in chicken egg white ovomucin. *Agricultural and Biological Chemistry, 37,* 2479–2485.
Kato, A., Imoto, T., & Yagishita, K. (1975). The binding groups in ovomucin–lysozyme interaction. *Agricultural and Biological Chemistry, 39,* 541–544.
Kato, A., Nakamura, R., & Sato, Y. (1970). Studies on changes in stored shell eggs. Part VI. Changes in the chemical composition of ovomucin during storage. *Agricultural and Biological Chemistry, 34,* 1009–1013.
Kato, A., Nakamura, R., & Sato, Y. (1971). Studies on changes in stored shell eggs part VII. Changes in the physicochemical properties of ovomucin solubilized by treatment with mercaptoethanol during storage. *Agricultural and Biological Chemistry, 35,* 351–356.
Kato, A., Oda, S., Yamanaka, Y., Matsudomi, N., & Kobayashi, K. (1985). Functional and structural properties of ovomucin. *Agricultural and Biological Chemistry, 49,* 3501–3504.
Kato, A., Ogata, S., Matsudomi, N., & Kobayashi, K. (1981). A comparative study of aggregated and disaggregated ovomucin during egg white thinning. *Journal of Agricultural and Food Chemistry, 29,* 821–823.
Kato, A., Ogino, K., Matsudomi, N., & Kobayashi, K. (1977). Separation of ovomucin into carbohydrate rich and poor components by chromatography on lysozyme-sepharose 4B. *Agricultural and Biological Chemistry, 41,* 1925–1929.
Kato, A., Osako, Y., Matsudomi, N., & Kobayashi, K. (1983). Changes in the emulsifying and foaming properties of proteins during heat denaturation. *Agricultural and Biological Chemistry, 47*(1), 33–37.
Kato, A., & Sato, Y. (1971). The separation and characterization of carbohydrate rich component from ovomucin in chicken eggs. *Agricultural and Biological Chemistry, 35,* 439–440.
Kato, A., & Sato, Y. (1972). Studies on changes in stored shell eggs. VIII the release of carbohydrate rich component from ovomucin gel during storage. *Agricultural and Biological Chemistry, 36*(5), 831–836.
Kato, A., Tanaka, A., Matsudomi, N., & Kobayashi, K. (1986). Deamidation of ovalbumin during S-ovalbumin conversion. *Agricultural and Biological Chemistry, 50,* 2375–2376.
Kato, A., Wada, T., Kobayashi, K., Seguro, K., & Motoki, M. (1991). Ovomucin-food protein conjugates prepared through the transglutaminase reaction. *Agricultural and Biological Chemistry, 55*(4), 1027–1031.
Kato, A., Yoshida, K., Matsudomi, N., & Kobayashi, K. (1976). The interaction between ovomucin and egg white proteins. *Agricultural and Biological Chemistry, 40,* 2361–2366.
Kelm, S., & Schauer, R. (1997). Sialic acids in molecular and cellular recognition. *International Reviews in Cytology, 175,* 137–240.
Kobayashi, K., Hattori, M., Kudo, H. Y., Okubo, T., Yamamoto, S., Takita, T., et al. (2004). Glycopeptide derived from hen egg ovomucin has the ability to bind enterohemorrhagic *Escherichia coli* O157:H7. *Journal of Agricultural and Food Chemistry, 52,* 5740–5746.
Kodama, Y., & Kimura, N. (1999). Inhibitor of *Helicobacter pylori* colonization. *US Patent, 6,*235,709 B1.
Lanni, F., Sharp, D. G., Eckert, E. A., Dillon, E. S., Beard, D., & Beard, J. W. (1949). The egg white inhibitor of influenza virus hemagglutination. I. Preparation and properties of semipurified inhibitor. *The Journal of Biological Chemistry, 179,* 1275–1287.
Li-Chan, E., & Nakai, S. (1989). Biochemical basis for the properties of egg white. *CRC Critical Reviews in Poultry Biology, 2,* 21–58.
Mac-Donnell, L. R., Lineweaver, H., & Feeney, R. E. (1951). Chemistry of shell egg deterioration: effect of reducing agents. *Poultry Science, 30,* 856–863.
Mine, Y. (1995). Recent advances in the understanding of egg white protein functionality. *Trends in Food Science & Technology, 6,* 225–232.
Mine, Y., & Kovacs-Nolan, J. (2006). New insights in biologically active proteins and peptides derived from hen egg. *World's Poultry Science Journal, 62*(1), 87–95.
Nagaoka, S., Masaoka, M., Zhang, Q., Hasegawa, M., & Watanabe, K. (2002). Egg ovomucin attenuates hypercholesterolemia in rats and inhibits cholesterol absorption in Caco-2 cells. *Lipids, 37,* 267–272.
Nakamura, R., & Sato, Y. (1964). Studies on the foaming property of chicken egg white. X. On the role of ovomucin (B) in egg white foaminess (The mechanism of foaminess (2)). *Agricultural and Biological Chemistry, 28,* 530–534.
Nguyen, L. T. H., & Smith, M. B. (1984). S-ovalbumin in eggs – a review. *CSIRO Food Research Quarterly, 44*(2), 44–48.
Oguro, T., Watanabe, K., Tani, H., Ohishi, H., & Ebina, T. (2000). Morphological observations on antitumour activities of 70 kDa fragment in α-subunit from pronase treated ovomucin in a double grafted tumor system. *Food Science and Technology Research, 6,* 179–185.
Ohami, H., Ohishi, H., Yokota, T., Mori, T., & Watanabe, K. (1993). Cytotoxic effect of sialoglycoprotein derived from avian egg white ovomucin on the cultured tumor cell. *Medicine and Biology, 126,* 19–23.
Omana, D. A., & Wu, J. (2009a). A new method of separating ovomucin from egg white. *Journal of Agricultural and Food Chemistry, 57*(9), 3596–3603.
Omana, D. A., & Wu, J. (2009b). Effect of different concentrations of calcium chloride and potassium chloride on egg white proteins during isoelectric precipitation of ovomucin. *Poultry Science, 88,* 2224–2234.
Otani, H., & Maenishi, K. (1994). Effects of hen egg proteins on proliferative responses of mouse spleen lymphocytes. *Lebensmittel Wissenschaft Und Technologie, 27,* 42–47.
Prins, A. (1988). Principles of foam stability. In E. Dickinson, & G. Stainsby (Eds.), *Advances in food emulsions and foams* (pp. 91–121). London: Elsevier Applied Science.
Robinson, D. S. (1972). Egg white glycoproteins and the physical properties of egg white. In B. M. Freeman, & P. E. Lake (Eds.), *Egg formation and egg production* (pp. 65–86). Edinburgh: British Poultry Science Ltd.
Robinson, S. D., & Monsey, J. B. (1971). Studies on the composition of egg-white ovomucin. *Biochemical Journal, 121,* 537–547.
Robinson, S. D., & Monsey, J. B. (1972). Changes in the composition of ovomucin during liquefaction of thick egg white: the effect of ionic strength and magnesium salts. *Journal of the Science of Food and Agriculture, 23,* 893–904.
Ryoko, K., Makoto, H., Yukiko, K., Kazuo, K., Noriyuki, I., Tsutomu, O., & et al. (2004). Inactivation composition of food poisoning bacteria. *Japanese Patent,* 269420.
Schauer, R. (2004). Sialic acids: fascinating sugars in higher animals and man. *Zoology, 107,* 49–64.
Sim, J. S., & Sunwoo, H. H. (2006). In: J. S. Sim, & H. H. Sunwoo (Eds.), *The amazing egg: Nature's perfect functional food for health promotion* (pp. 519). Alberta: Dept. of Agricultural, Food and Nutritional Science, University of Alberta.
Sleigh, R. W., Melrose, G. J. H., & Smith, M. B. (1973). Isolation and characterization of hen egg white ovomucin. *Biochimica et Biophysica Acta, 310,* 453–460.
Smith, M. B., & Back, J. F. (1962). Modification of ovalbumin in stored eggs detected by heat denaturation. *Nature, 193,* 878–879.

Smith, M. B., & Back, J. F. (1965). Studies on ovalbumin. 2. The formation and properties of S-ovalbumin, a more stable form. *Australian Journal of Biological Sciences*, *18*, 365–377.

Strecker, G., Wieruszeski, J. M., Cuvillier, O., Michalski, J. C., & Moutreuil, J. (1992). ^{1}H- and ^{13}C-NMR assignments for sialylated oligosaccharide–alditols related to mucins. Study of thirteen components from hen ovomucin and swallow nest mucin. *Biochimie*, *74*, 39–52.

Strecker, G., Wieruszeski, J. M., Martel, C., & Moutreuil, J. (1987). Determination of the structure of sulfated tetra and pentasaccharides obtained by alkaline borohydride degradation of hen ovomucin. A fast atom bombardment-mass spectrometric and ^{1}H-NMR spectroscopic study. *Glycoconjugate Journal*, *4*, 329–337.

Strecker, G., Wieruszeski, J. M., Martel, C., & Moutreuil, J. (1989). Complete ^{1}H- and ^{13}C-N.M.R. assignments for two sulphated oligosaccharide alditols of hen ovomucin. *Carbohydrate Research*, *185*, 1–13.

Tanizaki, H., Tanaka, H., Iwata, H., & Kato, A. (1997). Activation of macrophages by sulfated glycopeptides in ovomucin, yolk membrane, and chalazae in chicken eggs. *Bioscience, Biotechnology and Biochemistry*, *61*, 1883–1889.

Tomimatsu, Y., & Donovan, J. W. (1972). Light scattering study of ovomucin. *Journal of Agricultural and Food Chemistry*, *20*, 1067–1073.

Toussant, M. J., & Latshaw, J. D. (1999). Ovomucin content and composition in chicken eggs with different interior quality. *Journal of the Science of Food and Agriculture*, *79*, 1666–1670.

Tsuge, Y., Shimoyamada, M., & Watanabe, K. (1996a). Binding of egg white proteins to viruses. *Bioscience, Biotechnology and Biochemistry*, *60*, 1503–1504.

Tsuge, Y., Shimoyamada, M., & Watanabe, K. (1996b). Differences in hemagglutination inhibition activity against bovine rotavirus and hen Newcastle disease virus based on the subunit in hen egg white ovomucin. *Bioscience, Biotechnology and Biochemistry*, *60*, 1505–1506.

Tsuge, Y., Shimoyamada, M., & Watanabe, K. (1997a). Structural features of hen Newcastle disease virus and anti-ovomucin antibodies binding glycopeptides from pronase treated ovomucin. *Journal of Agricultural and Food Chemistry*, *45*, 2393–2398.

Tsuge, Y., Shimoyamada, M., & Watanabe, K. (1997b). Bindings of ovomucin to Newcastle disease virus and anti-ovomucin antibodies and its heat stability based on binding abilities. *Journal of Agricultural and Food Chemistry*, *45*, 4629–4634.

Watanabe, K., Shimoyamada, M., Onizuka, T., Akiyama, H., Niwa, M., Ido, T., et al. (2004). Amino acid sequence of a-subunit in hen egg white ovomucin deduced from cloned cDNA. *DNA Sequence*, *15*(4), 251–261.

Watanabe, K., Tsuge, Y., Shimoyamada, M., Ogama, N., & Ebina, T. (1998). Antitumor effects of pronase-treated fragments, glycopeptides, from ovomucin in hen egg white in a double grafted tumor system. *Journal of Agricultural and Food Chemistry*, *46*, 3033–3038.

Yokota, T., Ohishi, H., & Watanabe, K. (1999a). *In vitro* studies of cytotoxic effect on sarcoma-180 cells of β-subunit from egg white ovomucin. *Food Science and Technology Research*, *5*, 273–278.

Yokota, T., Ohishi, H., & Watanabe, K. (1999b). Antitumor effects of β-subunit from egg white ovomucin on xenografted sarcoma-180 cells in mice. *Food Science and Technology Research*, *5*(3), 279–283.

Young, L. L., & Gardner, F. A. (1970). Preparation of egg white ovomucin by gel filtration. *Journal of Food Science*, *37*, 8–11.