Characterization of Haze-Forming Proteins of Beer and Their Roles in Chill Haze Formation[1]

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ABSTRACT

Four proteinaceous fractions responsible for chill haze of beer (haze-forming proteins I, II, III, and IV) were isolated from beer by ultrafiltration followed by ammonium sulfate precipitation, ion-exchange chromatography, and gel chromatography. The haze-forming proteins, constituting about one third of the nitrogenous substances of beer, had molecular weights of 1,000 to 40,000 and contained appreciable amounts of proline. The haze-forming proteins, particularly fraction II, had high affinities for polyphenols and readily combined with polyphenols to form chill haze. Because the contents of proline in the haze-forming proteins correlated well with their affinities for polyphenols, proline must be important in the combination of haze-forming proteins with polyphenols. Hydrogen bonding or hydrophobic bonding or both seemed to be involved in the combination. Immunological studies showed that the haze-forming proteins originated mainly from malt hordein and were mainly responsible for chill haze of beer.

Key words: *Beer, Chill haze, Hordein, Mechanism of reaction, Polyphenol, Protein*

Proteins are the main precursors of chill haze of beer. In aged beer, proteins combine with polyphenols to form protein-polyphenol complexes, and these complexes are thought to be responsible for the formation of chill haze. Kringstad and Damm (8) and, more recently, Fitchett[2] showed that the proteins adsorbed on silica gel formed typical chill haze in the presence of polyphenols. Nummi et al (12) suggested that acidic proteins derived from the albumin and/or globulin of barley had the strongest tendency to form chill haze. Narziss and Röttger (11) showed that proteinaceous fractions with molecular weight above 60,000 seemed to participate in formation of chill haze. Many attempts have been made to characterize chill haze of beer. Amino acid analyses have suggested that hordein may be involved in chill haze (13,15), whereas immunological studies have indicated that albumin or globulin or both were mainly responsible for chill haze (4). Thus, there appears to be an uncertainty about whether particular protein fractions are responsible for chill haze in beer.

We isolated and characterized the proteinaceous fractions of beer responsible for chill haze and demonstrated their roles in chill haze formation.

EXPERIMENTAL

Preparation of Beer Fractions

Beer fractions were prepared from unhopped and unstabilized beer as described previously (1).

Protein Preparations

Poly-amino acids and papain were purchased from Sigma Chemical Co., gliadin from Tokyo Kasei Kogyo Co., Ltd., and lysozyme from BDH Chemicals.

Measurement of Haze-Forming Capacity

Haze-forming capacities of beer fractions and protein preparations were measured in the following three ways: measurement in beer; reaction with beer polyphenols; and reaction with catechin.

Measurement in Beer. The sample dissolved in 2.0 ml of 0.02*M* sodium phosphate buffer, pH 4.2, containing 3.6% ethanol was added to 18.0 ml of degassed beer chill-proofed with papain. Two blanks were run simultaneously. One contained 2.0 ml of sodium phosphate buffer and 18.0 ml of beer, and the other contained 2.0 ml of the sample solution and 18.0 ml of sodium phosphate buffer. The mixtures were placed in glass tubes (1.8 × 7 cm) with a septum cap, stored at 50° C for 20 hr, and then chilled at 0° C for 4 hr. Chill haze was measured nephelometrically with a Zeiss-Pulfrich photometer. Readings in absolute units were corrected by subtracting blank values and converted to EBC formazin units.

Reaction with Beer Polyphenols. Beer polyphenols were prepared by adsorption on Polyamide Woelm® (Woelm Pharma GmbH and Co.) and elution with 70% aqueous acetone. Acetone in the effluent was removed under reduced pressure.

The sample dissolved in 2.0 ml of 0.02*M* sodium phosphate buffer was mixed with an equal volume of beer-polyphenol solution (160 mg/L) in a test tube (1.5 × 15.6 cm) capped with a glass ball. Two blanks were run simultaneously. One contained 2.0 ml of sodium phosphate buffer and an equal volume of beer-polyphenol solution, and the other contained 2.0 ml of the sample solution and an equal volume of sodium phosphate buffer. The mixtures were heated at 100° C for 20 min and then chilled at 0° C for 40 min. Chill haze was measured nephelometrically with a Zeiss-Pulfrich photometer. Readings in absolute units were corrected by subtracting blank values and converted to EBC formazin units.

Reaction with Catechin. Instead of beer-polyphenol solution (+)-catechin (Sigma Chemical Co.) dissolved in sodium phosphate buffer (800 mg/L) was used, and haze-forming capacity was measured in a fashion similar to that in the reaction with beer polyphenols.

The haze-forming capacities measured in these three ways correlated well with each other (measurement in beer:reaction with beer polyphenols, r = 0.923; measurement in beer:reaction with catechin, r = 0.966; reaction with beer polyphenols:reaction with catechin, r = 0.873).

Measurement of Foam

The head-forming capacity of beer was measured as described previously (1).

Molecular Weight Estimation

The sample (3–6 mg) dissolved in 0.05*M* NaCl was applied to a Sephadex G-50 or G-75 column (2 × 47 cm) equilibrated with 0.05*M* NaCl, and material was eluted with 0.05*M* NaCl at a flow rate of 40 ml/hr. Effluent was collected in 4-ml fractions, and protein and carbohydrate contents were measured by the methods of Lowry et al (10) and Molisch (2), respectively. The molecular weights of proteins were estimated by comparison of their elution volumes with those of the standard proteins such as bacitracin, cytochrome c, myoglobin, chymotrypsinogen A, ovalbumin, and bovine serum albumin.

Immunoelectrophoresis

Albumin and globulin were extracted from malt and rice with 0.5*M* NaCl, and hordein was then extracted from malt with 70%

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[2] Presented at the European Brewery Convention, Biochemistry Group Meeting, London, 1980.

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aqueous ethanol. Rabbit antisera toward albumin and globulin of malt and rice, hordein of malt, intact yeast cells, and foaming proteins (1) of beer were prepared in the Japan Immunoresearch Laboratories Co., Ltd.

Antiserum towards hordein was raised much slower than that towards albumin or globulin, and the activity of antihordein serum was less than one hundredth of that of albumin or globulin.

Rocket-immunoelectrophoresis was performed by the method of Laurell (9) at 8°C for 3 hr at 10 V/cm, using 1% agarose gel in Tris-barbiturate buffer, pH 8.6, containing each antiserum. After electrophoresis, the gel was washed with 0.1*M* NaCl to remove excess antiserum, and protein precipitates in the gel were stained with Coomassie brilliant blue R-250.

Chemical Analysis

The protein and carbohydrate contents of samples were determined by the method of Lowry et al (10) and the phenol-sulfuric acid method (7), with bovine serum albumin and glucose, respectively, as standards.

The amino acid composition of samples was analyzed with an amino acid analyzer, JEOL model JLC-6AH, after the samples (5 mg) had been hydrolyzed with 1 ml of 6*N* HCl at 110°C for 20 hr in evacuated sealed tubes.

Constituent sugars in samples were analyzed by the method of Sawardeker et al (14) after the samples (5 mg) had been hydrolyzed with 0.5 ml of 1*N* H_2SO_4 at 100°C for 3 hr.

Separation of Chill Haze from Stored Beer

Bottled beer prepared without any chill-proofing treatment was stored at 50°C for 3 days and then chilled to 0°C for 24 hr. Chill haze was separated from the beer by filtration on a membrane filter (Millipore®, pore size: 0.65 and 0.45 μ), washed with 3.6% aqueous ethanol at 0°C, dissolved in deionized water at 20°C, and lyophilized.

The beer from which chill haze was removed was further stored at 50°C for four days (seven days total), and the chill haze developed was again separated in the same way. These procedures were repeated at intervals of seven or 14 days for a total of 70 days.

RESULTS

Fractionation of Haze-Forming Proteins

The fractionation procedure for haze-forming proteins is summarized in Fig. 1. As shown in Table I, fraction 2 had the highest haze-forming capacity, followed by fractions 5 and 6, and fraction 4 did not form chill haze appreciably. Therefore, fractions 2, 5, and 6 were used for further fractionation.

Fractionation of Fraction 2. A solution of 240 mg of fraction 2 in 0.005*M* ammonium formate adjusted to pH 4.0 with 0.005*M* formic acid was applied to a carboxymethyl (CM) cellulose column (3.5 × 6 cm) equilibrated with 0.005*M* ammonium formate, pH 4.0. The column was first washed with 160 ml of 0.005*M* ammonium formate, pH 4.0, and then the material retained was eluted with 200 ml of 0.5*M* ammonium formate, pH 6.5. Each effluent was lyophilized to yield 224.9 and 12.3 mg of unretained and retained materials, respectively.

Fractionation of Fraction 5. A solution of 100 mg of fraction 5 in 0.1*M* ammonium bicarbonate was applied to a Bio-Gel P-150 column (3.2 × 58 cm) equilibrated with 0.1*M* ammonium bicarbonate. Material was eluted with 0.1*M* ammonium

TABLE I
Haze-Forming Capacities of Beer Fractions

Beer Fraction	Concentration in Beer (mg/L)	Haze-Forming Capacity,[a] EBC f.u.[b]		
		In Beer	Reaction with Beer Polyphenols	Reaction with Catechin
2	22,380	6.0	7.8	6.2
4	4,823	0.5	0	0
5	630	3.6	6.9	2.0
6	492	2.3	2.1	1.4
2 + 5 + 6	23,502	...	12.6	9.1
Original beer	...	...	10.0	9.5

[a] Measured at half the concentration of each fraction in beer.
[b] EBC formazin units.

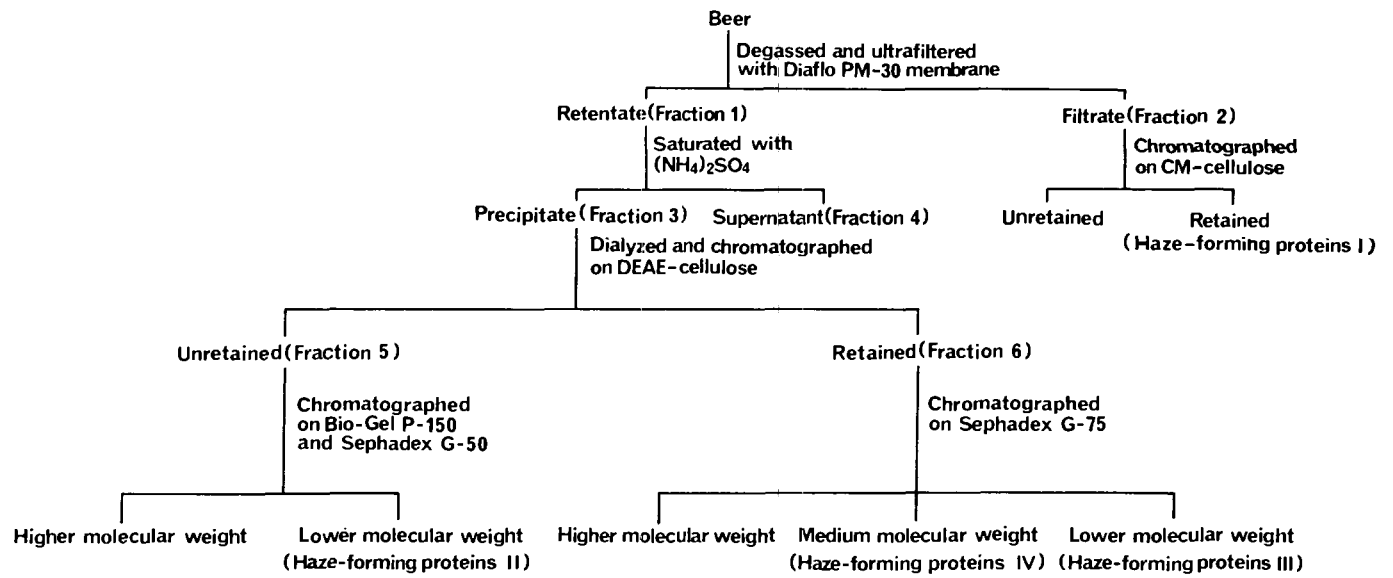

Fig. 1. Procedure for isolation and fractionation of haze-forming proteins of beer.

bicarbonate at a flow rate of 33 ml/hr, and the effluent was collected in 15-ml fractions. Fractions of effluent containing higher and lower molecular weight materials, respectively, were combined as shown in Fig. 2 and lyophilized to yield 46.5 and 35.5 mg of higher and lower molecular weight materials, respectively. The lower molecular weight material was purified on a Sephadex G-50 column (3.2 × 59 cm) with 0.1 *M* ammonium bicarbonate as eluent, at a flow rate of 25 ml/hr, as shown in Fig. 3.

Fractionation of Fraction 6. A sample of 100 mg of fraction 6 was fractionated into 21.3, 12.9, and 33.4 mg of higher, medium, and lower molecular weight materials, respectively, on a Sephadex G-75 column (5 × 54 cm) as described previously (1).

Table II shows that the material retained on CM cellulose of fraction 2 had the highest haze-forming capacity, followed by the lower molecular weight material of fraction 5 and the lower and

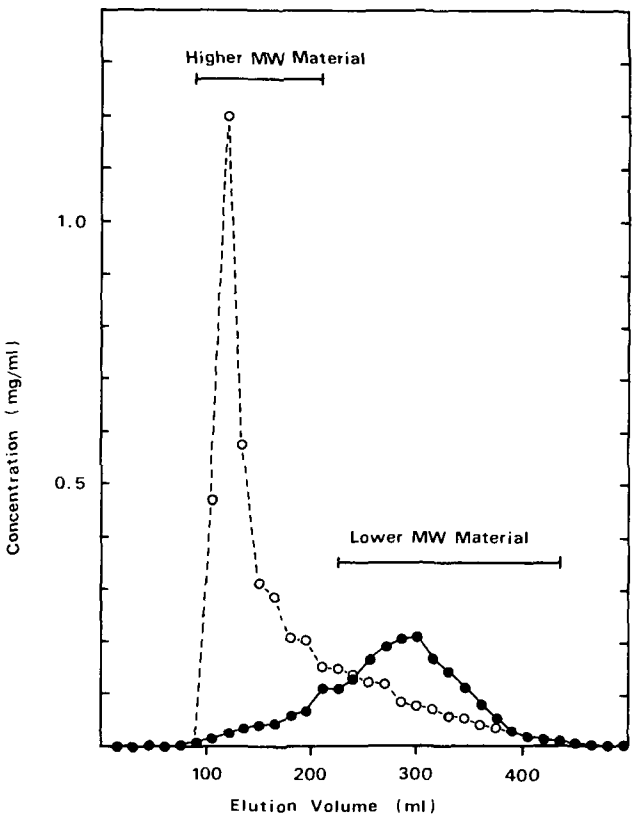

Fig. 2. Fractionation of fraction 5 by gel chromatography on Bio-Gel P-150. ● = Protein, o = carbohydrate.

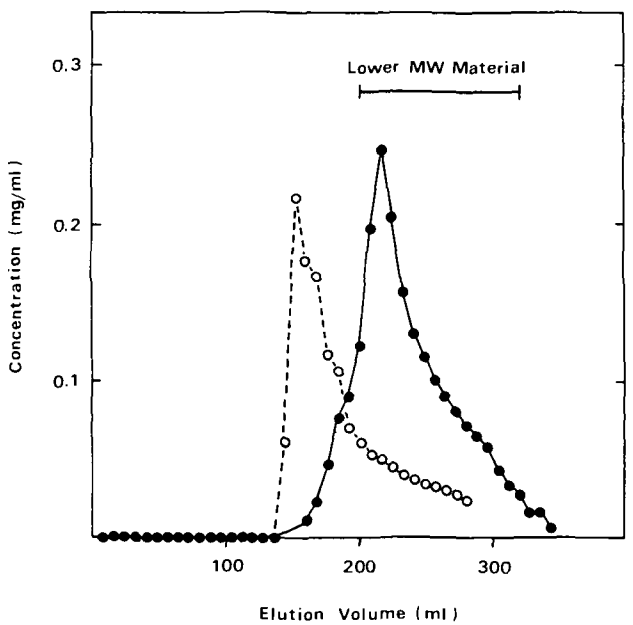

Fig. 3. Purification of the lower molecular weight material of fraction 5 by gel chromatography on Sephadex G-50. ● = Protein, o = carbohydrate.

TABLE II
Haze-Forming Capacities of Haze-Forming Proteins

Fraction	Concentration in Beer (mg/L)	Haze-Forming Capacity,[a] EBC f.u.		
		In Beer	Reaction with Beer Polyphenols	Reaction with Catechin
Fraction 2				
Retained on carboxymethyl cellulose (haze-forming proteins I)	1,161	8.3	7.8	7.3
Unretained on carboxymethyl cellulose	21,219	0	0.4	0.1
Fraction 5				
Lower molecular weight (haze-forming proteins II)	273	3.8	6.0	2.4
Higher molecular weight	357	0	0	0
Fraction 6				
Lower molecular weight (haze-forming proteins III)	207	3.5	3.1	1.9
Medium molecular weight (haze-forming proteins IV)	118	2.3	2.8	1.6
Higher molecular weight	167	0	0	0.1
Haze-forming proteins I + II + III + IV	1,759	...	12.7	8.4
Original beer	...	...	10.0	9.5

[a] Measured at half the concentration of each fraction in beer.

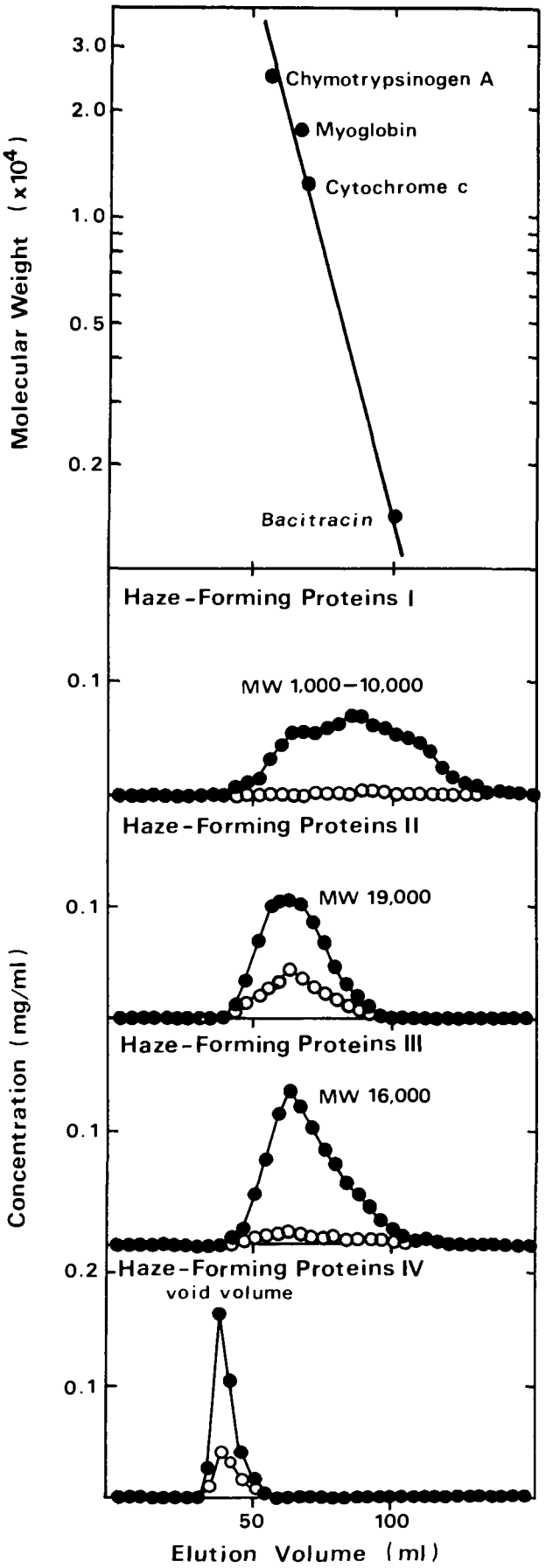

Fig. 4. Estimation of molecular weight of haze-forming proteins I, II, III, and IV by gel chromatography on Sephadex G-50. ● = Protein, o = carbohydrate.

medium molecular weight materials of fraction 6. Because these four materials, when recombined, showed a haze-forming capacity equivalent to that of the original beer, they seemed to be responsible for chill haze formation of beer, and so we named them "haze-forming proteins I, II, III, and IV," respectively.

Physical and Chemical Properties of Haze-Forming Proteins

Figures 4 and 5 show that haze-forming proteins II, III, and IV were almost homogeneous in molecular weight; their molecular weights were estimated to be 19,000, 16,000, and 40,000, respectively. On the other hand, haze-forming proteins I consisted of a number of lower molecular weight fractions with molecular weights of 1,000–10,000.

Table III shows that 69, 76, 65, and 75% of the haze-forming proteins I, II, III, and IV, respectively, were proteins or peptides. The haze-forming proteins, particularly the haze-forming proteins II, were rich in proline and glutamic acid.

On the other hand, 7, 23, 12, and 17% of haze-forming proteins I, II, III, and IV, respectively, were carbohydrates consisting mainly of glucose, especially in I, II, and III, with substantial arabinose and xylose, especially in fraction IV.

Origin of Haze-Forming Proteins

The haze-forming proteins were subjected to rocket-

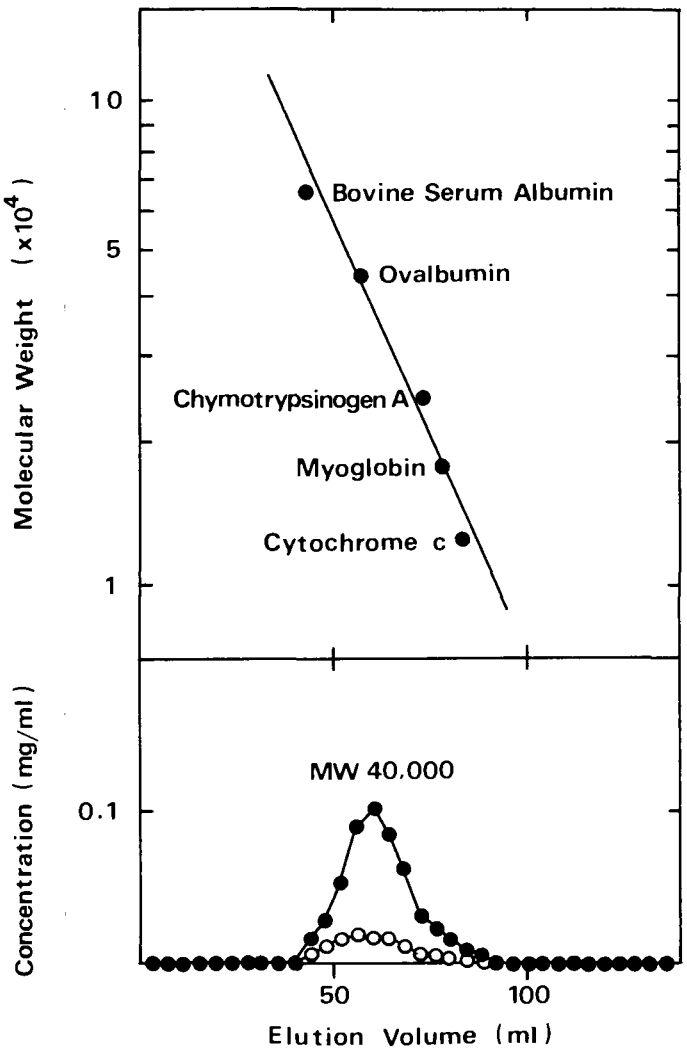

Fig. 5. Estimation of molecular weight of haze-forming proteins IV by gel chromatography on Sephadex G-75. ● = Protein, o = carbohydrate.

immunoelectrophoresis to identify their origins. As shown in Fig. 6, haze-forming proteins I and II formed immunoprecipitation peaks with antimalt-hordein serum, whereas haze-forming proteins III and IV formed immunoprecipitation peaks with both antimalt-hordein and antimalt-albumin and antimalt-globulin sera. None of the haze-forming proteins reacted with antirice-albumin nor antirice-globulin sera. These results suggest that the haze-forming proteins originated mainly from malt-hordein.

TABLE III
Chemical Composition of Haze-Forming Proteins

	Haze-Forming Proteins				Malt	
	I	II	III	IV	Hordein[a]	Albumin and Globulin[a]
Protein content (%)	69	76	65	75	...	...
Amino acid composition of protein (mol %)						
Gly	9.3	4.7	9.2	7.5	1.8	8.7
Ala	6.1	3.1	7.4	8.6	2.1	8.6
Val	3.6	2.2	5.5	6.4	3.1	6.4
Leu	3.3	2.3	5.3	8.3	4.7	6.5
Ile	2.2	2.3	2.8	3.4	2.8	3.2
Ser	4.6	3.4	5.9	7.5	3.3	6.0
Thr	0.4	2.0	4.1	3.9	1.4	3.5
Cys	1.2	0.9	1.9	0	0.6	1.5
Met	0.7	0.5	1.1	0	0.6	1.7
Phe	1.2	2.6	1.5	4.1	4.4	2.7
Tyr	1.6	2.0	1.7	1.1	1.6	2.7
Pro	5.5	19.9	10.3	8.7	18.2	7.3
Asp	6.6	3.0	8.5	6.6	1.2	9.3
Glu	12.1	20.9	14.3	14.2	29.1	9.6
Lys	3.9	1.3	2.5	3.3	0.3	3.6
Arg	4.2	1.4	3.6	3.4	1.3	4.3
His	2.3	0.6	2.7	3.4	0.9	1.7
Carbohydrate content (%)	7	23	12	17	...	...
Constituent sugars of carbohydrate (mol %)						
Ara	5	13	18	48	...	...
Xyl	5	13	19	25	...	...
Glc	79	66	60	21	...	...
Man	trace	trace	3	6	...	...
Gal	2	trace	trace	trace	...	...

[a] Albumin and globulin were extracted from malt with 0.5*M* NaCl, and hordein was then extracted with 70% ethanol.

Combination of Haze-Forming Proteins with Polyphenols

The mechanism of chill haze formation of the haze-forming proteins with polyphenols was examined in a model system composed of the haze-forming proteins (500 mg/L) and catechin

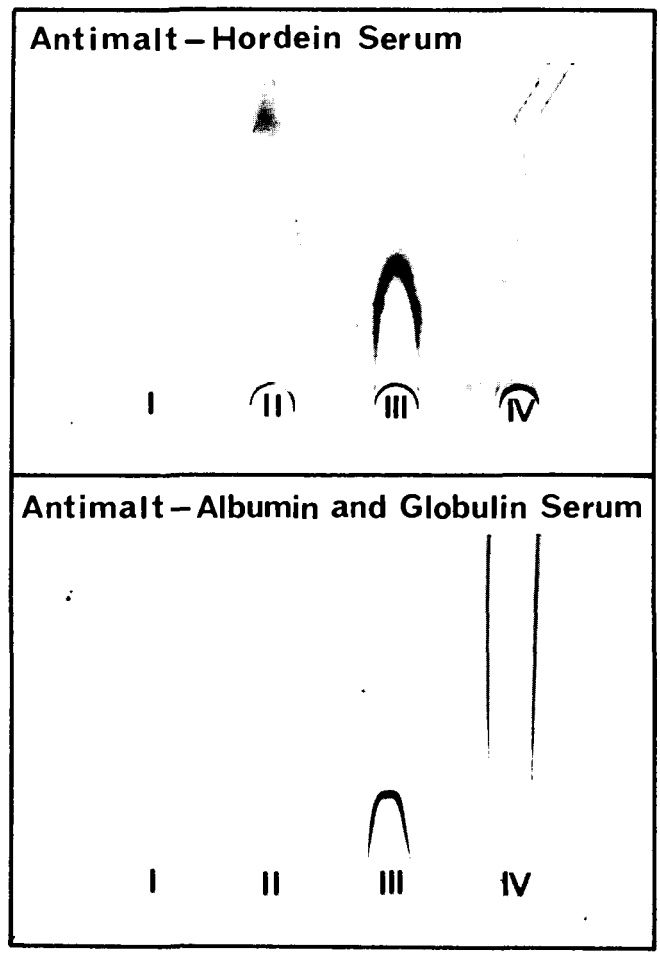

Fig. 6. Immunoelectrophoretic analysis of haze-forming proteins. Five hundred micrograms of haze-forming proteins I, II, and III and 200 μg of haze-forming proteins IV were subjected to rocket-immunoelectrophoresis with 64.1 μl/cm^2 of antimalt-hordein and 12.8 μl/cm^2 of antimalt-albumin and globulin sera. I, II, III, and IV represent haze-forming proteins I, II, III, and IV, respectively.

TABLE IV
Combination of Catechin with Haze-Forming Proteins

	Proline Content (mol %)	Haze-Forming Capacity (EBC f.u.)	Catechin Combined with Protein (mol/10^{4}g protein)	Inhibition of Chill Haze Formation		
				25% *N,N*-Dimethyl formamide	25% Dioxane	5% NaCl
Haze-forming proteins						
I	5.5	6.7	0.61	+	+	–
II	19.9	23.3	2.86	+	+	–
III	10.3	13.2	1.66	+	+	–
IV	8.7	10.1	1.52	+	+	–
Poly-L-proline	100.0	113.9	8.09	+	+	–
Gliadin	15.2	58.7	1.66	+	+	–
Papain	4.7	2.7	0.30	+	+	–[a]
Lysozyme	1.6	0	0	...	...	...
Poly-L-glutamic acid	0	0	0	...	...	...
Poly-L-lysine	0	0	0	...	...	...
Poly-L-serine	0	0	0	...	...	...

[a] Insoluble in 5% NaCl.

(400 mg/L) in 0.02*M* sodium phosphate buffer, pH 4.2, containing 3.6% ethanol. The solution containing the haze-forming proteins and catechin was heated at 100° C for 20 min and then chilled at 0° C for 40 min. The chill haze was measured with a Zeiss-Pulfrich photometer and then removed by centrifugation at 18,000 rpm at 0° C for 20 min. The content of catechin in the supernatant was measured according to Analytica EBC (3), and the amount of catechin combined with the haze-forming proteins was calculated from the decrease in the level of catechin in the supernatant. Table IV shows that haze-forming proteins II and to a lesser extent, haze-forming proteins III, IV, and I, combined with catechin to form chill haze. Because the amount of catechin combined with the haze-forming proteins correlated well with the content of proline in the haze-forming proteins ($r = 0.976$), proline seemed to be essential for the combination of the haze-forming proteins with catechin. To confirm this, we examined the combination of proteins of different proline contents with catechin in a similar way. As shown in Table IV, proline-rich proteins, particularly poly-L-proline, combined with a large amount of catechin to form a large amount of chill haze, whereas proteins with little or no proline, such as lysozyme and poly-amino acids other than poly-L-proline, did not combine with catechin and so did not form chill haze. A significant correlation was also obtained between the content of proline in the proteins and the amount of catechin combined with these proteins ($r = 0.998$).

The formation of chill haze by the combination of the haze-forming proteins with catechin was inhibited by the hydrogen bond acceptors such as *N,N*-dimethylformamide, as well as by nonpolar solvents such as dioxane, but was not inhibited by an ionic bond acceptor such as NaCl. These results suggest that hydrogen bonding and/or hydrophobic bonding were responsible for the combination of haze-forming proteins with catechin.

Participation of Haze-Forming Proteins in Chill Haze Formation During Storage of Beer

Chill haze was separated from beer stored at 50° C at intervals of three to 14 days, and its chemical composition and origin were analyzed. Table V shows that chill hazes contained 31–50% protein, 13–17% polyphenol, and 39–57% carbohydrate. Although the levels of most amino acids in chill haze did not change appreciably throughout the storage period, the level of proline in chill haze was highest initially and then decreased with increase in the storage period.

Figure 7 shows the immunoelectrophoretic profiles of chill hazes. The chill haze developed in the early stage of storage contained significant amounts of hordein fractions of malt, but their level decreased with increase in the storage period. In contrast, the level of albumin and/or globulin fractions of malt in chill haze was very low initially, but increased with increase in the storage period, and then decreased.

Figure 8 shows that the foaming proteins (1), which originated mainly from malt-albumin, globulin, or both, also participated in chill haze after long storage. Therefore, beers from which chill hazes had been removed gradually lost their head-forming capacities.

DISCUSSION

We isolated the haze-forming proteins responsible for chill haze of beer and obtained clear evidence that they originated mainly from malt-hordein and had specific affinities for polyphenols.

Because of the high contents of proline and glutamic acid of chill haze, the involvement of malt-hordein in chill haze was suggested (13,15), but the immunological studies of Grabar and Daussant (4) showed that the albumin and globulin fractions of malt were

TABLE V
Development of Chill Haze and Its Chemical Composition

	No. of Days						
Storage Period of Beer at 50°C	**0–3**	**4–7**	**8–14**	**15–28**	**29–42**	**43–56**	**57–70**
Chill haze developed in beer, EBC f.u.	10.0	15.0	17.0	16.7	16.2	14.5	13.2
Chill haze							
Amount (mg/L·beer)	29.1	30.8	42.7	57.9	46.1	35.0	23.8
Protein content (%)	31	42	35	43	49	48	50
Amino acid composition of protein (mol %)							
Gly	6.2	9.8	8.6	6.4	8.0	8.4	8.9
Ala	2.7	5.6	4.7	3.6	5.4	6.1	6.2
Val	3.7	4.3	3.9	4.3	4.2	4.4	4.2
Leu	3.8	4.9	4.3	4.5	4.5	4.9	4.9
Ile	1.9	2.3	2.1	2.1	2.4	2.6	1.7
Ser	4.7	6.1	5.6	6.2	5.3	5.2	5.2
Thr	2.6	3.6	3.2	3.2	2.8	2.9	2.9
Cys	0	1.1	1.0	1.2	1.1	0.1	1.1
Met	trace	0.8	0.7	0.7	0.7	0.2	0.6
Phe	1.0	2.3	2.3	2.4	2.1	2.1	2.0
Tyr	1.5	2.3	2.2	2.4	1.7	1.2	1.7
Pro	13.6	7.9	8.3	8.3	6.2	5.9	6.9
Asp	5.4	7.4	6.7	6.7	7.2	7.0	7.6
Glu	14.3	12.2	11.3	11.3	10.2	11.2	12.6
Lys	1.9	2.8	2.5	2.4	2.3	2.7	3.1
Arg	2.4	2.9	2.6	0.7	2.7	2.8	3.1
His	1.1	1.4	1.3	1.4	1.5	1.5	1.5
Polyphenol content (%)	17	15	16	13	15	16	15
Carbohydrate content (%)	50	57	46	48	42	39	51
Constituent sugars of carbohydrate (mol %)							
Ara	4.1	3.7	3.5	3.4	3.6	3.0	3.6
Xyl	3.8	3.5	3.1	3.1	3.6	3.3	3.6
Glc	86.4	84.2	87.5	87.1	86.1	89.2	88.5
Man	2.0	3.2	3.0	3.6	3.2	1.7	1.4
Gal	0.8	0.7	0.5	0.3	0.5	0.4	0.5

mainly responsible for chill haze. Our chemical and immunological studies on the haze-forming proteins and chill hazes confirmed that hordeins are important in chill haze formation. Because the antigenic activity of hordein is much weaker than that of albumin or globulin, as shown in this work, the antiserum prepared by Grabar and Daussant (4) seemed to have low activity for hordein and thereby did not react with hordein appreciably.

The haze-forming proteins derived from hordein were rich in proline and this amino acid seemed to be responsible for the specific affinity for polyphenols. As shown in Fig. 9, combinations of proteins with polyphenols are generally thought to result from: hydrogen bonding between oxygen atoms of peptide bonds and hydroxyl groups of polyphenols; hydrophobic bonding between hydrophobic amino acids such as proline, tryptophan, phenylalanine, tyrosine, leucine, isoleucine, and valine and the hydrophobic ring structure of polyphenols; and ionic bonding between positively charged groups of proteins, such as the ϵ-amino groups of lysine, and negatively charged hydroxyl groups of polyphenols. But in acidic conditions, such as in beer, hydroxyl groups of polyphenols have no charge and therefore ionic bonding is not involved in the combination of the haze-forming proteins with polyphenols.

Because of the pyrrolidine ring of proline, the proline-rich haze-forming proteins have unfolded molecular structures that facilitate the entry of polyphenols into them. Furthermore, as shown in Fig. 9-(1), the pyrrolidine ring of proline cannot form intramolecular and intermolecular hydrogen bonds with oxygen atoms of peptide bonds and, consequently, these free oxygen atoms readily form hydrogen bonds with hydroxyl groups of polyphenols. In addition, proline is a hydrophobic amino acid and thereby also participates in hydrophobic bonding between the haze-forming proteins and polyphenols. These two mechanisms of combination between the haze-forming proteins and polyphenols were probably responsible for chill haze formation of beer. Recently, Hagerman and Butler (6) also showed high affinities of proline-rich proteins for proanthocyanidins and proposed similar mechanisms for protein-proanthocyanidin interactions.

More than 10 years ago, Gramshaw (5) speculated that hordein-derived proteins in beer readily formed hazes with polyphenols and later that albumin- and globulin-derived proteins reacted with more polymerized polyphenols to form hazes, but this has not been

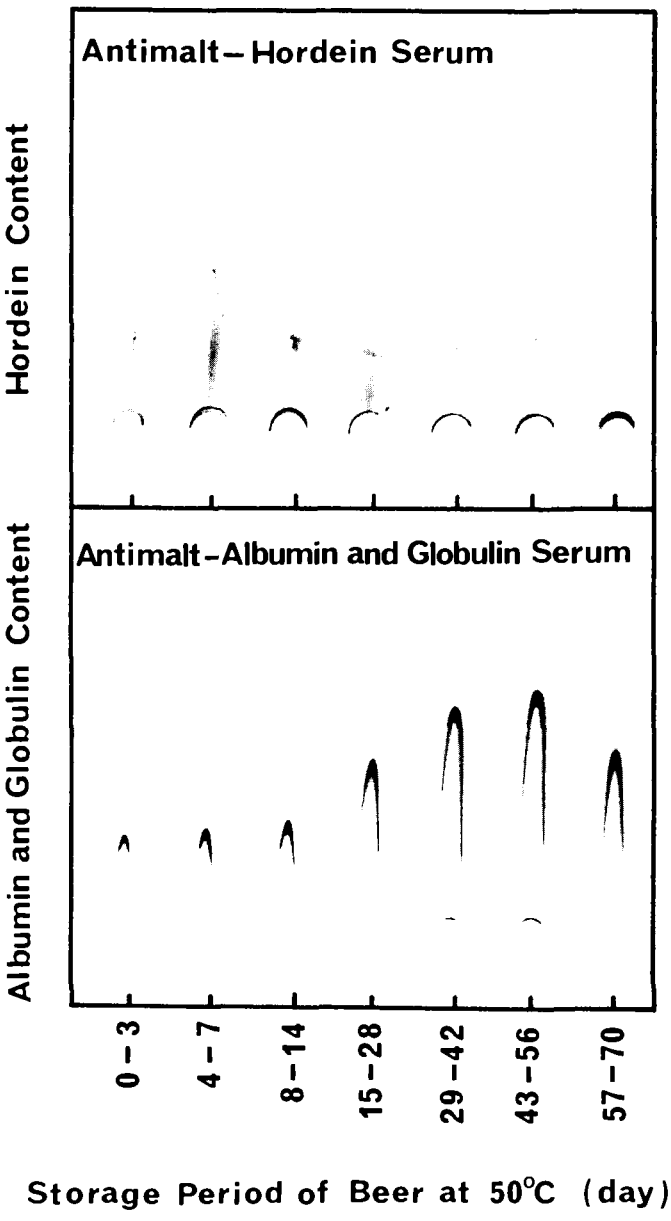

Fig. 7. Changes in contents of hordein and albumin and globulin fractions in chill haze. Chill hazes (100 and 50 μg) were subjected to rocket-immunoelectrophoresis with 64.1 and 12.8 μl/cm^2 of antimalt-hordein and antimalt-albumin and globulin sera, respectively.

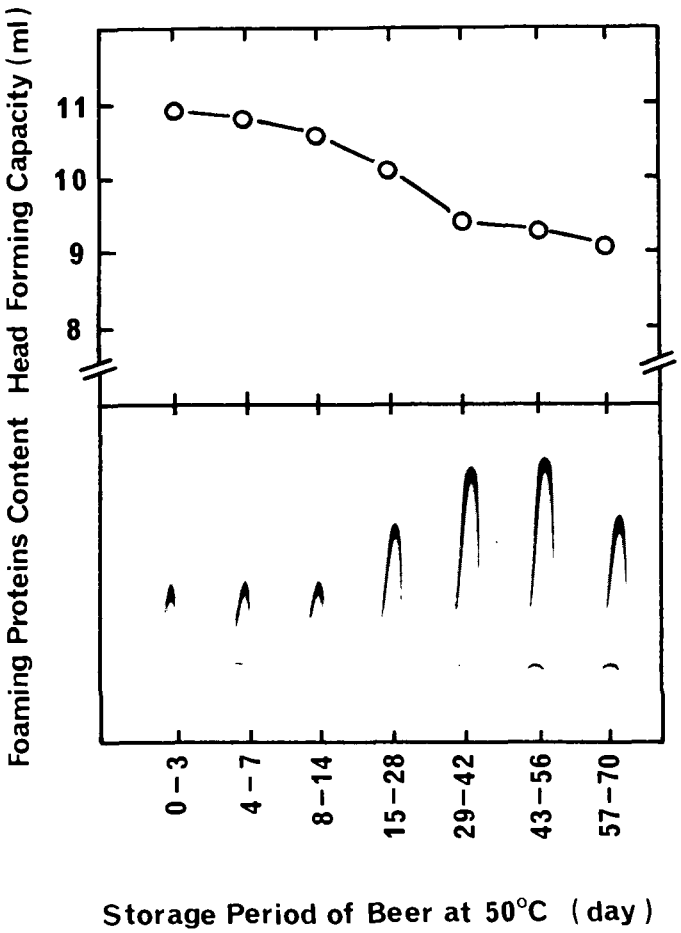

Fig. 8. Changes in contents of foaming proteins in chill haze. Chill haze 50 μg was subjected to rocket-immunoelectrophoresis with 12.8 μl/cm^2 of antifoaming protein serum.

demonstrated until now. We also obtained evidence that beer proteins derived from malt-hordein, such as the haze-forming proteins, preferentially form chill haze because of their high affinities for polyphenols, but that after these proteins were removed as precipitates, proteins derived from albumin or globulin or both, such as the foaming proteins (1), also participated in chill haze formation. Because chill hazes developed in the early stage of storage considerably shorten the shelf-life of beer, it is particularly important to reduce the level of haze-forming proteins in beer.

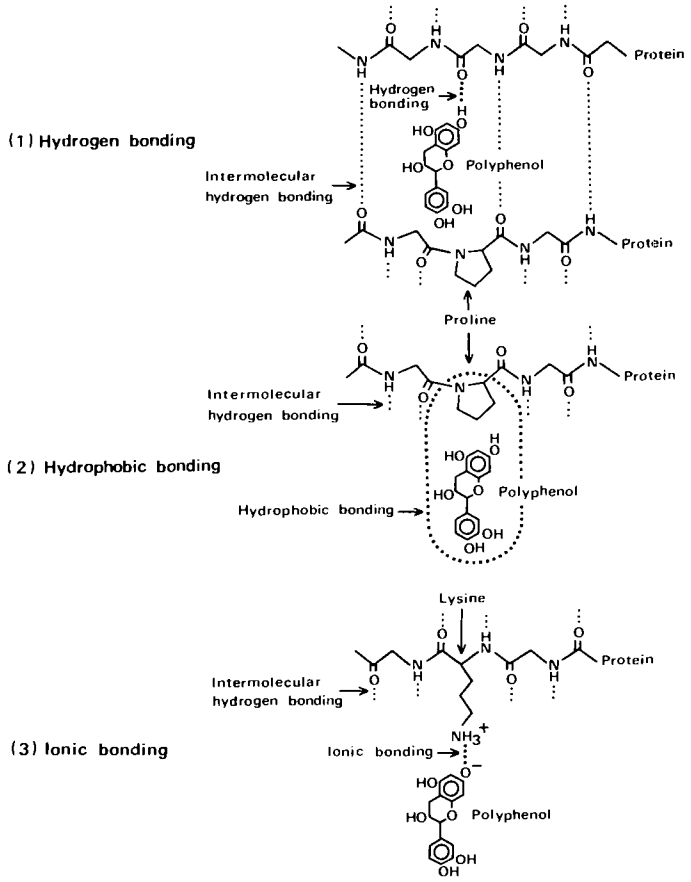

Fig. 9. Mode of combination of proteins with polyphenols.

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