Note

Effect of Heating Temperature on Sulfhydryl and disulfide Contents and State of Aromatic Amino Acid Residues in Soybean Protein

Tatsunori Yamagishi,* Mutsuko Takaya, Futoshi Ebina and Fumio Yamauchi

Department of Food Chemistry, Faculty of Agriculture, Tohoku University, Sendai 980, Japan
Received July 18, 1983

Sulfhydryl and disulfide residues play a great role in texture formation. Heating causes modification of rheological and functional properties. In order to detect quantitatively changes of rheological and functional properties, it seems to be necessary not only to measure physical properties, but also quantitatively the reactivity of sulfhydryl and disulfide residues. On heating, the change of their reactivity would follow that of the protein’s conformation. We paid attention to changes of locative sulfhydryl or disulfide residue contents and to structural changes of soybean protein with increasing heating temperature.

Defatted meal was prepared from soybeans cv Raiden by the method described previously. The lyophilized protein was dissolved to a concentration of 1% (determination of sulfhydryl and disulfide residues) or 0.1% (spectrophotometric measurement) in standard buffer (pH 7.6). After heating, the protein solution (1%) was centrifuged for 30 min at 16000 rev min. Available (A) and total (B) sulfhydryl (SH) residues were determined by the method of Ellman and the modified method, respectively. Disulfide residues (SS) were reduced by the method of Cavallini et al. Available residues (C, SH + SH due to SS) and total residues (D, SH + SH due to SS) were determined in the absence and presence of 1% SDS–8 m urea using the modified method of Cavallini et al., respectively. The number of disulfide residues was calculated with the following formula:

Number of the available SS = (C – A)/2
Number of the total SS = (D – B)/2
Number of masked SH or SS = Total SH or SS – available SH or SS

Total SH and SS residues in the precipitate were determined after the precipitate was dissolved in 1% SDS–8 m urea. SH or SS residues were expressed as each number of mole, occurring in the supernatant or precipitate formed on heating of 1 ml of 1% acid precipitated protein solution. Second derivative absorption spectra were obtained with a Yanagimoto high order derivative UV-visible spectrophotometer. Solutions of 8 m urea were prepared just before use, and used for urea denaturation of a protein.

With increasing heating time, each content of total, available and masked sulfhydryl residues in the super-

---

* Present address: Shokei Women’s Junior College, Sendai, Japan.
natant (buffer-soluble fraction) decreased (Fig. 1). On the other hand, available disulfide residues increased, and both total and masked disulfide residues decreased (Fig. 2). With increasing heating temperature from 70 to 80°C, total free sulfhydryl residue contents in the precipitate decreased, while total disulfide contents increased (Fig. 3). These results lead to the suggestions that (1) the decrease of total sulfhydryl residue content is due to the oxidation of sulfhydryl residues or the formation of disulfide bonds due to oxidation of sulfhydryl residues, and (2) the change of the reactivity (with Ellman's reagent in the absence of denaturant) of sulfhydryl and disulfide residues involves a conformational change in the neighborhood of the residues.

In order to determine the relationship between the change of the reactivity of sulfhydryl and disulfide residues and the conformational change of this protein, a change in the absorption of the chromophoric side chain was investigated by difference-derivative absorption spectrophotometry. Figure 4 shows that a denaturation blue-shift occurs markedly after heating at 70°C. The derivative difference intensity at 291 nm was more than that at 280 or 286 nm with increasing heating temperature. Judging from difference-derivative intensities due to a blue shift arising from an environmental change of three kinds of aromatic amino acid residue, heating may cause exposure of a more internal area in the neighborhood of a phenylalanine or tryptophan residue than that of a tyrosine residue. As a denaturant such as urea was used as a solvent perturbant, difference absorption spectrophotometry provides information as to the change of aromatic amino acid residues buried in a protein. The ratios of each intensity, \( d^2A/d\lambda^2 \), in the difference-second derivative absorption spectra of heated proteins in the presence of urea to that of the native protein in the presence of urea are listed in Table I. The ratio expresses the quantities of states of aromatic amino acid residues buried in a heat-treated protein. Table I shows that 90% each of the phenylalanine, tyrosine and tryptophan residues buried in the acid precipitated protein remained as the buried forms in the heated acid precipitated protein on heating at 70°C. Even on heating at 90°C, 60% of the phenylalanine or tryptophan residues and 80% of the tyrosine residues remain as the buried forms. This result seems not to conflict with that seen in Fig. 4. With increasing heating temperature, the change of the reactivity of sulfhydryl and disulfide residues seems to be coupled with that of the state of aromatic amino acid residues.

In the difference-second derivative absorption spectra of 7S and 11S globulin on denaturation with 6M urea, 7S globulin is free of the peak at 291~292 nm due to tryptophan residues, which was detected for 11S globulin. Accordingly, the conformational change around the tryptophan residue as the buried form may occur in 11S globulin rather than 7S globulin. Furthermore, SDS electrophoresis also suggested that the 11S globulin component in acid precipitated protein is concerned with thermal aggregation (data not shown). When the acid precipitated protein was heated, its insolubility was very

---

**Fig. 3.** Effect of Heating Temperature on Sulfhydryl and Disulfide Contents of Acid Precipitated Protein (Precipitate).

- , sulfhydryl residues; O-O, disulfide residues.

**Fig. 4.** Effect of Heating Temperature on Second Derivative Difference Spectra of Unheated and Heated Acid Precipitated Protein.

The derivative wavelength difference, \( \Delta \lambda \), was 5 nm. Acid precipitated protein was heated at various temperatures as samples and that at room temperature as a reference. Heating temperature: A, 30°C; B, 50°C; C, 70°C; D, 90°C. The spectra of the protein heated at 30, 50 and 70°C were measured with 2.5-fold the sensitivity for those of the protein heated at 90°C.
TABLE I. THE RATIOS OF INTENSITY, A(d²ΔI/dλ²)*, IN THE DIFFERENCE-
SECOND DERIVATIVE SPECTRA OF HEATED ACID PRECIPITATED PROTEIN 
DENATURED WITH UREA TO THAT OF NATIVE ACID PRECIPITATED 
PROTEIN DENATURED WITH UREA

<table>
<thead>
<tr>
<th></th>
<th>20°C</th>
<th>30°C</th>
<th>50°C</th>
<th>70°C</th>
<th>90°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>1.00</td>
<td>1.021</td>
<td>1.003</td>
<td>0.826</td>
<td>0.529</td>
</tr>
<tr>
<td>C-B</td>
<td>1.00</td>
<td>0.989</td>
<td>1.009</td>
<td>0.963</td>
<td>0.654</td>
</tr>
<tr>
<td>C-D</td>
<td>1.00</td>
<td>1.111</td>
<td>1.003</td>
<td>0.916</td>
<td>0.574</td>
</tr>
<tr>
<td>E-D</td>
<td>1.00</td>
<td>0.999</td>
<td>1.012</td>
<td>0.929</td>
<td>0.571</td>
</tr>
<tr>
<td>E-F</td>
<td>1.00</td>
<td>0.997</td>
<td>1.103</td>
<td>0.944</td>
<td>0.622</td>
</tr>
<tr>
<td>G-F</td>
<td>1.00</td>
<td>1.012</td>
<td>0.989</td>
<td>0.847</td>
<td>0.607</td>
</tr>
<tr>
<td>G-H</td>
<td>1.00</td>
<td>1.092</td>
<td>0.998</td>
<td>0.829</td>
<td>0.614</td>
</tr>
<tr>
<td>I-H</td>
<td>1.00</td>
<td>0.991</td>
<td>0.997</td>
<td>0.850</td>
<td>0.742</td>
</tr>
<tr>
<td>I-J</td>
<td>1.00</td>
<td>1.008</td>
<td>1.021</td>
<td>0.848</td>
<td>0.806</td>
</tr>
<tr>
<td>K-J</td>
<td>1.00</td>
<td>1.023</td>
<td>1.031</td>
<td>0.891</td>
<td>0.844</td>
</tr>
<tr>
<td>K-L</td>
<td>1.00</td>
<td>0.993</td>
<td>1.022</td>
<td>0.946</td>
<td>0.873</td>
</tr>
<tr>
<td>M-L</td>
<td>1.00</td>
<td>1.002</td>
<td>0.998</td>
<td>0.942</td>
<td>0.913</td>
</tr>
<tr>
<td>M-N</td>
<td>1.00</td>
<td>0.998</td>
<td>1.013</td>
<td>0.896</td>
<td>0.605</td>
</tr>
<tr>
<td>O-N</td>
<td>1.00</td>
<td>1.033</td>
<td>1.005</td>
<td>0.854</td>
<td>0.650</td>
</tr>
</tbody>
</table>

* Difference in d²ΔI/dλ² values between positive (A, C, E, G, I, K, M and O) 
and negative peaks (B, D, F, H, J, L and N). The position of peaks (positive 
peaks) and troughs (negative peaks): A, 252 nm; B, 245.5 nm; C, 257 nm; D, 
260 nm; E, 263 nm; F, 265 nm; G, 267 nm; H, 270 nm; I, 277 nm; J, 280 nm; K, 
283 nm; L, 287 nm; M, 289 nm; N, 292 nm; O, 295 nm. The positions scarcely 
differ for different heating temperatures. Values in the table are averages for at 
least 5 runs.

low (10%). On comparison of the changes of the reactivity 
and the quantity of SH and SS residues with that of the 
ratio of difference-derivative intensity with increasing 
heating temperature, it seems that the extent of conformational change is less than that of these sulphhydryl and 
disulfide residues. Damondaran et al.13) suggested that 7S 
globulin leads to formation of a soluble complex with US 
globulin. 7S globulin may repress thermal aggregation of 
11S globulin.

REFERENCES

12) J. W. Donovan, “Physical Principles and Techniques of Protein Chemistry,” Part A, ed. by S. J. Leach, 