to DKP and aspartylphenylalanine, respectively, are the major products formed in the sample after extended storage periods. L-Phenylalanine methyl ester (peak 1) was a minor product. A significant increase in the level of phenylalanine (peak 4), however, was observed between a period of 6–36 months. The term "APM equiv" in Table II stands for the aspartame equivalent of the amount of breakdown product on a molar basis. It is interesting to note that the same proportion of DKP (37% of the decomposed APM) was obtained at pH 8 and at the low pH of the soft drinks.

The pathways of decomposition of aspartame in the diet soft drinks are depicted in Figure 7. One of the major degradation product is 5-benzyl-3,6-dioxo-2-piperazineacetic acid (DKP) (2), obtained from cyclization of aspartame, with a resultant loss of methanol. The other degradative pathways involve the formation of phenylalanine methyl ester (1) and aspartylphenylalanine (3), which separately undergo further hydrolysis to give phenylalanine (4).

**Registry No.** PM, 2577-90-4; DKP, 55102-13-1; AP, 13433-09-5; PA, 63-91-2; aspartame, 22839-47-0.

LITERATURE CITED

Argoudelis, C. J. *J. Chromatogr.* **1984**, *303*, 256.
Cloninger, M. R.; Baldwin, R. E. *J. Food Sci.* **1974**, *39*, 347.
Cross, R.; Cunico, B. *Liq. Chromatogr.* HPLC Mag. **1984**, *2* (9), 678.
Daniels, D. H.; Joe, Jr., F. L.; Warner, C. R.; Fazio, T. *J. Assoc. Off. Anal. Chem.* **1984**, *67* (3), 513.
Homler, B. E. *Food Technol.* **1984**, *38* (7), 50.
Hussein, M. M.; D'Amelia, R. P.; Manz, A. L.; Jacin, H.; Chen, W. T. C. *J. Food Sci.* **1984**, *49*, 520.
Mazur, R. In "Encyclopedia of Chemical Technology", 3rd ed.; John Wiley & Son, Inc.: New York, 1983; Vol. 22, p 448.
O'Sullivan, D. *Chem. Eng. News* **1983**, *61* (4), 29.
Scherz, J. C.; Monti, J. C.; Jost, R. *Z. Lebensm.*-Unters. Forsch. **1983**, *177* (2), 124.
Stegink, L. D.; Filer, L. J., Jr. In "Aspartame: Physiology and Biochemistry"; Marcel Dekker: New York, 1984; Vol. 12.
Tyler, T. A. *J. Assoc. Off. Anal. Chem.* **1984**, *67* (4), 745.
Webb, N. G.; Beckman; D. D. *J. Assoc. Off. Anal. Chem.* **1984**, *67* (3), 510.

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# Polymerization of Deamidated Peptide Fragments Obtained with the Mild Acid Hydrolysis of Ovalbumin

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The mild acid treatment of ovalbumin brought about the deamidation of asparagin and glutamine and the hydrolysis of peptide bonds on either side of aspartic acid. The ovalbumin peptide fragments thus obtained were polymerized by being allowed to stand at room temperature in contact with air. It was suggested that the polymerization of ovalbumin peptide fragments was due to the formation of hydrophobic and disulfide bonds. The polymerized products indicated good functional properties, such as solubility, emulsifying, and foaming properties, because of the higher electrostatic repulsion as a result of deamidation, the better amphiphilic nature due to the proper hydrophilic–hydrophobic arrangement, and the polymerization through hydrophobic and disulfide bonds of ovalbumin peptide fragments.

## INTRODUCTION

Some investigators have reported that mild acid treatment of food proteins was very effective to improve their functional properties (Finley, 1975; Wu et al., 1976; Matsudomi et al., 1982). Finley (1975) suggested a mild acid treatment of wheat gluten to increase its solubility in fruit-based acidic beverages. Wu et al. (1976) found a significant improvement in the emulsifying and foaming properties of wheat gluten by mild acid hydrolysis. It is well-known that mild acid treatment of proteins results in the deamidation of glutamine and asparagine residues and a concomitant cleavage of peptide bonds. On either side of aspartic acid, peptide bonds may be cleaved at a rate at least 100 times greater than other peptide bonds under carefully controlled conditions in diluted acid (Schultz et al., 1962; Han et al., 1983). Therefore, this treatment appears to be more suitable for controlled hydrolysis of proteins than protease. A mild acid hydrolysis may bring about (1) increased solubility of proteins mainly due to the higher electrostatic repulsion as a result of deamidation of glutamine and asparagine, and (2) the higher amphiphilic nature due to the exposed hydrophobic amino acid residues as a result of either denaturation or cleavage of peptide bonds.

In addition to these advantages, we found some sort of polymerization of peptide fragments in the mild acid hydrolysates of ovalbumin. This reaction can be utilized as an useful tool to fabricate new functional proteins. The present paper describes the mechanism of polymerization and the properties of resultant peptide products.

## MATERIALS AND METHODS

Ovalbumin was prepared from egg white by a crystallization method in sodium sulfate and recrystallized five times (Kekwick and Cannan, 1936).

The mild acid hydrolysis of ovalbumin was carried out as follows. Ovalbumin was dissolved in a concentration of 1 mg per mL of 0.03 N HCl. Ovalbumin solution was sealed in 10-mL amples in vacuo and placed in an oven at 115 °C. At various time intervals, ampules were removed and cooled.

Ovalbumin solution (3 mL) was immediately applied to a Sephadex G-100 column (0.8 × 112 cm) equilibrated with 0.03 N HCl. Another 3 mL of ovalbumin solution was allowed to stand at room temperature in contact with air for a given time before application to a Sephadex G-100

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column (0.8 × 112 cm) equilibrated with 0.03 N HCl.

The analysis of amino acid released in ovalbumin hydrolysates was carried out with HLC-805 Automatic Amino Acid Analyzer (Toyo Soda Co., Japan) for samples hydrolyzed with 0.03 N HCl at 115 °C for 0.5, 1, and 2 h. Ovalbumin hydrolysates were directly injected into the column (7.5 × 75 mm) with TSK-Gel IEX-215 (Toyo Soda Co.) at 50 °C. The flow rate was 0.82 mL/min. Three types of buffers were used for elution as follows. Buffer 1 was 0.2 M citrate buffer, pH 3.30, containing 8% ethanol, 0.25% β-thiodiglycol, 0.01% *n*-caprylic acid, and 0.05% polyoxyethylene lauryl ether. Buffer 2 was 0.2 M citrate buffer, pH 4.25, containing 0.25% β-thiodiglycol, 0.01% *n*-caprylic acid, and 0.05% polyoxyethylene lauryl ether. Buffer 3 was 0.15 M citrate buffer, pH 9.7, containing 0.025 M sodium borate, 0.6 M NaCl, 0.01% capryic acid, and 0.05% polyoxyethylene lauryl ether. Buffers 1, 2, and 3 were eluted for 22 min, 15 min, and 20 min, respectively.

The rate of deamidation of ovalbumin was determined by measuring the amounts of ammonia released from samples. Ovalbumin hydrolysates dissolved in 5 mL of 3 N HCl were sealed in a 10-mL glass ampule and heated at 110 °C for 3 h to deamidate completely asparagine and glutamine in ovalbumin hydrolysates. The amounts of ammonia released from samples were determined by the microdiffusion method of Conway and O'Malley (1942).

The SH groups of ovalbumin hydrolysates were determined by the method of Beveridge et al. (1974). Ovalbumin hydrolysates were dialyzed against deionized water for 48 h at 4 °C and then lyophilized. The content of SH groups in ovalbumin hydrolysates was indicated as the ratio of native ovalbumin.

Ovalbumin hydrolysates were lyophilized after dialysis against distilled water and used in the experiments described below. The surface hydrophobicity of lyophilized ovalbumin hydrolysates was measured by the fluorometric method by using *cis*-parinaric acid (Kato and Nakai, 1980). Ten microliters of *cis*-parinaric acid solution was added to 2 mL of ovalbumin hydrolysate solution in 0.02 M phosphate buffer, pH 7.4. The *cis*-parinaric acid–protein conjugates were excited at 325 nm and the relative fluorescence intensity was measured at 420 nm in an Aminco-Bowman Spectrophotofluorometer, J4-8962. The reading of relative fluorescence intensity was adjusted to 1.0 when 10 μL of *cis*-parinaric acid solution was added to 2 mL of 0.02 M phosphate buffer, pH 7.4, in the absence of protein solution. The initial slope ($S_0$), fluorescence intensity, and percent protein were calculated from the fluorescence intensity vs. protein concentration plot.

The solubility of ovalbumin hydrolysates were determined in acetate buffer (pH 2–5.5), phosphate buffer (pH 6–8), and carbonate buffer (pH 9).

The emulsifying activity of lyophilized ovalbumin hydrolysates was measured by the method of Pearce and Kinsella (1978). To prepare emulsion, 1 mL of corn oil and 3 mL of 0.1% ovalbumin hydrolysate solution in 1/15 M phosphate buffer, pH 7.4, were homogenized in an Ultra Turrax (Hansen & Co., West Germany) at 12000 and rpm for 1 min at 20 °C. Emulsions ($^1/_{10}$ mL) were taken from the bottom of the container immediately after homogenization and were diluted with 0.1% SDS solution to $^1/_{50}$. The absorbance of diluted emulsions was measured at 500 nm. Emulsifying activity was indicated as the absorbance at 500 nm. Emulsion stability was determined from the decrease curves in absorbance of emulsion during standing time. Emulsion stability was indicated as half-life period of the absorbance of emulsion obtained immediately after homogenization.

**Table I. Changes in the Content of Free Aspartic Acid and the Rate of Deamidation during Mild Acid Hydrolysis**

| ovalbumin hydrolysates[a] (hydrolysis time, h) | free amino acids,[b] residues/mole: aspartic acid | free amino acids,[b] residues/mole: other amino acid | rate of deamidation,[c] % |
|---|---|---|---|
| 0.5 | 1.5 | 0 | 20 |
| 1.0 | 4.8 | 0 | 50 |
| 2.0 | 16.2 | 0 | 63 |

[a] Ovalbumin was hydrolyzed by 0.03 N HCl at 115 °C. [b] Aspartic acid released by mild acid hydrolysis of ovalbumin where the total aspartic acid residues per mole was determined to be 31. [c] The rate of deamidation was indicated as percent of free ammonia released by acid hydrolysis in 3 N HCl at 105 °C for 3 h.

The foaming properties of lyophilied ovalbumin hydrolysates were determined by measuring the conductivity of foams produced when air was introduced into 5 mL of 0.1% ovalbumin hydrolysate solution in 1/15 M phosphate buffer, pH 7.4 (Kato et al., 1983). The conductivity of foams was measured by using the electrode fixed in a glass column (2.4 × 30 cm) with a glass filter (G-4), connecting with conductivity meter (Kyoto Electric Industry Co., CM-07). The foaming power was calculated by measuring initial conductivity of foams produced immediately ater aeration. Foam stability was indicated as a ratio of the conductivity after 5 min against initial conductivity.

## RESULTS AND DISCUSSION

Table I shows the contents of free amino acid and the rate of deamidation during mild acid hydrolysis. 1.5, 4.8, and 16 mol of aspartic acid was released from ovalbumin by acid hydrolysis in 0.03 N HCl at 115 °C for 0.5, 1, and 2 h, respectively, although any other amino acid did not release. This result suggests that both peptide bonds of amino and carboxyl sides of aspartic acid in peptide chains are preferentially hydrolyzed by mild acid hydrolysis, as reported by Han et al. (1983). Calculating from the number of released aspartic acid, 2–3, 6, and 17 peptide fragments may be presumed to be cleaved by acid hydrolysis in 0.03 N HCl at 115 °C for 0.5, 1, and 2 h, respectively. In addition to this peptide cleavage, the deamidation from asparagine and glutamine proceeded at the same time during mild acid hydrolysis, as shown in Table I. The deamidation rate of ovalbumin attained to about 50% by mild acid hydrolysis for 1 h. The deamidation appears to occur in a rate at least 3–4 times greater than the cleavage of peptide bonds.

Figure 1 shows the elution patterns on a Sephadex G-100 column of ovalbumin hydrolyzed by 0.03 N HCl at 115 °C for 0.5, 1, and 2 h. After mild acid hydrolysis for a given time, ovalbumin hydrolysates were immediately applied to a Saphadex G-100 column equilibrated with 0.03 N HCl. As acid hydrolysis proceeded, the peak of native ovalbumin diminished and the peaks of cleaved peptide fragments emerged in a fraction number of 30–50. This suggests that ovalbumin was cleaved into smaller peptides with the progress of acid hydrolysis in 0.03 N HCl at 115 °C.

On the other hand, when ovalbumin hydrolysates were allowed to stand for several hours at room temperature in contact with air, the gel filtration patterns of ovalbumin hydrolysates changed remarkably. As shown in Figure 2, the gel filtration patterns on a Sephadex G-100 column of ovalbumin hydrolyzed by 0.03 N HCl at 115 °C for 30 min greatly changed with standing time at room temperature. The peaks of cleaved peptide fragments were mostly shifted to the void volume fraction during standing for 16 h. This result suggests that ovalbumin peptide fragment are gradually polymerized by being allowed to stand at

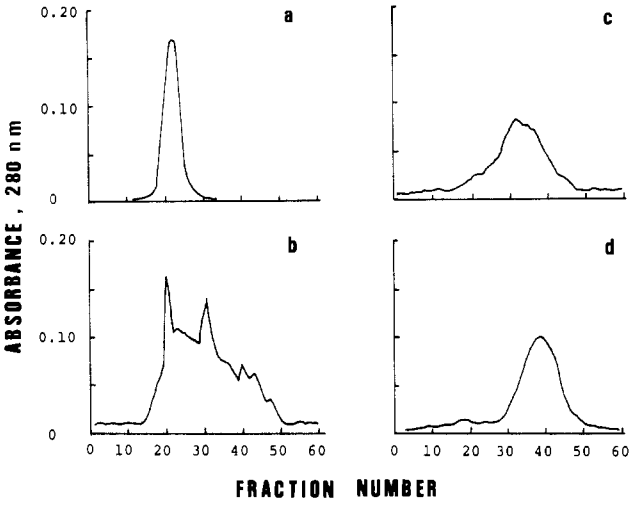


**Figure 1.** Elution patterns on a Sephadex G-100 column of ovalbumin hydrolyzed by 0.03 N HCl at 115 °C for 0.5, 1, and 2 h. Ovalbumin solutions (3 mL) were immediately after hydrolysis applied to a Sephadex G-100 column equilibrated with 0.03 N HCl: (a) untreated ovalbumin; (b) ovalbumin hydrolyzed for 30 min; (c) ovalbumin hydrolyzed for 1 h; (d) ovalbumin hydrolyzed for 2 h.

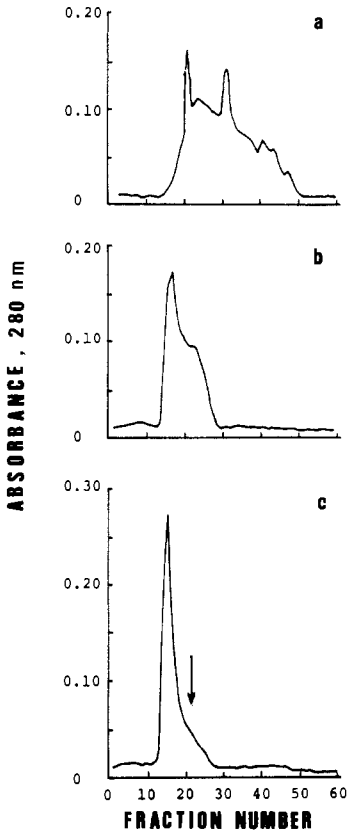


**Figure 2.** Changes in elution patterns on a Sephadex G-100 column of ovalbumin hydrolyzed by 0.03 N HCl at 115 °C for 30 min during standing time at room temperature. Ovalbumin (3 mL) hydrolyzed by 0.03 N HCl at 115 °C for 30 min was applied to a Sephadex G-100 column equilibrated with 0.03 N HCl after standing at room temperature for a given time: (a) immediately after hydrolysis; (b) after standing for 2 h; (c) after standing for 16 h. An arrow (↓) indicates the elution peak of untreated ovalbumin.

room temperature in contact with air. This formation of soluble macromolecular aggregates of peptide fragments was further confirmed with ovalbumin hydrolyzed by 0.03 N HCl at 115 °C for 1 and 2 h. Figure 3 shows the changes in the gel filtration patterns on a Sephadex G-100 column

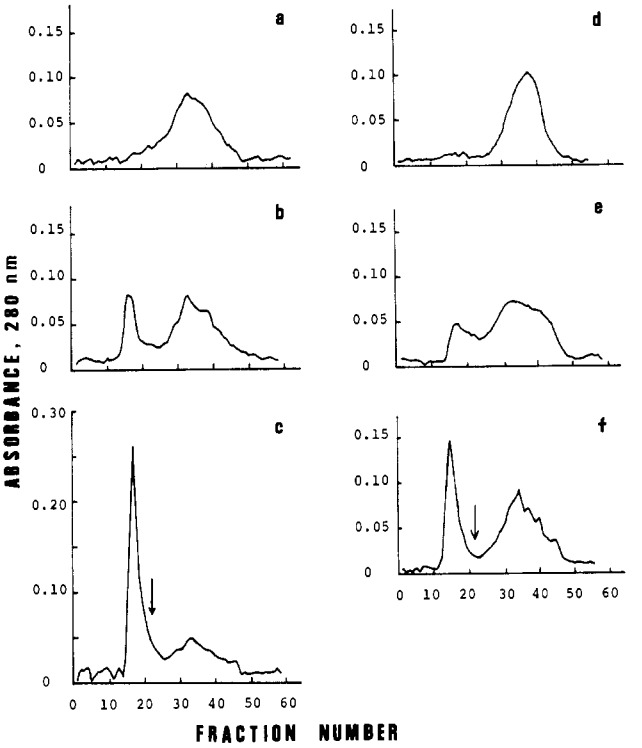


**Figure 3.** Changes in elution patterns on a Sephadex G-100 column of ovalbumin hydrolyzed by 0.03 N HCl at 115 °C for 1 and 2 h during standing time at room temperature. Ovalbumin (3 mL) hydrolyzed by 0.03 N HCl at 115 °C for 1 and 2 h was applied to a Sephadex G-100 column equilibrated with 0.03 N HCl after standing at room temperature for a given time: (a) immediately after hydrolysis for 1 h; (b) after standing the 1-h hydrolysates for 2 h; (c) after standing the 1-h hydrolysates for 24 h; (d) immediately after hydrolysis for 2 h; (e) after standing the 2-h hydrolysates for 2 h; (f) after standing the 2-h hydrolysates for 30 h. An arrow (↓) indicates the elution peak of untreated ovalbumin.

of ovalbumin hydrolyzed by 0.03 N HCl at 115 °C for 1 and 2 h during standing time at room temperature. The peak of void volume fraction increased correspondingly at a rate depending on the time for which ovalbumin hydrolysates were allowed to stand at room temperature in contact with air. As the hydrolysis is prolonged, a considerable amount of peptide fragments remained in smaller peptide fraction without polymerization. These results show that ovalbumin hydrolysates obtained from the prolonged hydrolysis are too small to polymerize. Therefore, the optimal condition is the hydrolysis by 0.03 N HCl at 115 °C for 0.5–1 h to obtain the macromolecular polymerized peptides.

The mechanism of polymerization of ovalbumin peptide fragments was investigated. Figure 4 shows the effect of sodium dodecyl sulfate, guanidine hydrochloride, and mercaptoethanol on the gel filtration patterns on a Sephadex G-100 column of the polymerized ovalbumin peptides obtained by acid hydrolysis in 0.03 N HCl at 115 °C for 30 min followed by standing at room temperature for 24 h. The peak of polymerized ovalbumin peptides emerging in void volume was not affected by the presence of 0.5% sodium dodecyl sulfate, while it mostly diminished and converted into two major peaks, smaller peptide fragments in the presence of 0.3 M mercaptoethanol in 0.5% SDS. In addition, the peak of polymerized ovalbumin peptides largely diminished without intermediate peak in the presence of 0.3 M mercaptoethanol in 4 M guanidine hydrochloride. This result suggests that the driving force for the polymerization of ovalbumin peptide fragments may

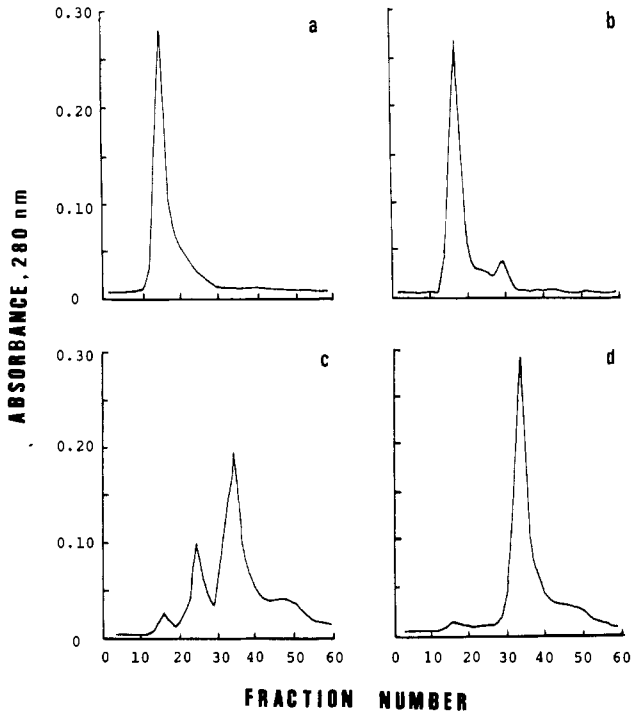


**Figure 4.** Elution patterns on a Sephadex G-100 column of polymerized ovalbumin peptides in the presence of sodium dodecyl sulfate, guanidine hydrochloride, and mercaptoethanol. The polymerized ovalbumin peptides were obtained by acid hydrolysis in 0.03 N HCl at 115 °C for 30 min followed by standing at room temperature for 24 h in contact with air: (a) polymerized ovalbumin peptides; (b) added 0.5% sodium dodecyl sulfate; (c) added 0.5% sodium dodecyl sulfate and 0.3 M mercaptoethanol; (d) added 4 M guanidine hydrochloride and 0.3 M mercaptoethanol.

**Table II. Sulfhydryl Content and Surface Hydrophobicity of Polymerized Ovalbumin Peptides**

| ovalbumin | sulfhydryl content, mol/mol | surface hydrophobicity |
|---|---|---|
| untreated | 4.0 | 20 |
| polymerized[a] | | |
| sample I (0.5) | 2.2 | 1980 |
| sample II (1.0) | 2.6 | 1780 |
| sample III (2.0) | 2.8 | 1720 |

[a] The values in parentheses indicate the hydrolysis time (h). The polymerized samples were obtained by lyophilizing the dialyzates against water after the mild acid hydrolysis of ovalbumin for a given time.

be hydrophobic and disulfide bond formation.

Table II shows the SH content and surface hydrophobicity of the polymerized ovalbumin peptides. The SH contents of polymerized ovalbumin peptides decreased to almost one-half. As the hydrolysis progressed, the SH contents gradually increased. This may be because of the loss of small peptides during the dialysis causing a gradual increase in the SH containing large peptides. However, the loss of small peptide fragments during dialysis was not observed in the polymerized ovalbumin peptides obtained by acid hydrolysis for 30 min, as shown in Figure 2. Therefore, it is presumed that disulfide bond formation is concerned in the polymerization of ovalbumin peptides. The surface hydrophobicity of polymerized ovalbumin peptides remarkably increased, suggesting the exposure of internal hydrophobic residues due to the cleavage of peptide bonds. A slight decrease in the surface hydrophobicity as the hydrolysis progressed also may be because of the loss of hydrophobicity small peptides during dialysis. Since the polymerized ovalbumin peptides were shown to have high surface hydrophobicity, the functional properties

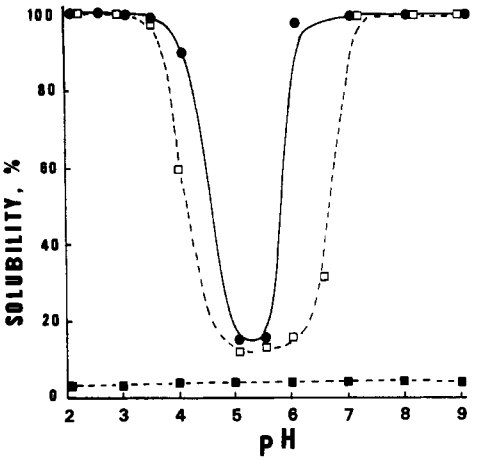


**Figure 5.** Effects of pH on solubility of polymerized ovalbumin peptides. The polymerized ovalbumin peptides were obtained by acid hydrolysis in 0.03 N HCl at 115 °C for 30 min followed by standing at room temperature for 24 h in contact with air. The polymerized ovalbumins were dialyzed against distilled water and then lyohilized: (●–●) 0.01 M acetate buffer (pH 2–5.5)–phosphate buffer (pH 6–8)–carbonate buffer (pH 9) system; (□- - -□) 0.1 M acetate, phosphate, and carbonate buffer system; (■- - -■) heat-denatured ovalbumin.

may be expected to be good.

We have proposed in a previous paper (Kato et al., 1983; Kato, 1984) that the soluble ovalbumin aggregates during heat denaturation were formed mainly by hydrophobic interaction in the first step and then slowly by disulfide bond formation in the second step. This is probably the case for acid treated ovalbumin. That is, the driving force for aggregation in an early step is hydrophobic and then after a proper hydrophilic–hydrophobic arrangement takes place, further increase in the polymerization number may occur by disulfide bond formation for which closer proximity is a necessity.

The functional properties of polymerized ovalbumin peptides were investigated. As shown in Figure 5, the polymerized ovalbumin peptides were soluble except the range between pH 4.0 and pH 6.5 despite a remarkable increase in the surface hydrophobicity, while the lyophilized heat denatured ovalbumin was insoluble at all pH range. This may be because a proper hydrophilic–hydrophobic arrangement took place during mild acid hydrolysis followed by standing at room temperature and lyophilizing ovalbumin peptide fragments. In addition, the electrostatic repulsion resulting from the deamidation of asparagine and glutamine may contribute to solubilize the polymerized ovalbumin peptides. The solubility between pH 4.5 and pH 6.5 decreased with an increase in ionic strength, diminishing the electrostatic repulsion of polymerized ovalbumin peptides.

Table III shows the emulsifying and foaming properties of polymerized ovalbumin peptides. These functional properties of polymerized ovalbumin peptides were greatly improved. It is interesting to see if the improvement of functionality is due to the hydrolysis alone or the polymerization of ovalbumin peptides. This was ascertained in Table IV. The functional properties of ovalbumin peptide fragments were measured immediately after acid hydrolysis. In this condition ovalbumin peptide fragments were not polymerized. As shown in Table IV, the functional properties of ovalbumin peptides obtained immediately after hydrolysis were not so good as those of polymerized ovalbumin peptides obtained by standing for 24 h in contact with air after hydrolysis. Therefore, it is probable that the polymerization of ovalbumin peptides is necessary

**Table III. Emulsifying and Foaming Properties of Polymerized Ovalbumin Peptides**

| ovalbumin | emulsifying activity, $OD_{500}$ | emulsion stability, min | foaming power, $\mu\Omega$/cm | foam stability |
|---|---|---|---|---|
| untreated | 0.25 | 0.6 | 110 | 0.02 |
| polymerized[a] | | | | |
| sample I (0.5) | 0.92 | 4.1 | 1350 | 0.25 |
| sample II (1.0) | 0.93 | 4.2 | 1500 | 0.19 |
| sample III (2.0) | 0.91 | 4.0 | 1440 | 0.19 |

[a] The values in parentheses indicate the hydrolysis time (h). The polymerized samples were obtained by standing at room temperature for 24 h in contact with air after the mild acid hydrolysis of ovalbumin for a given time.

**Table IV. Effect of Polymerization of Ovalbumin Peptides on Emulsifying and Foaming Properties**

| ovalbumin | emulsifying activity, $OD_{500}$ | emuslion stability, min | foaming power, $\mu\Omega$/cm | foam stability |
|---|---|---|---|---|
| peptide fragments[a] | 0.71 | 1.8 | 260 | 0.05 |
| polymerized[b] | 0.93 | 4.2 | 1500 | 0.25 |

[a] Peptide fragments were obtained immediately after acid hydrolysis (0.03 N HCl) for 1 h. [b] Polymerized ovalbumin were obtained by standing for 24 h in contact with air after acid hydrolysis (0.03 N HCl) for 1 h.

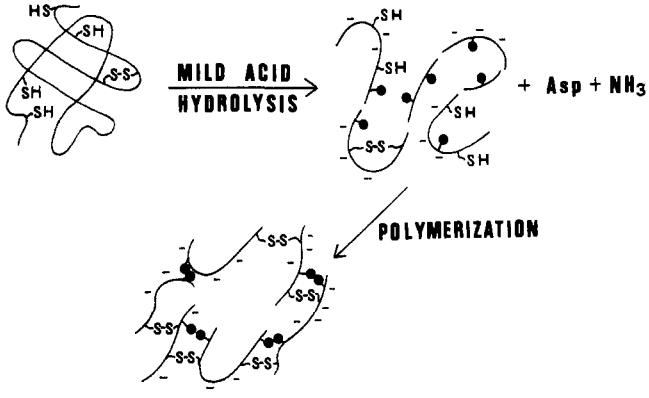


**Figure 6.** Scheme of polymerization of ovalbumin peptide fragments during mild acid hydrolysis followed by standing at room temperature in contact with air: (–) negative charge; (●) hydrophobic residues.

for the improvement of the functional properties. The improvement of functional properties of ovalbumin by acid treatment may be caused by (1) increased solubility due to the higher electrostatic repulsion as a result of deamidation and the hydrophilic N-terminal and C-terminal free amino acid formed by peptide cleavage, (2) better amphilic nature due to the proper hydrophilic–hydrophobic arrangement, and (3) polymerization through hydrophobic and disulfide bond formation.

We have reported that the emulsifying and foaming properties of ovalbumin were significantly improved by heat denaturation because of the increased hydrophobicity (Kato et al., 1981). However, the heat denaturation of ovalbumin causes the coagulation by various factors, such as pH, protein concentration, and lyophilization. On the other hand, mild acid hydrolysis of ovalbumin causes the improvement of functional properties without the loss of solubility below pH 3.5 and above pH 7.0. In addition, this acid treatment is superior to the alkaline treatment and the chemical modification with regard to the safety for food application, because this does not cause any degradation of amino acid and a contamitant production of toxic chemicals (Wu et al., 1976). Ovalbumin has about 3% carbohydrates consisting of mannose and *N*-acetylglucosamine. Since the free carbohydrates were not liberated under the experimental conditions employed, Maillard reaction products, undesirable in food preparations, may be absent in this system.

In conclusion, the polymerization scheme of ovalbumin peptide fragments was shown in Figure 6 during mild acid treatment. A mild acid treatment of ovalbumin brings about (1) deamidation of asparagine and glutamine, (2) hydrolysis of peptide bonds on either side of aspartic acid, and (3) polymerization of peptide fragments due to the hydrophobic interaction and SH oxidation. This polymerization system during acid treatment may be utilized to develop a new functional food protein.

## LITERATURE CITED

Beveridge, T.; Toma, S. J.; Nakai, S. *J. Food Sci.* **1974**, *39*, 49.
Conway, E. J.; O'Malley, E. *Biochem. J.* **1942**, *36*, 655.
Finley, J. W. *J. Food Sci.* **1975**, *40*, 1283.
Han, K. K.; Richard, C.; Biserte, G. *Int. J. Biochem.* **1983**, *15*, 875.
Kato, A. *New Food Ind.* **1984**, *26*, 69.
Kato, A.; Nagase, Y.; Matsudomi, N.; Kobayashi, K. *Agric. Biol. Chem.* **1983b**, *47*, 1829.
Kato, A.; Nakai, S. *Biochim. Biophys. Acta* **1980**, *624*, 13.
Kato, A.; Tsutsui, N.; Matsudomi, N.; Kobayashi, K.; Nakai, S. *Agric. Biol. Chem.* **1981**, *45*, 2755.
Kato, A.; Takahashi, A.; Matsudomi, N.; Kobayashi, K. *J. Food Sci.* **1983a**, *48*, 62.
Kekwick, R. A.; Cannan, R. K. *Biochem. J.* **1936**, *30*, 227.
Matsudomi, N.; Kato, A.; Kobayashi, K. *Agric. Biol. Chem.* **1982**, *46*, 1583.
Pearce, K. N.; Kinsella, J. N. *J. Agric. Food Chem.* **1978**, *26*, 716.
Schultz, J.; Allison, H.; Grice, M. *Biochemistry* **1962**, *1*, 694.
Wu, C. H.; Nakai, S.; Powrie, W. P. *J. Agric. Food Chem.* **1976**, *24*, 504.

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