Preparation of Blood Glue from Porcine Plasma Protein and Cross-linking Reaction of Plasma Protein with Formaldehyde

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Blood glue was prepared to reutilize porcine blood. Plasma proteins after lyophilization were treated by addition of wood flour, sodium hydroxide, sodium silicate, and hydrated lime to make blood glue with a suitable adhesivity. Characteristics of the prepared blood glue was monitored by measuring the viscosity with time, and the relationship between degree of hydrolysis of plasma proteins by addition of various amounts of sodium hydroxide and adhesivity was studied. To prevent the emission of formaldehyde during manufacturing of plywood by blood glue, the cross-linking reaction of plasma protein with formaldehyde was also examined. Fourier transform infrared, circular dichroism, and fluorescence spectroscopy study showed that blood plasma proteins react with formaldehyde, resulting in removal of formaldehyde by cross-linking reaction.

Key words : *plasma protein, formaldehyde, cross-linking.*

Considerable amount of blood waste from slaughtered animals have caused many problems. Disposal of blood causes serious water pollution since blood is mostly discharged without undergoing suitable wastewater treatment. Therefore, appropriate utilization of blood should be considered. Previously, we have reported on ways to reutilize blood as source of ACE inhibitory peptides and blood glue.[1-3]

Although blood glue had been used as a protein adhesive in wood products industry until early 1980's, it has been mostly replaced by petroleum-based adhesives which are more economical.[4-6] However, it still remains important in certain areas due to its unique bonding characteristics. Therefore, it is desirable to overcome high production cost of blood glue to compete with amino resin such as UF resin and UMF resin.

In this study, to utilize blood isolated from slaughtered pigs and prevent water pollution, blood glue was prepared, and its characteristics were investigated. Prevention of the emission of formaldehyde in plywood industry by cross-linking reaction was also examined.

Materials and Methods

Materials. Whole porcine blood was freshly collected right after slaughter and immediately used for plasma preparation. All chemicals used were of analytical grade.

Isolation of blood plasma protein. After collecting the blood from the slaughterhouse, EDTA (2 g/L) was immediately added to prevent coagulation and centrifuged at 11590×g for 30 min to remove blood cells. To the supernatant, 2% TCA was added to precipitate the plasma protein and was lyophilized.

SDS-PAGE. SDS-PAGE was performed according to the method of Laemmli.[7]

Preparation of blood glue. Blood glue was prepared according to the modified method of Lambuth.[6]

Viscosity measurement. Viscosity of blood glue treated at various sodium hydroxide concentrations was measured using a Brookfield viscometer (Brookfield Engineering Lab Inc., Model DV-1).

FTIR measurement. Porcine plasma protein was dissolved in sodium borate buffer (50 mM, pH 8.0) and filtered through a microfilter (0.45 μm, Whatman Co.). The solution was then mixed with equal volume of 0.01 M HCHO solution and incubated with agitation for 30 min. To stop the reaction, the solution was mixed with tetrahydrofurfural solution dropwise and centrifuged at 5000×g for 10 min. The precipitated protein was washed with acetone, dried using a speed vacuum concentrator, and prepared as a FTIR sample of KBr pellet. FTIR (Bomem MB100) was used where transmission resolution was 4 cm^{-1} and scan number was 16.

CD study. Far-UV CD spectrum was obtained at 25°C with a JASCO J-720 spectropolari-meter.[8] A cell of 1-mm-pathlength was used. The reported CD spectrum was an average of five scans and smoothed by polynomial

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Abbreviations: ACE, angiotensin converting enzyme; CD, circular dichroism; FTIR, fourier transform infrared; UF, urea formaldehyde; UMF, urea melamine formaldehyde.

curve fitting program.

Fluorescence spectroscopy. Emission spectrum was obtained by measuring the emission intensity of the protein solution after excitation at 280 nm using a spectrofluorometer (SLM 4800S). The pathlength was 1 mm.

Results and Discussion

Preparation of blood glue. A simple and rapid method of purification of plasma protein was previously reported.[1] SDS-PAGE showed that the prepared porcine blood plasma protein consisted mainly of serum albumin and globulin fractions (Fig. 1). The major fraction for blood glue was serum albumin.

Blood glue was prepared using porcine blood plasma proteins. The change of viscosity with time was measured to determine the adhesivity of blood glue (Fig. 2). To obtain a consistent finished glue viscosity, preparation of blood glue requires alkaline dispersion, appropriate agitation rate for mixing, and good control of water temperature after addition of wood flour, sodium hydroxide, sodium silicate, and hydrated lyme.[6] The optimum value of the viscosity of glue is a very important factor in that if too low, adhesivity of the glue becomes poor, and if above 40,000 cP, the glue cannot be applied to the wood. In the case of porcine blood glue, the viscosity value was between 15,000~20,000 cP for the first 3 hr, and increased very steeply from then on, indicating that the glue has a reasonably stable shelf life.

The adhesivity of protein adhesives depends on the degree of hydrolysis of proteins by sodium hydroxide. Full adhesive potential of the blood glue is developed only through alkaline dispersion. Therefore, at various concentrations of sodium hydroxide, the viscosity of blood plasma protein solution was monitored with time using a Brookfield viscometer (Table 1).

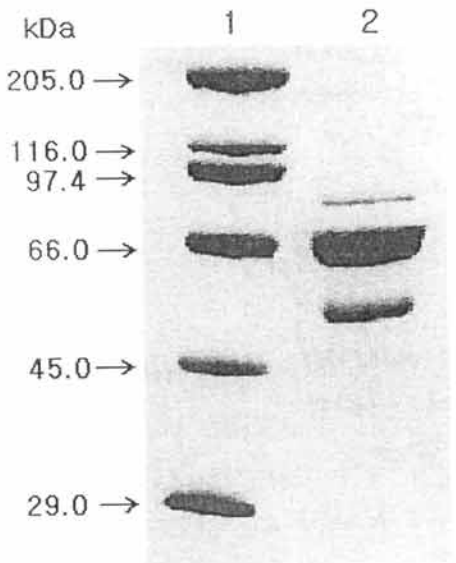

Fig. 1. SDS-PAGE profile of blood plasma protein. Lane 1, molecular weight marker; 2, porcine plasma.

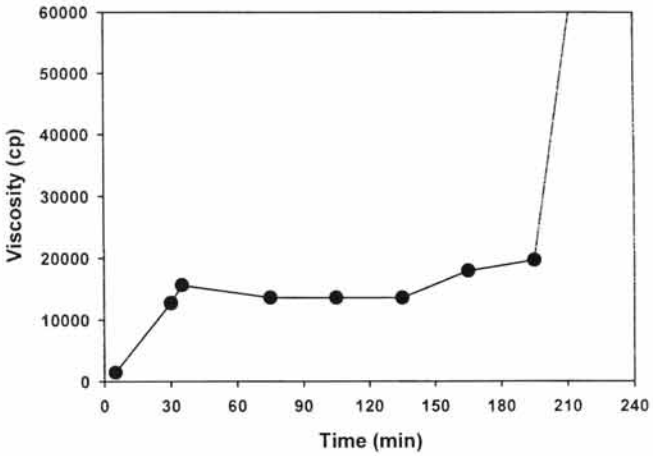

Fig. 2. Changes in viscosity of porcine blood glue with time.

Table 1. Changes in viscosity of porcine blood plasma protein treated by various sodium hydroxide concentrations.

unit (cP)

Time (min)	2.5% NaOH	5% NaOH	7.5% NaOH	10% NaOH
5	2.9	2.9	3.1	2.8
30	2.6	2.8	2.7	2.6
60	2.4	2.5	2.4	2.6
90	2.2	2.2	2.4	2.3
120	2.0	2.1	2.2	2.3
150	1.9	2.0	2.0	2.2
180	1.9	2.0	2.0	2.2
210	1.7	1.9	2.0	2.2
240	1.7	1.8	1.9	2.0

Viscosity values of all the samples decreased with time up to 4 hr. Also, among the treatments, there were not much significant differences in the ranges of sodium hydroxide used in this study. However, considering the strength and shelf life of blood glue, it is recommended that 10% of sodium hydroxide be used although the experiment was performed for blood plasma proteins and not blood glue. Also, it should be mentioned that preparation of blood glue involves several materials which affect the adhesivity of glue.

Cross-linking reaction with formaldehyde. Blood glue prevents the emission of formaldehyde by cross-linking reaction. Air pollution is caused by formaldehyde emitted during the process of making plywoods using a formaldehyde-based resin. Blood plasma protein, able to entrap formaldehyde by cross-linking reaction, was examined using CD, spectrofluorometer, and FTIR.

The conformational change of proteins by crosslinking was examined using FTIR spectroscopy. IR spectroscopy may be a useful tool for studying the conformational change of proteins.[9-11] In IR spectroscopy, protein molecules show NH streching where there is change in 3400, 3300, and 3100 cm^{-1}. Since the intermolecular interaction of the protein molecules by formaldehyde occurs around the amino group, FTIR spectrum shows that cross-linking of the polypeptide changes two broad peaks at 3400 cm^{-1} into a single peak (Fig. 3). This strongly indicates the change in the molecular

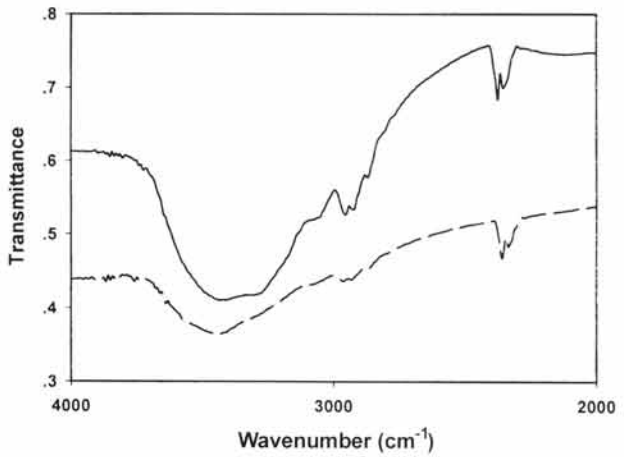

Fig. 3. FTIR spectrum of porcine blood plasma protein cross-linked. –, control; ---, cross-linked.

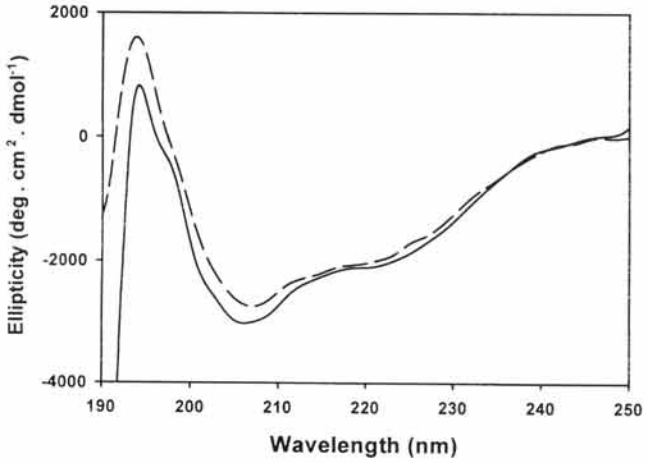

Fig. 4. CD spectrum of porcine blood plasma protein cross-linked. –, control; ---, cross-linked.

property of the protein cross-linked by formaldehyde.

Far-UV CD spectrum shows the conformational change on the secondary structure of proteins. Especially, in the case of change in the local environment of ordered structure of a polypeptide chain, it is easily differentiated from the native one. To determine the change of secondary structure of plasma protein by formaldehyde cross-linking, Far-UV CD spectrum was obtained (Fig. 4). The CD spectrum of the plasma protein showed a typical ordered structure pattern where there were negative minimum ellipticity values at 221 and 207 nm and a positive maximum value at 193 nm. Cross-linking by formaldehyde caused the change in ellipticity values. Cross-linking decreased the ellipticity value in the range between 205 nm and 225 nm, indicating a decrease of ordered structure of the protein

When the tertiary structure of the protein changes, it can be monitored using an emission spectroscopy since it reflects the change in the local environment around the aromatic amino acid residues. To examine the change of tertiary structure of plasma protein by cross-linking reaction, emission spectrum was obtained using a spectrofluorometer (Fig. 5).

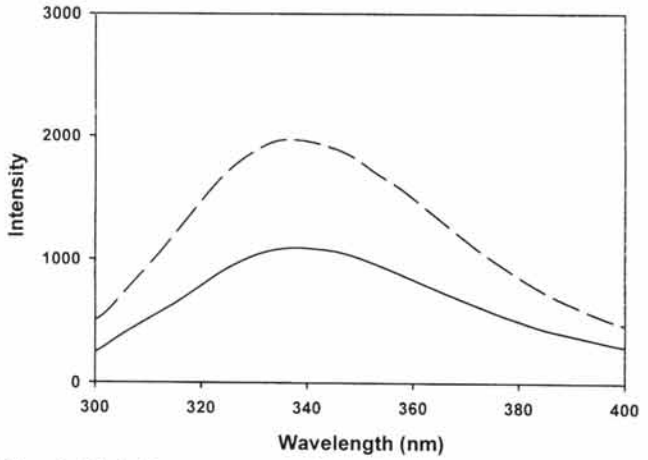

Fig. 5. Emission spectrum of porcine blood plasma protein cross-linked. –,control; ---, cross-linked.

The emissiom spectrum of the protein solution showed that the plasma protein had a maximum emission intensity at 340 nm and cross-linking reaction increased the relative intensity of emission. This indicates that cross-linking of the protein by formaldehyde altered the local environment around tryptophanyl residues

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