

Peptic digestibility of raw and heat-coagulated hen's egg white proteins at acidic pH range

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Allergenicity in food proteins is generally dependent on their heat stability and resistance to digestive enzymes together with the presence of IgE-recognizing epitopes on the molecules. Using sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting, we assessed peptic digestibility of raw and heat-coagulated hen's egg white proteins at acidic pH range (1.5–4.0). Ovalbumin in raw egg white was slightly digested by pepsin at pH 1.5 and pH 2.0, and was almost resistant to the enzyme at pH 2.5 and over, which was altered in heat-coagulated egg white at the pH range from 1.5 to 2.5 where the protein was well digested against the enzyme. Peptic digestibility of ovomucoid in raw egg white was good at the pH range from 1.5 to 2.5, but almost non-existent at pH 3.0 and over where the improvement of the digestibility of the protein was not found even in heat-coagulated egg white. As the stomach in new born infants shows a low amount of secretory pepsin and an out of optimum pH of peptic activity, low digestibility of ovalbumin and ovomucoid in raw and heat-coagulated egg white at over pH 3.0 is supposed to be responsible for their allergenicity and delayed outgrowth from hen's egg allergy in patients with delayed maturation of stomach functions.

Introduction

Hen's egg is widely used for cooking and processed foods due not only to its reasonable price, but also the good balanced nutrients for humans. On the contrary, hen's egg has been frequently identified as an allergen in food allergy patients, especially in infants aged younger than 3 years (Sampson & McCaskill, 1985; Bocks *et al.*, 1988; Bocks & Atkins, 1990). The major allergenicity

in hen's egg is involved in egg white rather than in egg yolk. To date, ovalbumin (OVA) (Gal d I), ovotransferrin (OT) (Gal d II), ovomucoid (OM) (Gal d III) and hen's egg lysozyme (HEL) (Gal d IV) are identical major allergenic proteins in egg white (Langeland, 1982; Hoffman, 1983; Holen & Elsayed, 1990). These proteins show consensus criteria for allergenic proteins, including

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resistance to digestive enzymes, heat stability and/or the presence of allergenic epitopes on the molecule. Ingested food proteins are mostly degraded to amino acids by digestive enzymes in the gastrointestinal tract, but some of them that escape from the degradation are absorbed into the intestinal epithelium and induce the production of their specific antibodies including IgE, a key molecule of anaphylaxis.

The stomach functions in newborn infants are immature, with a low amount of secretory pepsin and an out of optimum pH for peptic activity (Nagita *et al.*, 1994, 1996). Therefore, the digestive activity in the gastrointestinal tract is a critical factor in infants whether allergenicity of food proteins is reduced in the process of absorption into the intestinal mucosa. In the present paper, we investigated the peptic digestibility of allergenic proteins in hen's egg white in the acidic pH range, which is discussed in relation to the frequent development of hen's egg allergy in infants.

Methods

Hen's eggs were purchased from a grocery store, stored at 4°C and used in this experiment within 4 days. Egg white was separated from the whole egg and passed through a metal mesh three times to homogenize dense and thin egg white. Then, 500 µl of the egg white was heat-coagulated by boiling for 10 min and was homogenized with 19.5 ml of distilled water for 60 sec at room temperature in a Polytron homogenizer (Kinematica, Lucerne, Switzerland). The pH value in the samples was then adjusted to 1.5, 2.0, 2.5, 3.0, 3.5 or 4.0 with concentrated HCl. Porcine pepsin (20 mg/ml) (Sigma Chemical Co., St Louis, MO, USA) was added to the samples at the final concentration of 0.2 µg/ml, and they were incubated in a shaking water bath at 37°C for 30, 60 or 120 min. Then, 150 µl of each sample at the indicated time was removed and mixed with an equal volume of 5 mM Tris buffer (pH 8.3) containing 2% sodium dodecyl sulfate (SDS), 10% 2-mercaptoethanol, 10% glycerol and 2% bromophenol blue (2 × SDS-loading buffer). For preparation of peptic digestion

for 0 min, 2 × SDS-loading buffer was mixed with the samples before adding pepsin. Control samples were prepared in the same manner as already described except for adding distilled water to the mixtures instead of pepsin. The preparations were boiled for 5 min and 10 µl each subjected to 12.5% SDS-polyacrylamide gel electrophoresis (SDS-PAGE), stained with Coomassie Brilliant Blue R-250 (Laemmli, 1970). Immunoblotting was carried out with the samples subjected to SDS-PAGE followed by being transferred electrophoretically to a supported nitrocellulose membrane (Towbin *et al.*, 1979). The membrane was then soaked in 3% gelatin at room temperature for 2 h to block non-specific protein binding, and was incubated at room temperature with 2000 times diluted anti-OVA rabbit serum (Sigma) or 1000 times diluted anti-OM rabbit serum as described previously (Sakai *et al.*, 1998, 1999). Bound antibodies were detected by the color development system of goat anti-rabbit IgG conjugated with alkaline phosphatase (Sigma) and 5-bromo-4-chloro-3-indolyl phosphate/nitro-blue tetrazolium tablet (Sigma). Stained gels and immunoblots were directly scanned using an image scanner GT-7600S (Seiko Epson Co., Nagano, Japan). SDS-PAGE low-range protein standards (Pharmacia LKB Biotechnology AB, Upssala, Sweden) and pre-stained protein standards (APRO Science, Inc., Naruto, Japan) were used as molecular weight markers for SDS-PAGE and immunoblots, respectively.

Results

Peptic digestibility of raw egg white proteins at the pH range from 1.5 to 4.0 was analyzed by SDS-PAGE with Coomassie brilliant blue staining (Figure 1A). Intense bands of major egg white proteins including OT (76.6 kDa), OVA (38 kDa), OM (35–45 kDa) and HEL (14.4 kDa), all of which have been identified as allergens in hen's egg (Hoffman, 1983; Holen & Elsayed, 1990; Langeland, 1982), were visualized on the gels before peptic digestion. OT was rapidly digested by pepsin within 30 min at all the pH ranges tested in this experiment except for pH 4.0, where the

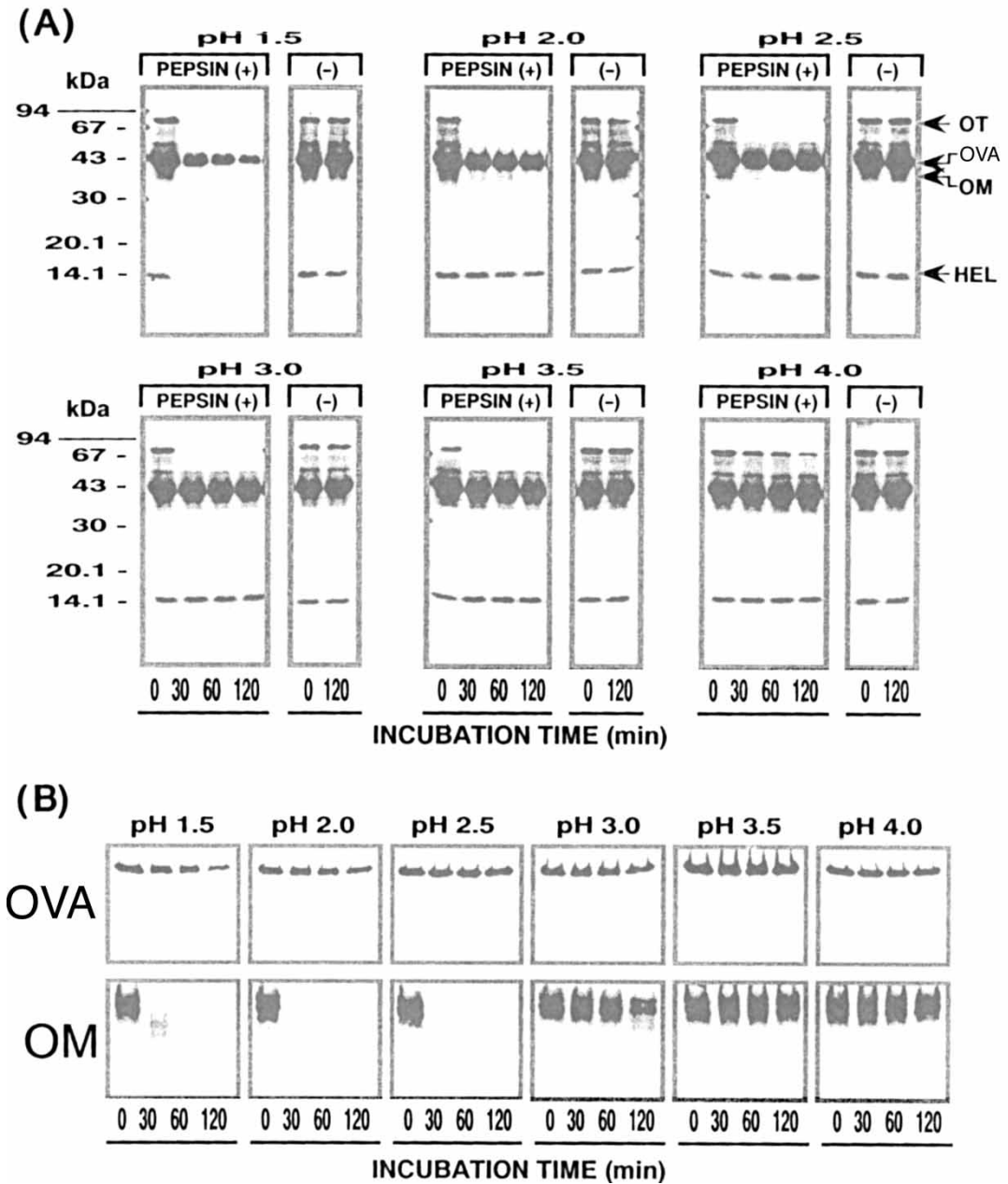


Figure 1. Profile of peptic digestibility of raw hen's egg white proteins. (A) SDS-PAGE of raw hen's egg white proteins digested by porcine pepsin at the pH range from 1.5 to 4.0. Sample preparations were analyzed by SDS-PAGE under reducing conditions and the gels were then stained with Coomassie Brilliant Blue R-250. Molecular weight standards are shown on the left. (B) Immunoblots of ovalbumin (*OVA*) and ovomucoid (*OM*) in raw egg white proteins digested by porcine pepsin at the same conditions in (A) using antibodies against *OVA* and *OM*, respectively. OT, Ovotransferrin; HEL, hen's egg lysozyme.

protein was gradually degraded during the incubation. HEL showed good peptic digestibility at pH 1.5, in contrast to the other pH

range where the protein was exclusively resistant to the enzyme. We could not assess the profiles of *OVA* and *OM* against peptic

digestion with SDS-PAGE because of not only diffuse shape of OM, but also both showing a similar mobile phase overlapping each other on the gels. Therefore, immunoblotting was carried out to assess the peptic digestibility of OVA and OM in raw egg white (Figure 1B). OVA was slightly degraded by pepsin at pH 1.5 and pH 2.0, and was resistant to the enzyme at pH 2.5 and over. OM was rapidly digested by pepsin within 30 min at the pH range from 1.5 to 2.5, but was almost resistant to the enzyme at pH 3.0 and over.

Peptic digestibility of heat-coagulated egg white proteins was analyzed in the same manner (Figure 2). On SDS-PAGE, the mobility of heat-coagulated egg white proteins before peptic digestion was almost the same as that of raw egg white proteins (Figure 2A). OT showed good digestibility by pepsin at the pH range from 1.5 to 3.0 but was resistant to the enzyme at pH 3.5 and 4.0. HEL was rapidly digested by pepsin at pH 2.5 and below, in contrast to pH 3.0 and over where the protein was resistant to the enzyme. For the same reason as the assessment of peptic digestibility of OVA and OM in raw egg white, immunoblotting of these proteins in heat-coagulated egg white was carried out (Figure 2B). Smooth peptic digestion was observed in OVA at the pH range from 1.5 to 2.5 and in OM at pH 1.5 and 2.0, while at the other pH ranges each protein was resistant to the enzyme.

Discussion

Food allergy frequently occurs in infants who sometimes show immature gastrointestinal functions such as a low amount of secretory pepsin with an out of optimal pH around 3–4 in the stomach (Nagita *et al.*, 1994, 1996). Therefore, the immaturity and delayed maturation of the digestive activity in the gastrointestinal tract lead some of ingested food proteins to escape from the degradation and to be absorbed into the intestinal epithelium. This could result in the sensitization against allergenic food proteins with the production of their specific antibodies including IgE, a key molecule of anaphylaxis.

We have investigated the allergenicity and antigenicity of some dietary food proteins at the point of digestibility by several gastrointestinal enzymes (Sakai *et al.*, 1997, 1999, 2000). To add some evidence to this project, in the present study we investigated the effects of graded acidic pH conditions on peptic digestibility of hen's egg white proteins. Among the allergenic proteins in hen's egg, OVA and OM have been the most considerable in the clinic because of frequent high levels of their specific IgE in serum from hen's egg allergy patients. In the present study, OVA in heat-coagulated egg white showed good peptic digestibility at the pH range from 1.5 to 2.5, unlike the protein in raw egg white, being almost resistant to the enzyme at all the acidic pH ranges tested in this experiment. This result suggested that OVA in heat-coagulated egg white is supposed to be well digestive with low allergenicity for infants whose stomach functions are well developed. On the contrary, OM in raw egg white was well digestive by pepsin at the pH range from 1.5 to 2.5, but not at pH 3.0 and over where the improvement of the digestibility of the protein was not found even in heat-coagulated egg white. Although heat treatment is useful tool for reducing allergenicity and enhancing enzymatic digestibility of dietary food proteins (Urisu *et al.*, 1997; Sakai *et al.*, 2000), the present data showed that OM is out of the role against peptic digestion.

Urisu *et al.* (1997) reported that patients who are delayed from outgrowth of hen's egg allergy frequently show positive by the oral challenge test against heat-coagulated egg white, but show negative against that with depletion of OM. Therefore, they concluded that monitoring of the sensitivity of OM is clinically useful to whether hen's egg allergy patients grow out of the allergic symptom in the future. On the contrary, ingested dietary proteins are digested by pepsin in the stomach, followed by pancreatic proteases including trypsin, chymotrypsin and carboxypeptidase in the small intestine. Hen's egg OM is resistant to tryptic digestion due to the presence of three homologous domains as a human trypsin inhibitor in the molecule (Kato *et al.*, 1987; Sampson & Cooke,

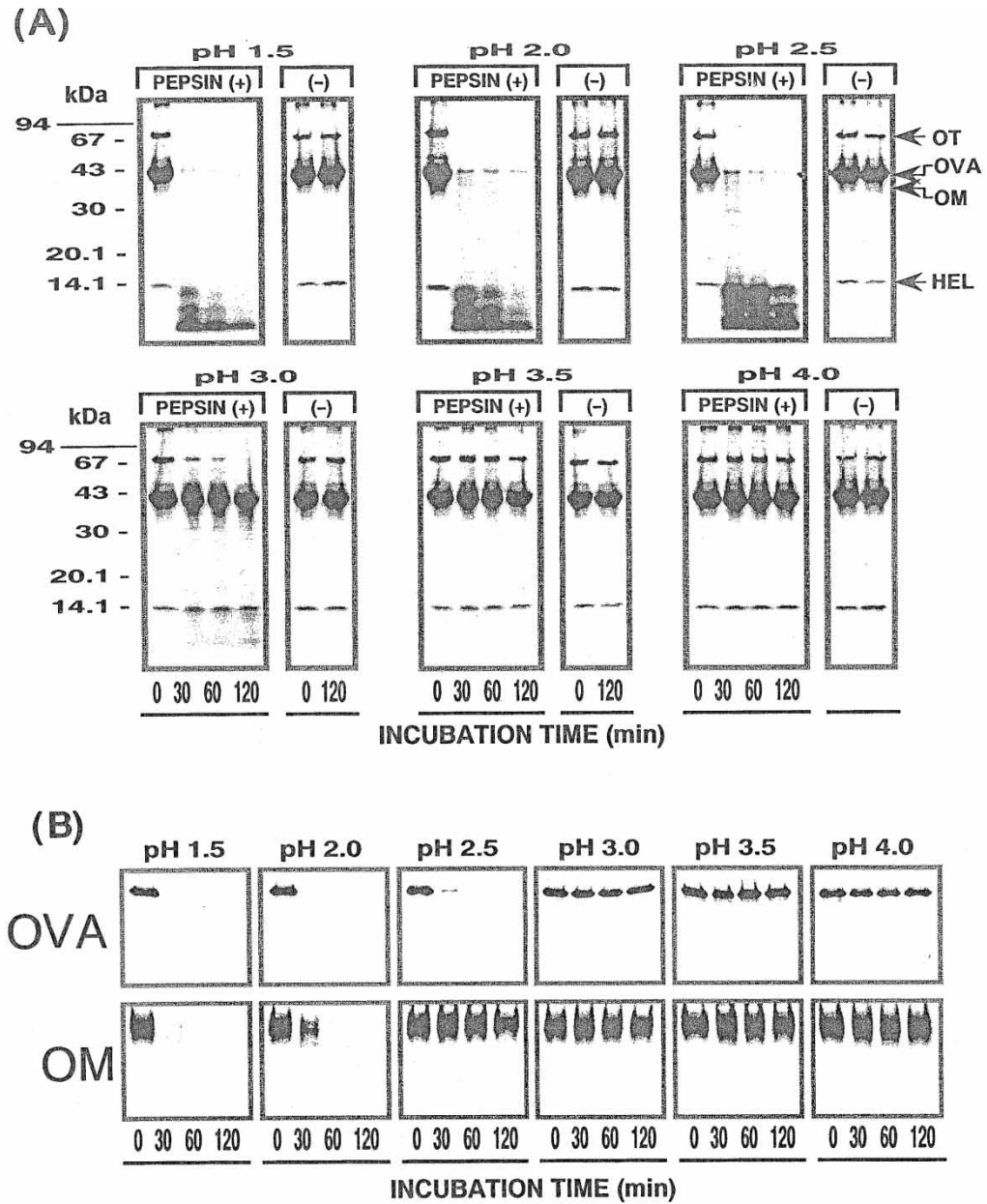


Figure 2. Profile of peptic digestibility of heat-coagulated hen's egg white proteins. The panels in SDS-PAGE (A) and immunoblots (B) are the same manner as shown in Figure 1. OT, Ovotransferrin; HEL, hen's egg lysozyme.

1997). Together with this information and our present data, development of peptic activity for suitable digestibility of OM in the stomach are critical for destroying its allergenicity, which may have important

consequences for release from the sensitization of the protein in hen's egg allergy patients.

Hen's egg allergy is frequently found in infants rather than in adults. About 40% of

hen's egg allergy patients in infants are outgrown from the disease by 3 years old, but the remainder of these is not (Eggleston, 1987). The present findings of peptic digestibility in graded acidic pH conditions in dietary food proteins could show that the power of peptic digestion in the stomach

affects whether there is the chance of suffering food allergy against dietary allergenic proteins.

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