KOREAN J. FOOD SCI. TECHNOL.
Vol. 21, No. 5, pp. 714~720 (1989)

Effect of Acetylation on Conformation of Glycinin

Kang Sung Kim and Joon Shick Rhee
Department of Biological Science and Engineering, Korea Advanced Institute of Science and Technology

Abstract

Effects of acetylation on conformational changes of glycinin was studied using solvent perturbation, second derivative spectroscopy, near uv circular dichroism spectra and viscosity. Glycinin with purity of more than 93% was used for the experiment. Modification was carried out with acetic anhydride and glycinin with lysine residue modification of 0%, 28%, 65%, 85%, and 95% were used for the experiment. The result of solvent perturbation using some selected perturbants, such as glycerol, ethylene glycol, and dimethyl sulfoxide revealed that acetylation has caused increase in solvent accessibility of tyrosine residues from less than 40% in native protein to more than 70% for 95% acetylated glycinin. This was confirmed by second derivative spectroscopy. Near ultraviolet circular dichroism revealed that the spectra of native and acetylated glycinin were almost identical differing only in intensity and no other useful information could be derived from it. However, in the case of 95% acetylated glycinin the influence of tryptophan on the spectrum was more pronounced. Specific viscosity of glycinin also increased by modification, the extent of which depended upon the degree of acetylation. These results supported that acetylation had caused globular conformation of glycinin to be expanded and denatured.

Keywords: glycinin, conformation, acetylation

Introduction

Chemical modification improves functional properties of food proteins as the result of changes in conformation and physicochemical properties of the protein.[1-3] As the result, elucidating conformations of food proteins and correlating them to functionalities should be of much interest to food chemists.

Conformational changes of the protein can be monitored by spectrophotometric techniques,such as circular dichroism, ultraviolet, and flourescence spectroscopy. Spectroscopic studies on glycinin have been performed by numerous researchers[4,5]. From optical studies of soy 11S globulin, it has about 5% α-helix, about 35% β-sheet with the remainder being random coil[6,7]. The glycinin is believed to be composed of six basic and six acidic subunits packed as two identical hexagons facing each other[4]. Glycinin is of special interest since this protein exhibits some interesting properties in the presence of salts[8,9], and heat[10,11]. Also, wider application of the protein as food ingredient in industry requires further research.

In the previous reports, we have reported effects of acetylation on physicochemical properties of glycinin[12]. In this work, we report effect of acetylation on conformation of glycinin and discuss in correlation with functionality change. Solvent perturbation, second derivative spectroscopy, viscosity, and near uv circular dichroism were employed in probing the changes.

Corresponding author; Joon Shick Rhee, Department of Biological Science and Engineering,Korea Advanced Institute of Science and Technology, P.O. Box 150, Chongryang, Seoul 130-650

Materials and Methods

Materials

Soy bean flour was purchased from Sigma Chemical Company (St. Louis, MO). Acetic anhydride used for acetylation reaction was also of Sigma brand. All other reagents used were of analytical grade.

Methods

11S Purification

Glycinin (11S protein) rich fractions obtained by the methods of Thanh and Shibasaki[14] was applied to a DEAE-Sephadex A-50 column. Buffer used for eluting the protein was 30 mM phosphate buffer, pH 7.5, with NaCl gradient of 0.25-0.45 M. Purity of the glycinin fraction was found to be more than 93% by a densitometric scanning of SDS polyacrylamide electrophoresed gel of the protein.[15]

Acetylation

The acetylation procedure used was similar to that of Riordan and Vallee[16]. Acetic anhydride was added slowly to the protein dissolved in 30 mM potassium phosphate buffer of pH 7.6 (designated as the "standard buffer" hereafter) and the pH 7.6 was maintained by using at pH stat with 1N NaOH. After the reaction, the sample was exhaustively ultrafiltered to get rid of salts before lyophilization. Lysine residue modified was determined using ninhydrin reagent and samples of 0, 28, 65, 85, and 95% lysine residue modifications were used for the subsequent experiments. Samples of native and acetylated proteins were quantified using microkjehldal nitrogen analysis.

Solvent Perturbation

The general procedures for preparing matched solution for solvent perturbation have been described by Herskovits and Laskowski[17]. It this experiment, β-mercaptoethanol was used as reducing agent instead of glycolic acid. Protein concentrations were determined spectrophotometrically with the use of the following extinction coefficients: 0% modified protein, 8.1; 28% modified protein, 8.1; 65% modified protein, 7.5; 85% modified protein, 6.06; 95% modified protein, 5.94% Protein concentration used were 0.08-0.12%. Perturbants employed were 20% ethylene glycol, glycerol and dimethyl sulfoxide[18]. Measurements were determined with a Jasco Spectrophotometer with slit width of 5 mm.

Second derivative spectroscopy

Second derivation spectroscopy of 0.1-0.05% samples were obtained on a Shimadtzu Spectrophotometer. Second derivative adsorption spectra were obtained by placing the native and 6M guanidium hydrochloride denatured protein solutions in the sample compartments[19,20]. The derivative wavelength difference, $\Delta\lambda$, was 1 nm.

Near ultraviolet circular dichroism

CD measurments were carried out with a Jasco spectropolarimeter equipped with a Xenon arc lamp photomultiplier[23,23]. Measurement were made at room temperature. Protein concentrations of 0.3% in the standard buffer were used for the near uv measurement with 10 mm light path length.

Measurement of viscosity

The specific viscosity of protein solutions (1% on the phosphate buffer) was determined using Oswald type viscometer[21]. Measurement was made at 25 °C and the time for the sample to flow through the capillary tube under gravity was used to calculate specific viscosity according to the following equation:

$$\text{specific viscosity} = t_s/t_b - 1$$

where t_s is the time for the sample and t_b is the time for the buffer solution flow through the capillary tube.

Results and Discussion

Purification and Acetylation of Glycinin

Glycinin of more than 93% purity from soybean flour was obtained after isoelectric point precipitation and DEAE-Sephadex A 50 column chromatography. Sodium chloride gradient applied was 0.25-0.45 M (Fig. 1). Purity of the protein was checked by a densitometric scanning of SDS PAGE of glycinin. Acidic and basic subunits of molecular weight of 35,000 and 20,000 are shown, respectively. Fig. 2 shows SDS PAGE of purified glycinin. To obtain glycinin with different

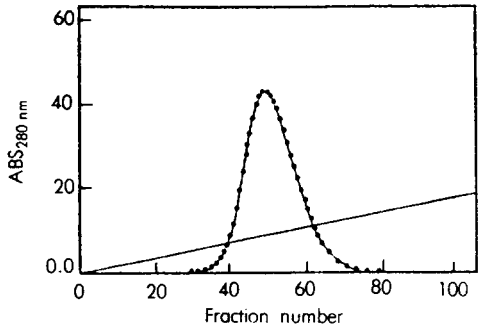

Fig. 1. Elution profile of 11S soy globulin. Buffer used was 30 mM phsphate buffer, pH 7.5, and NaCl gradient was 0.25-0.45 M. Column was 8×15 cm, flow rate 16 ml/hr.

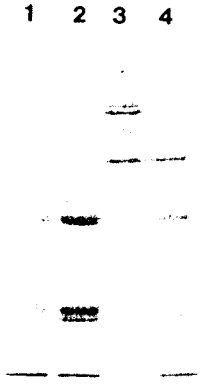

Fig. 2. SDS-PAGE of glycinin
1, 4; soluble protein of soybean meal, 2; glycinin, 3; conglycinin

degree of acetylation, acetic anhydride was added slowly to the protein solution with the pH maintained at 7.6 using a pH stat with 1N NaOH. Lysine residue modified was determined using ninhydrin reagent and samples of 0, 28, 65, 85, and 95% lysine residue modification were obtained. Native and acetylated samples were quantified using microkjedahl nitrogen analysis and correlated to 280 nm.

Solvent perturbation and second derivative spectroscopy

Fig. 3 shows solvent perturbation spectra of native and 85% modified glycinin, when ethylene glycol was used as the perturbant. In the solvent

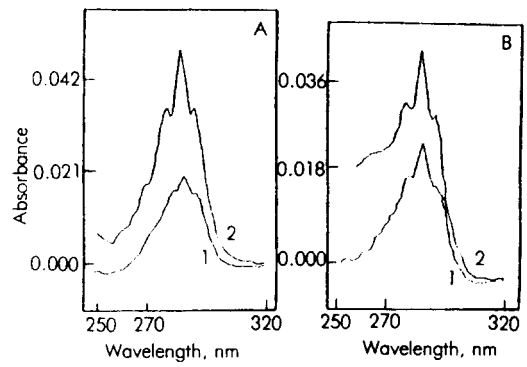

Fig. 3. Solvent perturbation difference spectra of A) native and B) 85% acetylated 11S, in buffer (1) and in 6M urea (2), due to ethylene glycol.

perturbation method of probing the surface of protein molecules, advantage is taken of the fact that the spectra of chromophoric residue coming freely in contact with the solvent are sensitive to changes in the physical properties of the solvent, such as dielectric constant and refractive index[17,18]. Mild nonaqueous substances, in concentration mild enough to cause measurable shifts on the spectra of chromophoric residues, but mild enough not to affect the conformation of the protein, are employed as perturbants. In this experiment 20% ethylene glycol, glycerol, and DMSO were employed as the perturbants. The fraction of the total shift is relative measure of the fraction of the chromophoric groups exposed to the solvent. Total exposure of the chromophore of the protein was achieved by treating the protein with 6M urea and 0.1 M β-mercaptoethanol. Table 1 shows ex-

Table 1. Effects of various perturbants of the accessiblity of tyrosine and and tryptophan residues of native and acetylated glycinin.

Perturbant	Native Glycinin		85% Acetylated Glycinin	
	Tyrosine	Tryptophan	Tyrosine	Tryptophan
Ethylene glycol	41	38	74	65
Glycerol	37	30	62	—
Dimethyl sulfoxide	34	37	78	70

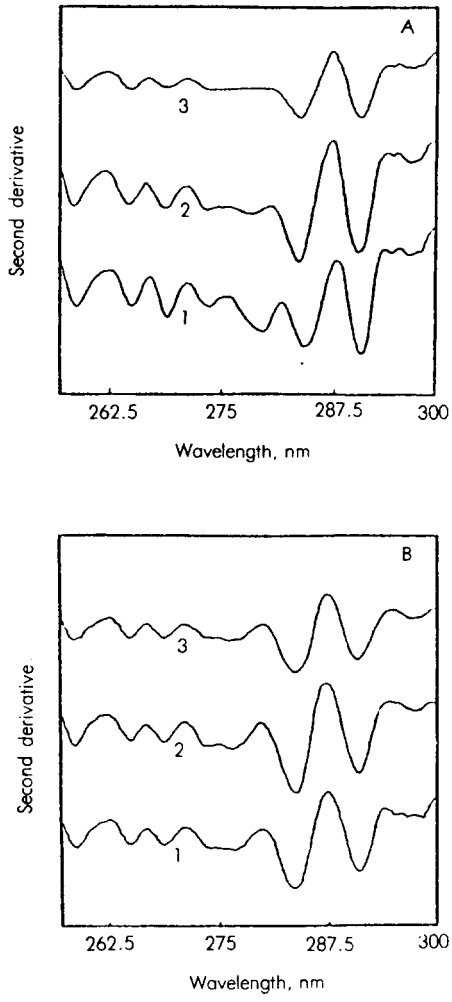

Fig. 4. Second derivative spectra of native and acetylated 11S soy protein. A) in the 30 mM phosphate buffer and B) in 6M Gdn.HCl. Degree of acetylation: 1) 0% 2) 65%, and 3) 95%. Protein concentration were 0.05-0.07%.

posure of tyrosine and tryptophan residues of the protein upon acetylation, using three different perturbants. In native protein about 40% of tyrosine were found to be accessible to the solvent, whereas in the 85% modified sample, 70% tyrosine residues were solvent accessible.

Second derivative spectroscopy

In order to confirm the result obtained in the

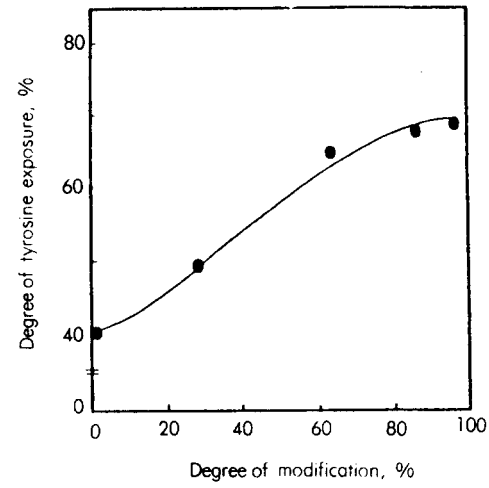

Fig. 5. Tyrosine accessibility due to acetylation as obtained from second derivative spectra.

solvent perturbation experiment, second derivative spectra was performed. Second derivative spectroscopy has proved to be an effective tool because of its ability to resolve overlapping bands in normal spectrum. This technique has been utilized to resolve the complex protein absorption spectrum into individual contribution of the three aromatic amino acids and to carry out relatively simple method for their quantitative estimation. Fig. 4 shows that acetylation caused a progressive blue shift in all the peaks and troughs, indicating exposure of hydrophobic chromophores to aqueous surroundng. This result coincides with the result of uv spectra and solvent perturbation. Using the equation developed by Servillo *et al.*[19] denaturation effects of acetylation on tyrosine residue was quantitatively calculated (Fig. 5). At native state tyrosine exposure was less than 40% but the level increased to about 70% upon excessive acetylation of 95%. Initially, tyrosine is rapidly exposed to the hydrophilic environment but above lysine modification of 80%, the increase in solvent accessibility of tyrosine resides were less rapid, indicating less structural or refolding to a new conformation. Our previous report on uv absorption spectra and fluorescence spectra had revealed that tyrosine residues and tryptophan

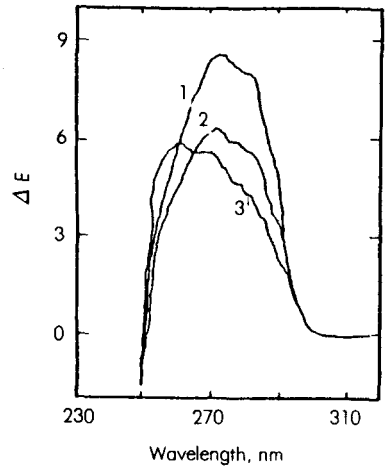

Fig. 6. CD spectra of native and acetylated glycinin. The degree of acetylation: (1) 0%, (2) 65%, (3) 95%.

residues were shifted to a more polar environment following acetylation[12,13]. These results indicated that the compact structure of glycinin was ruptured by chemical modification at lysine residues, the extent of which depended upon the acetylation degree.

Near ultraviolet CD spectra

Fig. 6 shows near uv CD spectra of native and acetylated glycinin. The near ultraviolet spectra of the samples in 30 mM phosphate buffer, pH 7.6, all showed the contributions of aromatic groups. Because side chain chromophores produce relatively weak CD bands between 250 and 320 nm and identical residues can have either positive and negative CD band depending on their spatial arrangement, interpretation of these near ultraviolet CD bands is extremely complex and at present only qualitative description could be presented.

As a main feature, the native and acetylated glycinin have quantitatively nearly identical positive spectra: signals displaying about the same shape, showing the same substructures and located at the same wavelengths and differing only in the intensities. Generally CD band above 290 nm can be assigned to tryptophan residues, these between 270 and 288 nm are mostly due to tyrosine

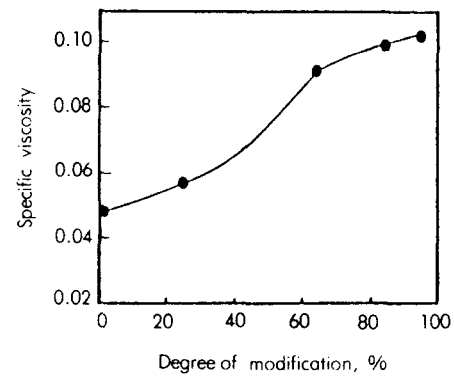

Fig. 7. Specifiv viscosity of native and acetylated glycinin. Specific viscosity measurement was done at protein concentration of 1% in the standard buffer.

residues, and the two negative peaks near 269 and 261 nm can be attributed to phenylalanine residues[22,23]. This the spectra suggest strong influence of tryptophan for all the protein samples, especially the 95% acetylated glycinin, and little other information could be drawn from the result.

Specific viscosity

The specific viscosity of native and acetylated glycinin at 1.0% protein concentration (Fig. 7) showed that with increasing levels of acetylation viscosity of the protein increased, the highest level being the glycinin with 95% acetylation.

According to Kim[21], the increase in the hydrodynamic volume of the protein and an increased member of particles had occurred as the result of succinylation at lysine residues. The alteration of charge of lysine groups from positive to neutral with acetylation destabilized the oligomeric structure of native glycinin and the native globule dissociated into intermediate subunits. Accompanying dissociation, the change of charge caused unfolding of intermediate subunits, which also increases the hydrodynamic volume.

Nakai *et al.*[24] reported that there exists direct relationship between surface hydrophobicity and emulsifying properties of the protein. Increase in accessibility of hydrophobic residues, tyrosine and

tryptophan, means surface hydrophobicity of glycinin is increased by acetylation and thus emulsifying properties of the protein could be improved by the modification. Surface hydrophobicity and emulsifying properties were indeed found to be improved by acetylation[12,14], prompting usefulness of the modification in functionalities of food protein, where surface properties are important. Also increased in viscosity suggests that acetylation might be applicable to food such as gravies and soups, where viscosity is important.

References

1. Li-Chan, E., Helbig, N., Holbek, E., Chan, S. and Nakai, S.: Covalent attachment of lysine to wheat gluten for nutritional improvement. *J. Agric. Food Chem.*, **27**, 877 (1979)
2. Childs, E.A. and Park, K.K.: Functional properties of acylated glandless cotton flour. *J. Food Sci.*, **41**, 713 (1976)
3. Kella, N.K.D., Barbeau, W.E. and Kinsella, J.E.: Effect of oxidative sulfitolysis of disulfide bonds of glycinin on solubility, surface hydrophobicity, and *in vitro* digestibilty. *J. Agric. Food Chem.*, **34**, 215 (1986)
4. Badley, R.A., Atkinson, O., Oldani, D., Green, J.P. and Stubbs, J.M.: The structure, physical and chemical properties of the soy bean protein glycinin. *Biochim. Biophys. Acta*, **412**, 214 (1975)
5. Kitamura, K., Takagi, T. and Shibasaki, K.: Subunit structure of soybean 11S globulin. *Agric. Biol. Chem.* **40**, 1837 (1976)
6. Koshiyama, I. and Fukushima, D.: Comparison of conformations of 7S and 11S soybean globulins by optical rotatory dispersion and circular dichroism studies. *Cereal Chem.*, **50**, 114 (1973)
7. Catsimpoolas, N., Campbell, T.G. and Meyer, E.W.: Association-dissociation phenomena in glycinin. *Arch. Biochem. Biophys.* **131**, 577 (1969)
8. Appurao, A.G. and Narashingarao, M.S.: Binding of Ca(II) by the 11S fraction of soybean proteins. *Cereal Chem.*, **52**, 21 (1975)
9. Sakakibara, M. and Noguchi, H.: Interaction of 11S fraction of soybean protein with calcium ion. *Agric. Biol. Chem.*, **41**, 1575 (1977)
10. Damodaran, S. and Kinsella, J.E.: Effect of conglycinin on the thermal aggregation of glycinin. *J. Agric. Food Chem.*, **30**, 812 (1982)
11. Wolf, W.J. and Tamura, T.: Heat denaturation of soybean 11S protein. *Cereal Chem.*, **46**, 331 (1969)
12. Kim, K.S. and Rhee, J.S.: Effects of acetylation on physicochemical and functional properties of glycinin. *J. Food Biochem.* (in press)
13. Kim, K.S. and Rhee, J.S.: Effect of acetylation on emulsifying properties of glycinin. *J. Agric. Food Chem.* (submitted for publication)
14. Thanh, V.H. and Shibasaki, K.: Major proteins of soybean seeds. A straightforward fractionation and their characterization. *J. Agric. Food Chem.*, **24**, 1117 (1976)
15. Weber, K., Osrobn, M.: The relability of molecular weight determination by dodecyl surlfate-polyacrylamide gel electrophoresis. *J. Biol. Chem.*, **244**, 4406 (1969)
16. Riordan, J.F. and Vallee, B.L.: *Acetylation. Methods Enzymol.*, **25**, 494 (1971)
17. Herskovits, T.T. and Laskowski, M., Jr.: Location of chromophoric residues in proteins by solvent perturbation. *J. Biol. Chem.*, **237**, 2481 (1962)
18. Herskovits, T.T.: Conformation of proteins and polypeptides. *J. Biol. Chem.*, **240**, 628 (1965)
19. Servillo, L., Colonna, G., Balestrieri, C., Ragone, R. and Irace, G.: Simultaneous determination of tyrosine and tryptophan residues in proteins by second-derivative spectroscopy. *Anal. Biochem.*, **126**, 251 (1982)
20. Ruckpaul, K., Rein, H., Ballou, D.P. and Coon, M.J.: Analysis of interactions among purified components of the liver microsomal cytochrome p-450-containing monooxygenase system by second derivative spectroscopy. *Biochim. Biophys. Acta*, **616**, 41 (1980)
21. Kim, S.H.: Structure, surface properties and foam stability of native, reduced and succinylated 11S globulins. Ph.D. Thesis, Cornell University, New York (1985)
22. Yutani, K., Ogasahara, K., Suzuki, M. and Sugino, Y.: Effect of a single amino acid substitution on the near ultraviolet CD spectra of tryptophan synthase α-subunit. *J. Biochem.*, **87**, 117 (1980)
23. Kubota, S., Yeung, H.W. and Yang, J.T.: Conformation of abortifacient proteins: trichosanthin, β-momorcharin and -momorcharin. *Biochim. Biophys. Acta*, **871**, 101 (1986)
24. Nakai, S.: Structure-function relationships of food proteins with an emphasis on the importance on protein hydrophobicity. *J. Agric. Food Chem.*, **31**, 679 (1983)

(Received Sep. 18, 1989)

아세틸화가 Glycinin의 구조에 미치는 영향

김강성·이준식
한국과학기술원 생물공학과

콩의 주요 저장 단백질인 glycinin의 라이신 잔기를 적당량의 acetic anhydride를 이용하여 28, 65, 85, 95%로 아세틸화시켰다. 아세틸화에 의한 구조적 변화를 solvent perturbant 방법으로 측정한 결과 자연상태의 단백질에 있어서는 타이로신 잔기의 약 40% 미만이 단백질 표면에 노출되어 있었으나 85% 아세틸화 glycinin에 있어서는 70% 이상이 표면에 노출되어 용매에 대해 접근이 용이하게 되었다. 이와 같은 현상은 second derivative spectroscopy에 의해 서로 동일하게 나타났으며, 따라서 아세틸화에 의해 타이로신과 같은 소수성 아미노산이 단백질 표면으로 이동하여 단백질 구조가 변형되었음을 알 수 있었다. 한편 near UV circular dichroism의 결과 자연상태의 glycinin과 아세틸화가 일어난 glycinin 모두 유사한 모양의 spectra를 나타내었으나 95% 아세틸화 glycinin의 경우에는 tryptophan의 영향이 두드러졌다.

Specific viscosity의 경우 아세틸화가 일어날수록 급격히 증가하였는데 이는 아세틸화에 의해 구형의 glycinin이 변형되어 분자의 부피가 커졌을 뿐 아니라 subunit의 분리에 의해 입자수가 증가했기 때문이다[12].