Properties of Starches Conjugated with Lysine and Poly(lysine) by the Maillard Reaction

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Native potato starch (PS), carboxymethyl potato starch (CMS), and corn starch phosphate monoester (PCS) were conjugated with lysine (Lys) and poly(lysine) (PL) by the Maillard reaction. The increased yellowness of each reaction mixture showed the development of browning, suggesting the conjugation of starch and Lys or PL. The Lys and PL contents in the conjugates were 0.12−0.68% and 2.8−4.3%, respectively. Conjugation with PL reduced the swelling and solubility of each starch. The CMS and PCS conjugates gelatinized at higher temperatures than either CMS or PCS alone and were hard to retrograde. The digestibility of each conjugate with α-amylase was lower than that of the original starch. The functional changes to the starches were most marked in the cases of starches with partial distortion of the internal structure as indicated for CMS and PCS.

Keywords: *Starch; functional change; amino acid; neoglycoconjugate; Maillard reaction*

INTRODUCTION

The conjugation of nonstarchy components with starch granules is considered to be one of the most effective methods to improve the functionality of starch. We have reported that a whey protein isolate and six kinds of amino acids (Glu, Cys, Lys, Gly, Leu, and Phe) could be conjugated to carboxymethyl starch granules with acid−amide bonding by using water-soluble carbodiimide, resulting in a marked decrease in the solubility, swelling power, retrogradation degree, and digestibility with α- or β-amylase and an increase in the thermal stability (Hattori et al., 1995; Yang et al., 1995).

However, to use such modified starches for food application, it is necessary to find a new method to achieve substantial functional changes to starch without applying any chemical reagents. The Maillard reaction is well-known as an amino−carbonyl reaction which occurs through glycosylamine formation during food processing and storage. Because the Maillard reaction has been used to improve the functions of proteins such as gluten, lysozyme, and egg white proteins by conjugation with galactomannan and dextran (Kato et al., 1991, 1993; Nakamura et al., 1992), we expected that the amino groups of amino acids or peptides could be conjugated with the reducing-end groups of starch by the Maillard reaction. However, the internal structure of native starch granules is thought to be tight due to their granular nature. This suggested an unfavorable environment for conjugation by the Maillard reaction, so we used starches with a partially distorted structure such as carboxymethyl potato starch and corn starch phosphate monoester in addition to native potato starch. We chose L-lysine (Lys) and ε-poly(L-lysine) (PL) for conjugation with these starches. Lys, one of the essential amino acids, is the most reactive amino acid for the Maillard reaction, and PL consisting of about 30 Lys residues obtained from the culture broth of *Streptomyces abulus* is a polycationic antibacterial substance.

The objective of this work was to conjugate native potato starch (PS), carboxymethyl potato starch (CMS), and corn starch phosphate monoester (PCS) granules with lysine (Lys) or poly(lysine) (PL) to achieve the functional changes to starch by the Maillard reaction.

MATERIALS AND METHODS

Materials. PS (Hokuren Research Institute, Japan) was used after repeated washings with Milli-Q water at 24 °C and air-drying. CMS (the degree of modification was 34 carboxymethyl residues/1000 glucose residues) was prepared as previously described (Hattori et al., 1995; Yang et al., 1995). PCS (9.5% moisture; 0.34% phosphate content) was obtained from Oji Corn Starch Co. (Tokyo, Japan). L-Lysine (Lys) was purchased from Ajinomoto Co. (Tokyo, Japan), and ε-poly(L-lysine) (PL; the average degree of polymerization was 30) was supplied by Chisso Co. (Yokohama, Japan). All other reagents were of reagent grade commercially available.

Preparation of the Conjugates. PS, CMS, or PCS (1 g) was dispersed in 1 mL of a Lys or PL solution (100 mg/mL, pH7.5) and stored overnight at 4 °C. After lyophilization, the dried mixture was incubated at 50 °C (PCS) or 60 °C (PS and CMS) at a relative humidity of 79% for 3 weeks (PS and CMS) or 4 weeks (PCS). The reaction mixture was washed with distilled water by centrifuging eight times at 5000 rpm for 10 min, and the conjugate was obtained by air-drying.

Measurement of the Yellowness Index. The yellowness index (N) of each conjugate was measured by a digital color-difference meter (Toyo Rika Instruments, Tokyo, Japan) to evaluate the progress of the Maillard reaction. N is defined by the following equation, where X, Y, and Z are the CIE tristimulus values (Method 6131 of Fed. Test Method Std. No. 141c, 1986):

$$N = (1.250X - 1.038Z)/Y$$

Determination of Lys and PL. The content of Lys or PL in each starch conjugate was determined according to the method of Jaenicke et al. (1974). A sample containing 0.002−0.2 μmol of nitrogen was mixed with 34 μL of 70% $HClO_4$ in a test tube (12 × 100 mm). This test tube was placed in a dry

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block bath (DM-8; Scinics Corp., Tokyo, Japan), and the temperature was maintained at 205−215 °C. After the water had been evaporated, the test tube was sealed by a glass marble. The sample was then heated for 20 min. This perchloric acid digestion was carried out in a fume cupboard, and to avoid contact with the skin and eyes, eye/face protection and suitable gloves were used. After the contents of the test tube had cooled to room temperature, 0.5 mL of distilled water was added and mixed well with 0.5 mL of phenol reagent (2% phenol containing 0.01% sodium nitroprusside). Hypochlorite reagent (0.2 mL; 0.02 M NaOCl in 2.5 M NaOH) was then added, and the mixture was thoroughly mixed and kept for 20 min at room temperature. The absorbance was measured in a 1-cm silica cuvette at 578 nm against a nitrogen-free blank. An ammonium sulfate solution (10^{-2} M) was used as a nitrogen standard.

Microscopic Observation. CMS and each CMS conjugate (10 mg) were dispersed in 1 mL of distilled water and heated at 50 °C for 10 min, the other samples being heated at 70 °C. After cooling to room temperature, each sample was stained with 1 mL of a Coomassie Brilliant Blue solution (CBB, 10 mg/mL) for 15 min and then washed with distilled water by centrifuging three times at 3000 rpm for 5 min. Each sample thus obtained was observed through a polarizing microscope (model PM-10AD sp; Olympus, Tokyo, Japan).

Solubility Measurement. A sample (10 mg) was suspended in 5 mL of distilled water and heated at a constant temperature (45 or 95 °C) for 1 h with stirring at 500 rpm. The solubility was evaluated by determining the saccharide concentration of the centrifuged supernatant (18 000 rpm for 10 min at 20 °C) according to the phenol−sulfuric acid method (Dubois et al., 1956).

Differential Scanning Calorimetry (DSC). DSC of each starch was performed with an SSC-5020 DSC 100 apparatus (Seiko, Tokyo, Japan) as previously described (Takahashi et al., 1988). About 5 mg of a sample and 10 μL of distilled water were sealed in an anodized aluminum sample cell. Water was used as a reference, and DSC was carried out from 5 to 100 °C at a heating rate of 2 °C/min. These DSC curves were used to evaluate the gelatinization temperatures [onset temperature (T_o), peak temperature (T_p), and conclusion temperature (T_c)] and gelatinization enthalpy (ΔH) as characteristics of the gelatinization process.

The sample heated to 80 °C in the DSC apparatus was preserved at 4 °C for 7 days and analyzed again by DSC. The ratio of the second gelatinization enthalpy to the first is regarded as the degree of retrogradation (Nakazawa et al., 1985).

Digestion with α-Amylase. The digestibility of each starch with α-amylase (EC 3.2.1.1; Sigma, St. Louis, MO) was measured as described previously (Hattori et al., 1995; Yang et al., 1995). A sample (2 mg) dispersed in 1.8 mL of a 0.02 M sodium citrate buffer at pH 6.5 containing 0.1 M sodium chloride (the citrate buffer) was heated at 100 °C for 10 min. After the mixture had cooled to room temperature, 0.01 unit of α-amylase in 0.2 mL of the citrate buffer was added, and the reaction mixture was incubated at 30 °C for 120 min. Digestibility was evaluated by determining the saccharide concentration of the filtrate passing through a membrane filter with a 0.45-μm pore size (cellulose nitrate A045A025A; Advantec, Tokyo, Japan) according to the phenol−sulfuric acid method (Dubois et al., 1956).

RESULTS AND DISCUSSION

Conjugation of Starch and Lys or PL. After incubation for 3 weeks (PS and CMS) or 4 weeks (PCS), the development of browning in each reaction mixture was apparent from the increased yellowness, especially in the cases of PS and CMS (Figure 1), suggesting the conjugation of starch and Lys or PL by the Maillard reaction. Because the conjugated starch granules showed apparent birefringence similar to those of each native starch (data not shown), it is thought that no extensive

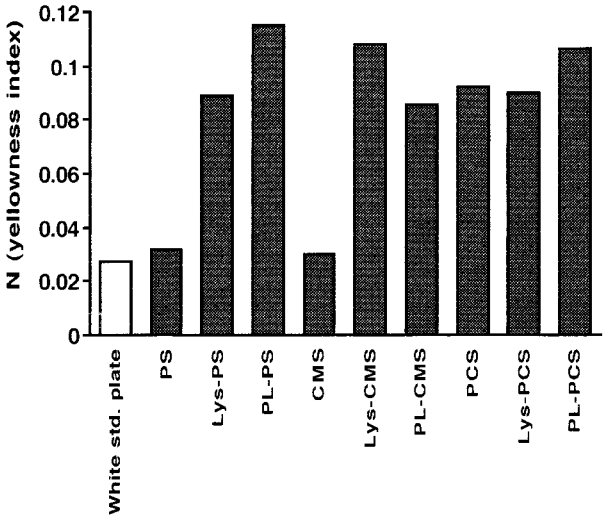

Figure 1. Yellowness (N) of Lys−starch and PL−starch conjugates. N is defined by the following equation, where X, Y, and Z are the CIE tristimulus values (Method 6131 of Fed. Test Method Std. No. 141c, 1986): $N = (1.250X - 1.038Z)/Y$.

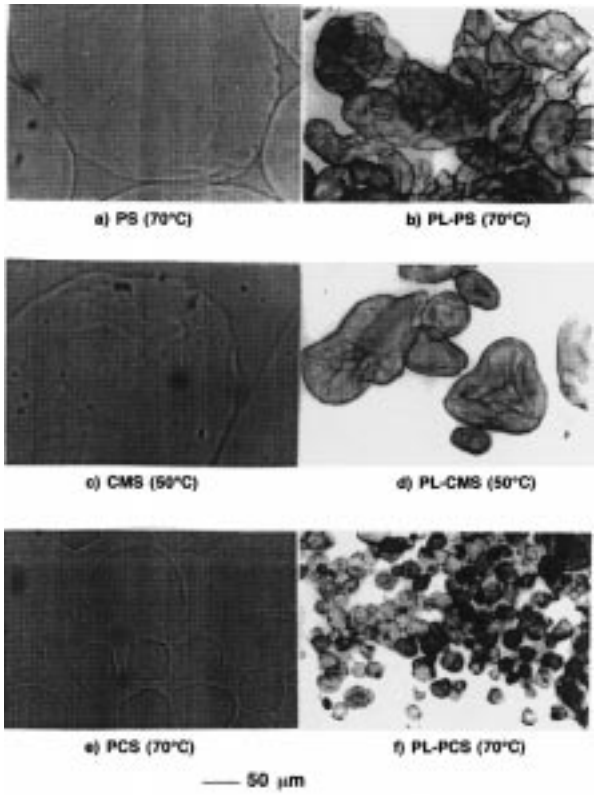

Figure 2. Polarizing micrographs of PL−starch conjugates after heating at 50 or 70 °C for 10 min and staining with CBB: (a) PS (70 °C); (b) PL−PS (70 °C); (c) CMS (50 °C); (d) PL−CMS (50 °C); (e) PCS (70 °C); (f) PL−PCS (70 °C).

distortion in the granular structure occurred by conjugation with Lys or PL through the Maillard reaction. The gelatinized conjugates with PL were stained with CBB (Figure 2), while the conjugates with Lys could not be stained (data not shown), demonstrating that PL could be conjugated with the starch granules by the Maillard reaction. The Lys and PL contents in the conjugates were 0.12−0.68% and 2.8−4.3%, respectively (Table 1).

Changes in Swelling and Solubility. The conjugates swelled to a limited extent after heating at 50 °C (PL−CMS) or 70 °C (PL−PS and PL−PCS) for 10 min,

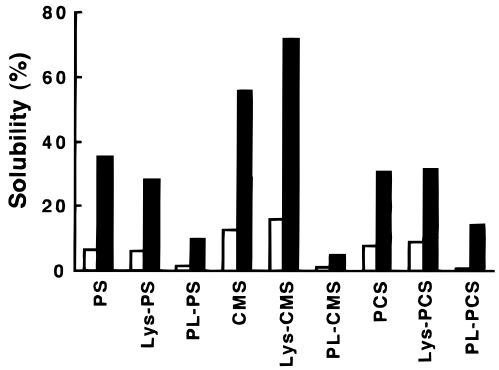

Figure 3. Solubility of Lys−starch and PL−starch conjugates. Each sample was suspended in 5 mL of distilled water and heated at 45 (□) or 95 °C (■) for 1 h with stirring. The solubility was evaluated by determining the saccharide concentration of the centrifuged supernatant (18 000 rpm for 10 min) according to the phenol−sulfuric acid method (Dubois et al., 1956).

Table 1. Contents of Lys and PL in the Lys−Starch and PL−Starch Conjugates

conjugate	Lys content (%)	conjugate	PL content (%)
Lys-PS	0.12	PL-PS	4.3
Lys-CMS	0.68	PL-CMS	2.8
Lys-PCS	0.41	PL-PCS	3.9

while the original starches swelled extensively (Figure 2). When heated at higher temperatures (>80 °C), the granules of CMS and PCS disappeared by completely dissolving, while each conjugate with PL remained without any marked morphological changes. It is considered that conjugation with polycationic PL could increase the stability of the starch structure by depressing the anionic repulsion due to carboxyl groups or phosphorus groups and restricting the mobility of the starch chains. Thus, conjugation by the Maillard reaction is thought to be effective for depressing the swelling of starch granules with partial distortion in the internal structure such as indicated for CMS and PCS. However, the Lys−starch conjugates had swelling properties similar to those of the original starches (data not shown). It is thus suggested that conjugation with a sequence with a suitable length or charge content is required to depress the swelling.

The solubility of each conjugate with PL was lower than that of the other starch samples (Figure 3). The decrease in the solubility of the PL−CMS conjugate was particularly remarkable. It is thus thought that conjugated PL could inhibit the migration of starch chains from the inside of the granules and that the PL−starch conjugates were stable to shearing stress induced by stirring. Another possible reason for the changes in swelling and solubility could be that a PL containing ~31 amino groups has conjugated with more than one starch molecule, the starch chains thus being cross-linked. Conjugation with PL can be expected to improve the physical properties such as viscosity or stickiness of starchy foods. The solubility of each conjugate with Lys was not significantly different from that of the original starch, corresponding to the morphological changes just described.

Gelatinization and Retrogradation Properties. Data from DSC indicate that the CMS and PCS conjugates gelatinized at about a 2−10 °C higher temperature (onset temperature, T_o) than did the original CMS and PCS and that the thermal transition became sharp

Table 2. Gelatinization Temperature, Enthalpy, and Retrogradation Degree of the Lys−Starch and PL−Starch Conjugates

sample	gelatinization temp (°C) T_o	T_p	T_c	ΔH (mJ/mg)	retrogradation degree (%)
PS	55.4	59.6	66.0	14.7	57.7
Lys−PS	54.7	60.6	68.2	16.2	48.1
PL−PS	52.7	58.6	65.0	13.0	74.6
CMS	35.1	42.5	61.9	12.5	27.6
Lys−CMS	39.5	44.6	56.1	8.2	10.8
PL−CMS	45.4	49.0	55.3	8.7	11.1
PCS	47.8	57.7	74.2	8.3	53.0
Lys−PCS	50.1	57.7	69.5	9.7	18.6
PL−PCS	55.6	63.7	71.2	8.9	15.7

(Table 2). The temperature increase was particularly marked in each conjugate with PL. Since conjugated cationic PL could effectively depress the electrostatic repulsion by the negative charge of CMS/PCS and the free motion of starch chains, the structural stability of the conjugates was considerably greater than that of original CMS and PCS and of all the conjugates with Lys. In the cases of the Lys−PS and PL−PS conjugates, the gelatinization temperatures (T_o) were, respectively, about 1 and 3 °C lower than that of original PS. The addition of a positive charge by conjugation with Lys or PL is considered to have resulted in partial distortion of the internal structure of PS. However, there was no distinct change in gelatinization enthalpy between PS/PCS and its conjugates, and CMS conjugates showed lower enthalpies than CMS. The influence of lyophilizing treatment might have resulted in these data. Ahmed and Leliever (1978) reported the degree of crystallinity of wheat starch decreased markedly after lyophilization. In our study, since the gelatinization enthalpy of CMS without addition of Lys or PL decreased markedly as compared with those of other starches after lyophilization (data not shown), the CMS conjugates showed low enthalpies.

The conjugates gelatinized by the DSC apparatus were preserved at 4 °C for 7 days prior to a second analysis by DSC. The ratio of the second gelatinization enthalpy to that from the first analysis can be regarded as the degree of retrogradation. There were no distinct changes in regelatinization temperatures of each starch and its conjugates after retrogradation (data not shown). The conjugates generally showed a low degree of retrogradation, except for PL−PS (Table 2). In particular, the PL−CMS and PL−PCS conjugates showed a low degree of retrogradation (about 11% and 17%, respectively). This indicates that these conjugates resisted retrogradation, suggesting that the bonding of Lys or PL to CMS and PCS by the Maillard reaction could restrict the rearrangement of starch chains.

Digestibility with α-Amylase. CMS, PCS, and Lys−CMS conjugate showed higher digestibility than the original starches (Figure 4). However, Wootton et al. (1979, 1981) reported the digestibility of gelatinized substituted and cross-linked starches is lower than that of the original starches. This is considered to result from the difference of method to measure the digestibility. Wootton et al. used the gelatinized starch solutions to estimate the digestibility by increase in reducing power and by decrease in blue value. We used gelatinized starch suspension without mechanical stirring and determined the saccharide concentration of the filtrate according to the phenol−sulfuric acid method as the digestibility, so the high swelling and solubility of these starch granules is thought to bring about the

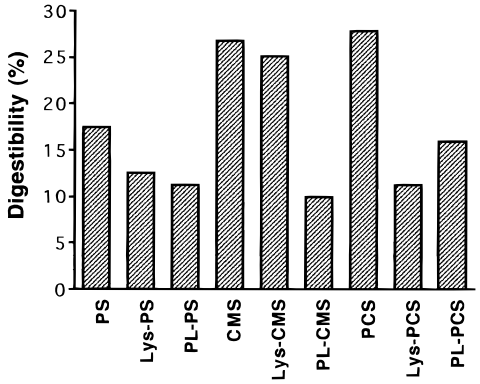

Figure 4. Digestibility of Lys−starch and PL−starch conjugates with α-amylase. Each sample was heated at 100 °C for 10 min. After the sample cooled to room temperature, α-amylase was added and the reaction mixture was incubated at 30 °C for 120 min. Digestibility was evaluated by determining the saccharide concentration of the filtrate after each period of digestion according to the phenol−sulfuric acid method (Dubois et al., 1956).

high digestibility. The digestibility of each conjugate with α-amylase was markedly lower than that of the original starch, except for Lys−CMS. The indigestibility of the conjugates with α-amylase is thought to have been caused by their low solubility and swelling and by their inhibiting effect on α-amylase, similar to the effects of other Maillard reaction products on α-amylase and trypsin (Miura et al., 1994; Hirano et al., 1996). From these results, it is expected that conjugation with amino acid or peptide by the Maillard reaction could endow starch with the characteristics of an indigestible polysaccharide. In particular, conjugation with peptide was more effective than that with amino acid. Since raw starches are generally of very low in vitro digestibility but are often digested quite effectively by animals, including humans, the conjugates may have a similar character. However, the low swelling and solubility and the conjugated Lys or PL are expected to lower the rate of digestion and affect the composition of digest. The in vitro digestibility for the conjugates should be further investigated in a separate study.

Concluding Remarks. In this study, Lys and PL conjugates of PS, CMS, and PCS were prepared by using the Maillard reaction. The swelling and solubility of the conjugates with PL were lower than those of the original starches. The CMS and PCS conjugates gelatinized at higher temperatures than CMS and PCS and were more resistant to retrogradation. The digestibility of each conjugate with α-amylase was lower than that of the orignal starch. These indicated the functionality of starch could be changed with this method, especially in the cases of starch with partial distortion of the internal structure such as CMS and PCS, and it is expected the conjugates will be used for the novel improvements of starchy foods.

ABBREVIATIONS USED

AA, amino acid; CMS, carboxymethyl starch; CBB, Coomassie Brilliant Blue; Lys, L-lysine; PCS, corn starch phosphate; PL, ϵ-poly(L-lysine); PS, potato starch; DSC, differential scanning calorimetry; T_o, onset temperature; T_p, peak temperature; T_c, conclusion temperature; ΔH, gelatinization enthalpy.

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