Interaction between Protein and Starch[1]

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ABSTRACT

The electrolytic conductance of mixed solutions composed of 5 ml. of alpha-casein solution and different levels of acidified potato starch solution was determined. When the results were represented by a straight line, an intersection appeared on the figure of electrolytic conductance against the volume of the starch solution. The results agreed well with the hypothesis of Terayama (J. Chem. Soc. Japan 78: 1261; 1957), who proposed that the equivalent point of opposite charges of two high-polymer ions could thus be shown. The equivalent value obtained by the electrolytic conductance measurement agreed well with the equivalent value calculated from the charges of the protein and the starch, defined by the colloid titration method. The combination of the protein and the starch by their opposite charges was thus verified.

This report is concerned with electrostatic interactions between protein and starch. These interactions have an important role in the rice fermentation industry in the brewing of sake and may also have some influence on bread dough.

Occasionally, the solution of some cereal proteins by acidic aqueous media or enzymatic hydrolysis is interfered with by the coexistence of protein and starch gel. To understand the mechanism of this interference, it is necessary to understand the nature of the attractive forces between starch and proteins in solution. Previously we supplied evidence concerning these interactions and two attributable forces by which the protein combined with the starch gel (1–5); these forces are of electrostatic and Van der Waals character. Van der Waals force was confirmed from studies of adsorption of protein and starch in aqueous solution (4). However, the study is not discussed in detail because this paper is concerned only with electrostatic forces.

An electrostatic interaction between protein and starch in acidic condition was confirmed by viscometric (2) and colloid titration methods (5).

An Ostwald-type capillary viscometer ($V = 2.10$ cc.; $r = 1.53 \times 10^{-2}$ cm.; $l = 10.48$ cm.; $[R] = 12.4$; $h = 15.1$ cm.; $k = 1.14 \times 10^{-3}$; flow time of water 698.8 sec. at 20 ± 0.01°C.) was employed to determine the ratio of the various flow times, where V is volume of the viscometer, r is radius of the capillary, l is length of the capillary, [R] is Reynolds number, h is average height of a liquid in the capillary, and k is correction of kinetic energy (6,7).

The ratio $\eta - \eta_0/\eta_0$ will be referred to as the specific viscosity, η_{sp}, of the solution, where η is the viscosity of the solution and η_0 the viscosity of the solvent. The intrinsic viscosity, $[\eta]$, is defined by the relationship,

$$[\eta] = \lim_{C \to o} \eta_{sp}/C,$$

where C is the concentration of total solid expressed in g. per 100 ml. of solution. Intrinsic viscosity of a mixed solution of two high polymers can be described by the following Mark and Whitbye equation (8),

$$[\eta] = \sum_{i}^{\eta} [\eta]_i w_i \qquad (1)$$

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where $[\eta]_i$ is the intrinsic viscosity of component i, and w_i is its weight fraction in the mixture. Conversely, when there are interactions between components, the measured and calculated values of $[\eta]$ will differ. This relationship has been shown to be valid for a mixed solution of protein and starch (2).

The intrinsic viscosity of each solution of protein and starch was measured and calculated by eq. 1. Good agreement was obtained between measured and calculated results at pH 6 (with alpha-casein) and pH 10.5 (with rice protein). However, at pH 3 the measured value was greater than calculated. These results might be interpreted as an indication of an interaction between protein and starch in acidic condition. Furthermore, the magnitude of interaction indicated that the volume of components seemed to be expanded.

Using an empirical equation which Fuoss and Strauss (9) developed for polyelectrolytes,

$$\eta_{sp}/\sqrt{c} = A + B\sqrt{c} \tag{2}$$

where c is the concentration in g. per 100 cu. cm. and A and B are coefficients of components, we plotted $\eta_{sp}/\sqrt{c}$ vs. $\sqrt{c}$ of protein or starch and obtained a linear relationship, which is a characteristic of a polyelectrolyte. Therefore, it is concluded that both protein and starch are typical polyelectrolytes and that the interaction in acidic condition must be caused by the attraction of opposite charges which are caused by the dissociation of some ionizable groups of the components.

The equivalence of electrostatic charges of protein and starch, in suspension, was investigated by the colloid titration method (5). This method involves titration of oppositely charged polymers in the presence of a metachromasic dye, such as toluidine blue, as indicator of the end point. (Metachromasy is the phenomenon of a change in color of a dye when combined with macromolecular ions.) Thus, toluidine blue, a basic dye, in combination with a minute excess of the negative polymer ion, changes from blue to a reddish purple at the equivalence point. The principle of the method is based on a stoichiometric combination between positively and negatively charged polymer ions, and the equivalence of the net charge can be estimated from that of a standard polymer. In our experiment, the potassium salt of polyvinylalcohol sulfate (PVSK) was used in the titer solution, and N-methylated chitosan iodide (macramin) was the standard positively charged polymer.

In titration of polymer ions, normality of the titer solution is defined by the number of equivalents of the dissociable groups of the polymer in 1 liter of solution. Mean molecular weight of the negative colloid (PVSK) was calculated from the sulfur content by the procedure of Krober and Howell (10).

In the experiments, 5 to 30 ml. of a solution of protein or starch, or mixture thereof, was mixed with 5 ml. of known concentration (normality) of a standard colloid solution (macramin). The pH of the mixture was then adjusted with either lactic acid or sodium hydroxide solution, and diluted to 40-ml. volume with water whose pH was also previously adjusted to that of the mixture. One drop of 0.05% toluidine blue solution was added as indicator, and the mixture was titrated with the standard colloidal solution of PVSK until the indicator changed color. After true pH was determined with a glass electrode, g. equivalence per unit g. of the sample was calculated by the following equation:

$$\text{Equivalence (g.)} = \frac{\text{normality of PVSK} \times \text{difference in titer}}{\text{amount of material (g.)}} \quad (3)$$

The g. equivalence per g. was then plotted against pH. The net charge of the mixed solutions of the protein and starch, at various pH values, were compared with the values calculated from each net charge of the protein and the starch, which had been predetermined by the same method. Both measured and calculated values coincided on the same curve. Thus, each component had a charge. However, because the titrating mixture consisted of four ionized polymers, including two kinds of standard polymers, protein and starch, it was not certain that the coincidence of measured and calculated values unequivocally established interaction between starch and protein. Moreover, it was impossible to titrate protein solution with starch suspension directly, because the combination did not manifest metachromasy at the end point. Therefore, it was necessary to sum the effects of two oppositely charged standard polymers.

Terayama (11) proposed that the equivalence point of two oppositely charged polymer ions could be determined from the intercept when electrolytic conductances of mixed solutions of the two polymers were plotted against concentration of either of the polymers. The value obtained by this method showed a remarkable coincidence with the one obtained by the colloid titration method.

MATERIALS AND METHODS

Alpha-Casein

The alpha-casein used in this study was prepared from Casein nach Hammersten (Merck) by the technique described by Hipp et al. (12). Two solutions containing 2 g. of alpha-casein were prepared. One casein preparation had 0.440 mg. N per ml. and was used for the calculations in "Results," sections 1 to 3; the other solution contained 0.480 mg. N per ml. and was used for "Results," section 4. Caseins were dissolved in 130 ml. of 0.01N hydrochloric acid by heating on a water bath; after cooling they were adjusted to pH 4.00 with 0.01N sodium hydroxide, then filtered through a No. 3 glass filter.

Potato Starch

Commercial potato starch was washed three times with distilled water and twice with methanol, and dried in air. Two solutions containing 4 g. of potato starch were prepared as follows: after boiling for 3 min. in 450 ml. of distilled water and cooling, the slurries were homogenized in a Waring Blendor, adjusted to pH 4.00 with 0.01N hydrochloric acid and 0.01N sodium hydroxide, and filtered through a No. 3 glass filter. One of the starch solutions contained 11.9 mg. of total reducing sugar as glucose per ml., and was used for the calculations in "Results," sections 1 to 3; the other solution (section 4) contained 11.7 mg. of total reducing sugar per ml.

Standard Polymers

N-Methylated chitosan iodide (macramin), as the standard positive colloid, and potassium salt of polyvinylalcohol sulfate (PVSK) as the standard negative colloid, were prepared as described by Yoshino and Matsumoto (13) and Terayama (14,15).

Preparation of Standard Solutions of Polymers and Samples

Macramin (1.213 g.) and PVSK (0.601 g.) were dissolved in 900 ml. of water and adjusted to pH 4.00 with 0.01N hydrochloric acid, and each solution was made up to 1,000 ml. with water at pH 4.00. The normality of macramin was 9.850×10^{-4} and that of PVSK was 14.096×10^{-4}. These solutions were used in "Results," sections 1 to 3.

Data for "Results," section 4, were obtained with macramin concentrations of 255.2 mg. per 1 (2.073×10^{-4} N) and 239.6 mg. per 1 of PVSK (5.619×10^{-4} N).

Colloid Titration Method

This method was reported in previous papers (5,13,14,15).

Electrolytic Conductance Measurement

Electrolytic conductance was determined on aliquots of a polymer solution, at pH 4.00, which contained a known volume of the other polymer in a total volume of 50 ml. of aqueous solution using an alternating-current bridge (50-cycle) and parellel-plate electrode of platinum black. Error of determination was less than 1%.

Determination of pH

A Hitachi-Horiba F-5-type pH meter (Hitachi Seisaku-sho Co., Ltd., Japan) was employed for determination of pH. Error of determination was less than ±0.002.

Preparation of Mixed Solutions of Two Polymers

Mixed solutions of polymers for electrolytic conductance measurement and adjustment of pH were prepared as shown in Table I.

TABLE I. PREPARATION OF MIXED SOLUTIONS OF TWO HIGH POLYMERS

	Solution Nos.							
	For Electrolytic Conductance Measurement[a]				For pH Measurement			
	1 ml.	2 ml.	3 ml.	4 ml.	1 ml.	2 ml.	3 ml.	4 ml.
Macramin	15	...	0.5–10	...	15	...	0.5–10	...
PVSK	1–20	1–20	...	...	1–20	3–27	...	...
Alpha-casein	...	20	...	5	...	15	...	10
Potato starch	...	...	30	5–40	...	...	60	10–100

[a]Made up to 50 ml. with water.

RESULTS AND DISCUSSION

The experiments reported herein involved the method employed by Terayama (11) for determining the equivalence point between oppositely charged polymers. Terayama hypothesized that since polyelectrolytes have free and bound ions (16), the admixture of oppositely charged polyelectrolytes results in formation of a complex through neutralization of charges, and there is a concomitant release of ions which are bound to both polymers. These free and newly released counter-ions can be determined by measuring the electrolytic conductance of the mixed solution. From a plot of the electrolytic conductance of the mixed solution against volume of added polymer solution, we can expect an inflection of the line at the equivalence point.

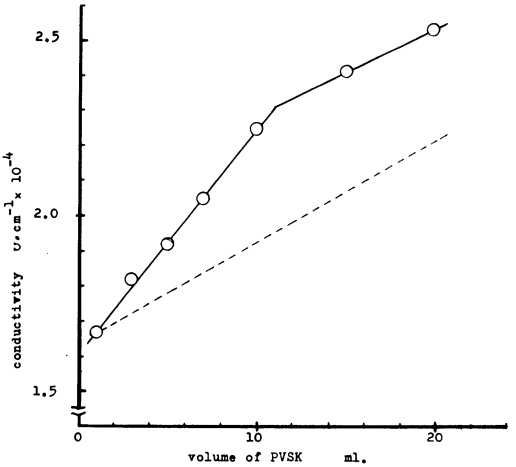

Fig. 1. Relation between the electrolytic conductance of a mixed solution of two polymers and the volume of either polymer solution. Polymers: macramin, 9.850 X 10^{-4}N, 15 ml.; PVSK, 14.096 X 10^{-4}N, 1 to 20 ml.; made up to 50 ml. with water, pH 4.00. pH 4.00: broken line indicates tangent of PVSK when electrolytic conductance is plotted against concentration.

1. Results with N-Methylated Chitosan Iodide and Potassium Salt of Polyvinylalcohol Sulfate

These results are shown in Fig. 1, where there is discontinuity in the electrolytic conductance at a volume of 10.6 ml. of PVSK. This value agrees well with the calculated theoretical to neutralize 15 ml. of macramin:

$$\frac{9.850 \times 10^{-4}\ (\mathrm{N}) \times (15/1{,}000)}{14.096 \times 10^{-4}\ (\mathrm{N}) \times (1/1{,}000)} = x$$

$$x = 10.48\ (\mathrm{ml.})$$

where 9.850 was the normality of the macramin, 14.096 the normality of the PVSK, and x the volume of the PVSK for neutralization. There was also good agreement with the colloid titration method where 10.55 ml. of PVSK was required to neutralize 15 ml. of macramin. These three values coincided well with each other, and the results support Terayama's hypothesis.

The data in Table II show that pH did not change more than 0.005 unit upon addition of PVSK to macramin. This observation indicates that an increase in electrolytic conductance due to release of the bound ions of the polymers is accompanied by a change in pH of the mixed solution. This situation can arise if freed counter-ions of polymers were hydrogen *and* hydroxyl, so they may combine to form water molecules immediately and the increase of released ions would not be related to electrolytic conductance. However, if counter-ions of either polymer were hydrogen *or* hydroxyl ions, and counter-ions of another polymer were of macramin or PVSK, the release of bound counter-ions by mixing of the two polymers may affect the pH of the mixed solution.

TABLE II. pH VALUES OF MIXED SOLUTIONS OF MACRAMIN (15 ml.) AND "PVSK"[a]

PVSK	pH of Mixed Solution	0.002N NaOH[b]	PVSK	pH of Mixed Solution	0.002N NaOH[b]
ml.	ml.	ml.	ml.	ml.	ml.
2	4.000	0.00	12	4.000	0.00
4	3.990	0.05	14	3.990	0.05
6	3.995	0.00	16	3.995	0.00
8	3.993	0.05	18	3.995	0.00
10	3.996	0.00	20	3.996	0.00

[a]Macramin: 9.850 X 10^{-4}N, pH 4.00; PVSK, 14.096 X 10^{-4}N, pH 4.00.
[b]Volume of alkaline solution necessary for adjustment of pH value of a mixed solution to pH 4.00.

2. Results with Alpha-Casein and Potassium Salt of Polyvinylalcohol Sulfate

The results (Fig. 2) differ markedly from the data in Fig. 1. If Fig. 2 is represented by a smooth curve, it resembles the type IV adsorption isotherm of Brunauer et al. (17,18). However, by drawing straight lines, we observe three inflections at volumes of 3.2, 6.5, and 11.0 ml. of PVSK. It is difficult to determine which of the three intercepts is the correct value of the equivalence point, but a value of 3.4 ml. of PVSK was obtained by colloid titration. Because the net charge of a protein varies with the pH of the solution, it is supposed that in acidic media the protein has hydroxyl ions as free ions and these are released when the protein combines with PVSK, which has potassium ions as free and bound ions. The presumption that released hydroxyl ions must have an influence on the pH values of the mixed solutions was confirmed by measuring the pH of the mixed solutions. The results (Table III) showed that at low levels of PVSK, the pH value of mixed solutions was increased, whereas at volumes greater than 12 ml. of PVSK there was only a slight increase in pH value.

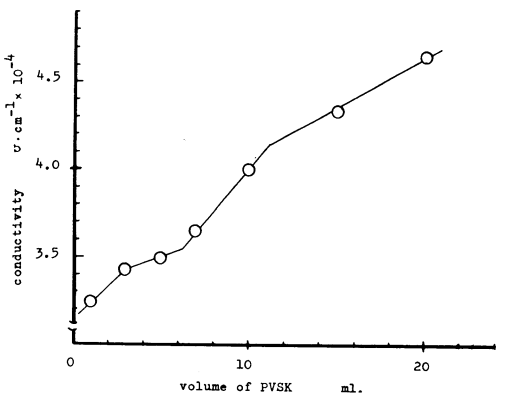

Fig. 2. Relation between the electrolytic conductance of a mixed solution of two polymers and the volume of either polymer solution. Polymers: alpha-casein, 0.440 mg. of nitrogen per ml., 20 ml.; PVSK, 14.096 X 10^{-4}N, 3 to 27 ml.; made up to 50 ml. with water, pH 4.00.

TABLE III. pH VALUES OF MIXED SOLUTIONS OF ALPHA CASEIN (15 ml.) AND "PVSK"[a]

PVSK ml.	pH of Mixed Solution	0.002N HCl[b] ml.	PVSK ml.	pH of Mixed Solution	0.00 HC ml
3	4.36	1.30	18	4.07	0.6
6	4.45	1.85	21	4.04	0.4
9	4.13	1.15	24	4.03	0.3
12	4.06	0.55	27	4.03	0.2
15	4.07	0.65			

[a]Alpha-Casein: 0.440 mg. of nitrogen per ml., pH 4.00; PVSK, 14.096 X 10^{-4}N, pH 4.00.
[b]Volume of acid solution necessary for adjustment of pH value of a mixed solution to pH 4.00.

The equivalence point may exist at approximately 3.5 ml. of PVSK; therefore, release of bound ions of alpha-casein and PVSK must occur at low levels of PVSK. The large increase in pH indicates that hydroxyl ions must be released from alpha-casein. It is not clear, however, why there is only a slight increase in pH at concentrations of PVSK which are greater than the equivalent value of PVSK. (Though a combination between polymers precedes a combination between a polymer and free ions, it is certain that a polymer can adsorb free ions.) A possible explanation may be that the hydroxyl ions which are ordinarily released at low levels of PVSK may adsorb or recombine with PVSK as its concentration is increased.

3. Results with Starch and N-Methylated Chitosan Iodide

Several different starches behaved as negatively charged polymers when we employed the colloid titration method (5); however, the procedure could not manifest the presence of counter-ions. Their presence was established by electrolytic conductance measurements (Fig. 3) where a linear relation was observed between conductance and starch concentration. These free counter-ions should be protons, whereas macramin releases iodide ions. When these ions combine, only the newly released hydrogen ions should have an influence on the pH value of a mixed solution. This hypothesis was confirmed (Table IV) where low levels of macramin caused the pH value of the mixed solutions to decrease. Thus, protons must be released. However, at higher levels of macramin, the released free

Fig. 3. Relation between the electrolytic conductance and the concentration of potato starch. Potato starch, 11.9 mg. of total reducing sugar per ml.; made up to 50 ml. with distilled water.

TABLE IV. pH VALUES OF MIXED SOLUTIONS OF POTATO STARCH (60 ml.) AND MACRAMIN[a]

Macramin ml.	pH of Mixed Solution	0.002N NaOH[b] ml.	Macramin ml.	pH of Mixed Solution	0.002N NaOH[b] ml.
0.5	3.960	0.30	4	3.990	0.10
1	3.993	0.05	5	3.995	0.05
2	3.985	0.10	7	3.985	0.10
3	3.990	0.10	10	3.990	0.10

[a]Potato starch: 11.9 mg. of total reducing sugar per ml., pH 4.00; macramin, 9.850×10^{-4}N, pH 4.00.

[b]Volume of alkaline solution necessary for adjustment of pH value of a mixed solution to pH 4.00.

ions must be adsorbed or recombined with macramin as described in section 2, above.

The electrolytic conductance of a mixture of macramin and starch at pH 4.00 is shown in Fig. 4. When the data are represented by straight lines, there are two intercepts at volumes of 1.9 and 6.8 ml. of macramin. However, the equivalent volume of macramin by the colloid titration method using macramin, starch, and PVSK was 1.7 ml.

If Fig. 4 is represented by a smooth curve, it resembles a type II adsorption isotherm. Thus the intersection of two tangents to the curve may be more suitable for determining the equivalence point rather than the intersection of straight lines.

It was very difficult to determine an end point of a titration of starch by the colloid titration method. A dye was used to indicate the end point of the titration of starch and macramin with PVSK, but metachromasy occurred very gradually and varied with the volume of the titer solution (PVSK) in the mixed solution. Thus, great care must be exercised in using the colloid titration method to determine the charge of starch.

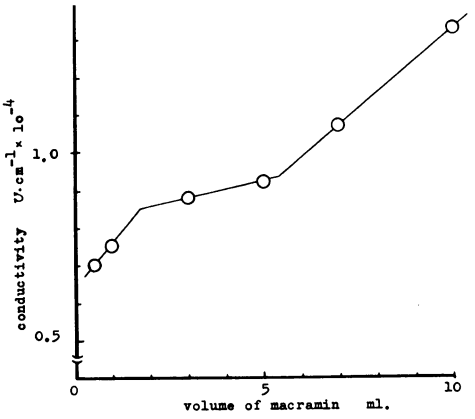

Fig. 4. Relation between the electrolytic conductance of a mixed solution of two polymers and the volume of either polymer solution. Polymers: potato starch, 11.9 mg. of total reducing sugar per ml., 30 ml.; macramin, 9.850×10^{-4}N, 0.5 to 10 ml.; made up to 50 ml. with water, pH 4.00.

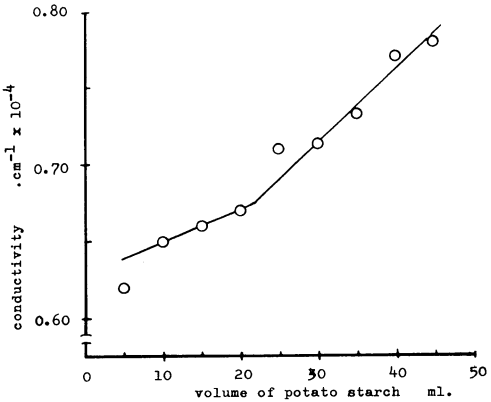

Fig. 5. Relation between the electrolytic conductance of a mixed solution of two polymers and the volume of either polymer solution. Polymers: alpha-casein, 0.480 mg. of nitrogen per ml., 1.5 ml.; potato starch, 11.7 mg. of total reducing sugar per ml., 5 to 45 ml.; made up to 50 ml. with water, pH 4.00.

4. Results with Protein and Starch

When protein was mixed with an oppositely charged starch, we found an intercept at 22.0 ml. of starch on the plot of electrolytic conductance of the mixed solution with concentration of polymer (Fig. 5). However, both protein and starch polymers have few counter-ions, i.e., hydrogen and hydroxyl, which could combine to form water molecules; so there is uncertainty as to whether the equivalent point is revealed by the intercept.

The net charge of both protein and starch was determined by the colloid titration method and expressed as a volume of PVSK. Alpha-casein had a positive net charge of 0.927 ml. of PVSK, and potato starch, 0.0633 ml. The equivalent volume of starch equal to 1.5 ml. of alpha-casein was calculated as follows:

$$\frac{\text{charge of alpha-casein} \times \text{volume of alpha-casein}}{\text{charge of starch}} = \frac{0.927 \times 1.5}{0.0633}$$

and a value of 21.97 ml. was obtained. The experimental value for the equivalent volume by electrolytic conductance agreed well with the calculated value. This observation suggests that protein-starch interaction is a charge-charge phenomenon.

SUMMARY

It was confirmed by the use of Terayama's postulate that a protein was able to combine with a starch. This hypothesis was based on the assumption that an increase in the number of released free ions can be detected by electrolytic conductance measurement, and can be used to estimate the equivalence point of two oppositely charged polymers. Since electrolytic conductance is a manifestation of the presence of counter-ions, it must be related to the interaction of the polymers. Therefore, a plot of electrolytic conductance of mixed solutions of two polymers against concentration of either polymer may correspond to an adsorption isotherm, because their interaction can be regarded as a form of adsorption between the polymers.

A search of the literature on Van der Waals adsorption of gases reveals that there

are five different types of adsorption isotherms. Our data corresponded to types I, II, and IV. Although it may have been desirable to plot our data as smooth curves so that the equivalence point would be represented as an intercept of two tangents to the curve, it was more convenient to use straight-line intercepts, such as those proposed by Terayama, and our data were in good agreement with his hypothesis. Thus, the equivalent value obtained by electrolytic conductance agreed well with that obtained by the colloid titration method and with the value calculated from the net charge of the polymers.

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