Gel Filtration of Starch–Iodine System

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The well-known starch-iodine reaction was taken up to study the applicability of gel filtration to the problem of physical chemistry. Gel filtration by the frontal analysis was carried out, the following results being obtained. (1) Soluble starch- and amylose-iodine systems give elution volumes of iodine containing constituents (V_{si} and V_{ai}, respectively) distinctly smaller than that of iodine, which decrease with increasing concentration of iodine, C, and starch, approaching the elution volume of starch. This is a direct experimental evidence of starch-iodine complex formation. (2) An equation derived for V_{si} *vs.* C and V_{ai} *vs.* C relations holds satisfactorily. The concentration of free iodine in equilibrium with the complex is calculated by the equation. (3) Presence of iodide ion facilitates the complex formation, but its decomposition is instantaneous when it is isolated from free iodine. (4) Some iodide ions are found to be incorporated in the complex. (5) Amylopectin also forms a complex with iodine, but the amount of iodide incorporated is not large. (6) Potentiometric titration of starch with iodine exhibits a similar behavior to the gel filtration measurement.

A number of studies have been made on starch–iodine complex formation by various methods such as potentiometry,[1] spectrophotometry,[2] electrophoresis[3] and X-ray diffraction,[4] but there do not seem to be many on the direct experimental confirmation of the binding between starch and iodine. The gel filtration method is suitable for such a measurement, since it enables us not only to identify the independent solute species as in the usual analytical procedure but also to study the solutes mutually in a rapid equilibrium such as

$$mA \rightleftharpoons A_m \quad \text{(a)} \qquad \text{or} \qquad A + B \rightleftharpoons AB \quad \text{(b)}$$

Case (a) has been reported for the system of aqueous solution of sodium dodecyl sulfate[5] and case (b) for polyvinylacetate–sodium dodecyl sulfate system.[6] The starch–iodine system belongs to the latter case, for which the gel filtration method is expected to give direct information on the formation of a starch–iodine complex.

Experimental

Material. Starch used included soluble starch, amylose and amylopectin. Commercial soluble starch (Wako Pure Chemicals Industries, Co., Ltd.) was used without further purification. Amylose and amylopectin were prepared from potato starch by selective precipitation with *n*-amyl and methyl alcohols.[7] Amylose and amylopectin solutions were prepared by dissolving purified sample in aqueous potassium hydroxide solution (0.5 M), neutralizing with dilute hydrochloric acid and desalting by gel filtration. Iodine was purified by repeated sublimation. Other reagents were of the commercial purest grade and were used without further purification. The gel used was Sephadex G-50 fine.

Apparatus. The apparatus used for gel filtration was similar to that described in a preceding report,[5] except for the flow cell for measuring the absorbance of blue starch-iodine mixture in place of the conductance cell. Absorbance was conventionally measured using a light source of an ordinary 40 W lamp without filter, the intensity being recorded as an electrical output of a photocell. The gel column used was Sephadex column K 15/30 (colorless) or brown glass column. The former, 1.5 cm in diameter, 30 cm long, and containing gel 11.4 cm in height, was used for the soluble starch-iodine system, and the latter, 1.1 cm in diameter, 30 cm long, and containing gel 20.0 cm in height, was used used for the amylose-iodine and amylopectin-iodine systems. The latter column was equipped with a water jacket thermostated at 30.0±0.05 °C. Soluble starch-iodine system was studied at room temperature (*ca.* 18 °C).

Method. The gel column was previously equilibrated with distilled water or 0.004 mol/l potassium iodide solution. A starch–iodine mixture of varying concentrations of starch and iodine as listed in each figure, and a constant iodide concentration of 0.004 or 0.025 mol/l was charged on top of the gel column. In order to carry out elution, the sample solution was charged in sufficient amount to ensure a wide plateau in the elution curve. The elution volume of starch, V_s, is determined by a slight depression appearing in the elution curve of soluble starch-iodine system shown in Fig. 2. The slight depression observed is further confirmed to be V_s since the outer volume of the gel as determined by blue dextran is in agreement with V_s obtained above, which would be expected from the gel used. This serves for the determination of the elution volume of amylose where the slight depression is lacking in the elution curve. The elution volume of free iodine, V_i, is determined by detecting yellow iodine eluted by the photocell. All experiments were carried out in a dark room to protect a light sensitive iodine–iodide solution.

Theoretical

When a sufficient amount of starch–iodine solution is subjected to gel filtration using an appropriate gel, a starch and starch–iodine complex, if any, moves faster than free iodine in the gel column. However, a part of starch–iodine complexes eluted in advance of free iodine decomposes and liberates starch and iodine at the front. Equilibrium is established between these substance and the remaining complexes, provided that decomposition is sufficienly rapid. As a result, starch is eluted first, followed by starch-iodine complexes and iodine. The behavior is shown schematically in Fig. 1. An imaginary elution front of starch–iodine complexes moves from left to right without decomposition (Fig. 1(A)). In this figure, □ACDH and □HDEG respectively represent the outer (or void) and inner

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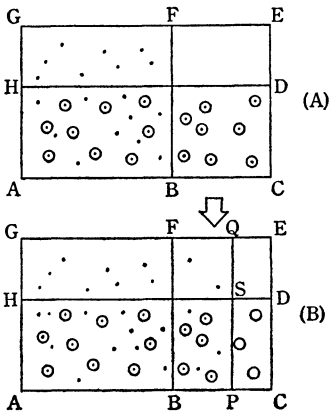

Fig. 1. Elution diagram of starch-iodine complex.
(A): Imaginary elution diagram without decomposition of starch-iodine complex
(B): Actual elution diagram
• Iodine, ⊙ Starch-iodine complex, ○ Starch
GH: Cross section area of inner space
HA: Cross section area of outer space

spaces of the gel column, AG and CE the upper and lower ends of the gel, AH and GH the cross sections of outer and inner gel spaces, CE and FB the imaginary elution fronts of starch–iodine complexes (⊙) and iodine (•). The actual front where the decomposition of the complexes takes place is shown in Fig. 1(B). The line CE indicates the front of starch (○) and PQ that of starch–iodine and iodine. The starch–iodine complexes in □SPCD decompose to liberate iodine of a constant equilibrium concentration to fill the space □FBPQ.

Such a decomposition is similar in nature to the case of the elution of sodium dodecyl sulfate above the critical micelle concentration, where the decomposition of micelles occur at the elution front.[5] Thus we obtain the equation

$$\frac{1}{V_{si}-V_s} = -\frac{1-\alpha}{(V_i-V_s)a} + \frac{C}{(V_i-V_s)a\alpha} \qquad (1)$$

from Eq. (8) for the micelle decomposition[5] by substituting $1/V_s$, $1/V_i$ and $1/V_{si}$ for R_f^m, R_f^s and R_f respectively. V_{si}, corresponding to the line PQ, represents the elution volumes of starch–iodine, V_s corresponding to EC the elution volume of starch, V_i corresponding to FB the elution volume of iodine, a the concentration of free iodine in equilibrium with starch–iodine complexes and α the rate parameter of the decomposition of starch–iodine complexes. $\alpha=1$ indicates that the decomposition of the isolated complexes is faster than the rate of elution and $\alpha=0$ no decomposition.[5] According to Eq. (1), V_{si} approaches V_s when C becomes large and C *vs.* $1/(V_{si}-V_s)$ plots are linear provided that a is constant. If the linear part of the plot when extrapolated passes the origin, we have $\alpha=1$ and decomposition of the complexes is rapid.

Results and Discussion

(1) Soluble starch. Elution volumes were determined by the sharp rise (V_{si}) and slight depression (V_s) of the elution curve shown in Fig. 2. In Fig. 3,

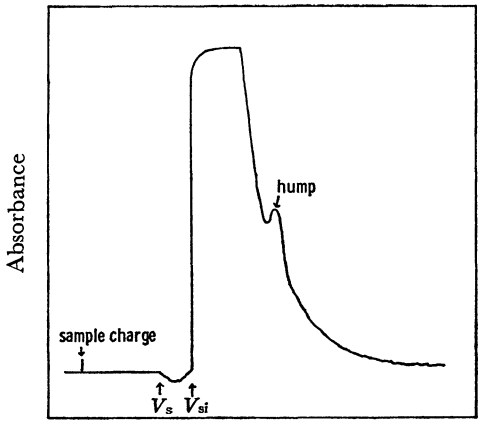

Fig. 2. The elution pattern of soluble-starch-iodine system.

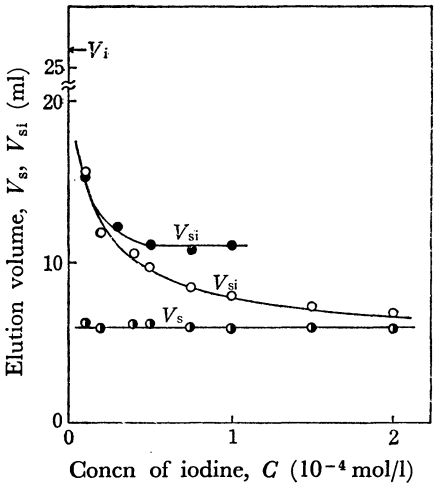

Fig. 3. V_s *vs.* C and V_{si} *vs.* C plots for soluble starch-iodine systems.
Sample solution: Concn of KI 0.025 mol/l
Concn of soluble starch ○ 0.5%, ● 0.05%
Gel column: Concn of KI 0 mol/l

V_{si} and V_s obtained are plotted against iodine concentration C. Here, the value V_s is constant (5.91 ml) as might be expected. The value V_{si}, being smaller than V_i (26.16 ml), decreases with C and approaches a constant for sufficiently large value of C. The constant is still a function of the concentration of soluble starch and approaches V_s for sufficiently high concentration as shown in Fig. 3.

The appearance of blue iodine front, V_{si}, moving faster than free iodine front is direct indication of the formation of large solute particles containing iodine, namely starch–iodine complexes. The tendency of V_{si} to approach a constant value distinctly larger than V_s for smaller starch concentration and larger iodine concentration as shown in the upper V_{si} *vs.* C curve of Fig. 3 indicates the saturation or a limit of binding capacity of iodine for starch.

The applicability of Eq. (1) was tested by plotting C against $1/(V_{si}-V_s)$. Figure 4 shows the results for soluble starch–iodine system. A straight line passing through the origin with a deviation in dilute and

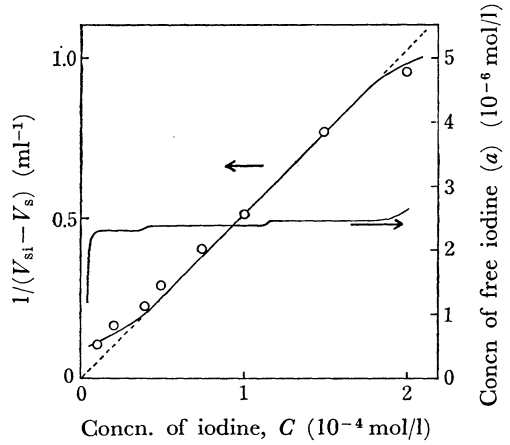

Fig. 4. $1/(V_{si}-V_s)$ *vs.* C plots and the potentiometric titration curve for soluble starch-iodine systems.
Gel filtration: Concn of KI 0.025 mol/l, V_s and V_{si} same as in Fig. 3
Potentiometry: Concn of KI 0.025 mol/l

concentrated iodine regions was obtained. The linear part shows the constancy of the concentration of free iodine in equilibrium with the complexes (a) and $\alpha=1$ obtained proves a rapid decomposition of the complexes at the elution front. From the inclination of the linear part of the plot and the values of V_s and V_i, a was calculated to be 9.6×10^{-6} mol/l.

The potentiometric titration of soluble starch (0.5%) with iodine in 0.025 mol/l KI solution was carried out for comparison. The results are shown in Fig. 4. Since soluble starch contains several hydrolysis products, the plateau part of the titration curve consists of several flat steps. The presence of hydrolysis products is also indicated in the elution curve as a hump in Fig. 2.

It is evident from Fig. 4 that the linear part of the gel filtration curve corresponding as a whole to the plateau part of the titration curve shows a constant value of a while the initial and final deviations from the linearity of the gel filtration curve indicate a smaller and larger concentration, respectively, of free iodine than a. The latter deviation also corresponds to the saturation of iodine in starch. A detailed potentiometric titration confirmed the value a to be *ca.* 2.5×10^{-6} mol/l. The smaller value as compared with the value obtained from gel filtration may be due to the absence of KI in the gel column in the former system as mentioned later.

(2) Amylose. The iodide concentration was kept constant to 0.004 mol/l, which was the largest possible concentration without the formation of amylose–iodine precipitate at high iodine concentration (2.0×10^{-4} mol/l). The gel column is equilibrated with distilled water as in the case of soluble starch. Elution curves obtained (not shown) lack the hump due to hydrolysis product shown in Fig. 2. Plots of the elution volume of blue amylose–iodine solution, V_{ai} and $1/(V_{ai}-V_a)$ against C shown in Figs. 5 and 6 respectively (called system II) resemble those of soluble starch. The observed V_a value of 4.50 ml was used. It is seen from Fig. 6 that $1/(V_{ai}-V_a)$ *vs.* C plots for different amylose concentration fall on a common straight line passing through the origin with similar deviations to

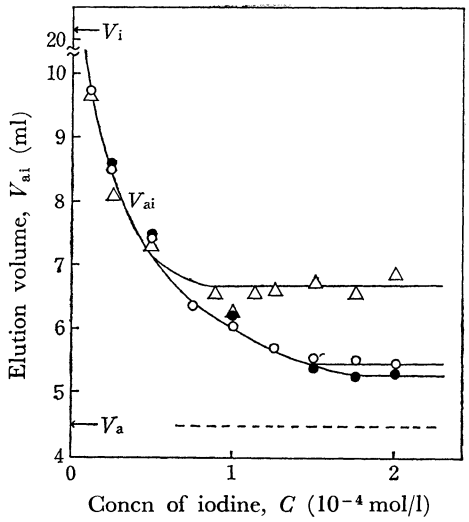

Fig. 5. V_{ai} *vs.* C plots for amylose-iodine systems.
Sample solution: Concn of KI 0.004 mol/l
Conc. of amylose 0.08%, 0.12%, 0.02%
Gel column: Concn of KI 0 mol/l

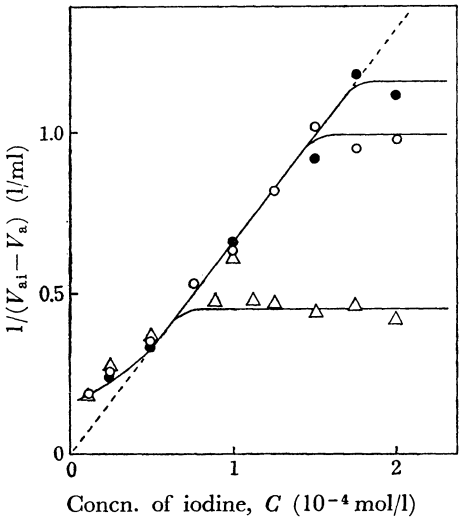

Fig. 6. $1/(V_{ai}-V_a)$ *vs.* C plots for amylose-iodine systems.
Sample solution: same as in Fig. 5
Gel column: same as in Fig. 5

those in Fig. 3.

From Fig. 5 it is clear that the constant V_{ai} for large C depends on the concentration of amylose. Such a tendency and similar behavior appearing in the potentiometric titration curve (not shown) also indicate the saturation of iodine in amylose as in the case of soluble starch. The value a calculated from Eq. (1) using the values $V_s(=V_a=4.50$ ml) and $V_i(21.17$ ml) is 9.1×10^{-6} mol/l.

While the binding of iodine with soluble starch or amylose is well accepted, the state of iodide ion in the solution has been precisely studied only in a few reports.[1,2] Although the blue amylose–iodine complexes are believed to form in the presence of iodide ions in the solution,[8] amylose–iodine complexes can be prepared without the addition of potassium iodide, *viz.*, as a solution almost free from iodide ions. Using

Table 1. Effects of iodide ion in amylose-iodine complex

System	Iodine species in solution	Gel matrix	a (mol/l)	V_{ai} at $C=1.0\times10^{-4}$ mol/l (ml)	Mol I_2 bound per g amylose at $C=1.0\times10^{-4}$ mol/l
I	I_2	—	19×10^{-6}	8.8	1.0×10^{-4}
II	I_2, I^-	—	9.1×10^{-6}	6.0	1.1×10^{-4}
III	I_2, I^-	I^-	1.1×10^{-6}	4.7	1.2×10^{-4}

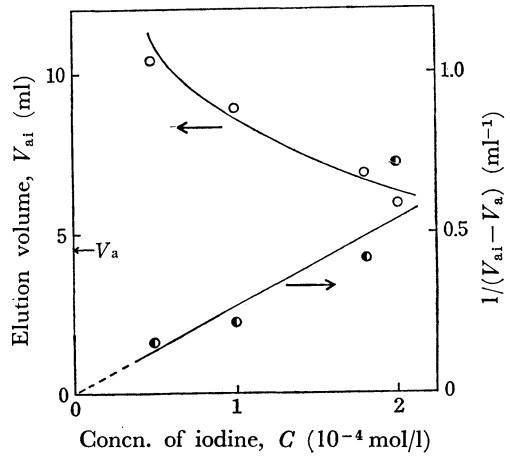

Fig. 7. V_{ai} and $1/(V_{ai}-V_a)$ *vs.* C plots for amylose-iodine systems.
Sample solution: Concn of KI 0 mol/l
Concn of amylose 0.08%
Gel column: Concn of KI 0 mol/l

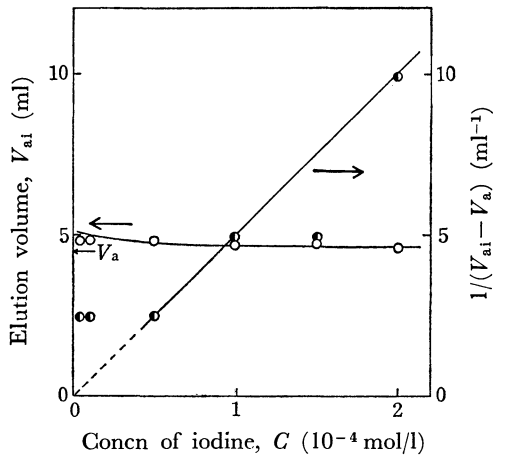

Fig. 8. V_{ai} and $1/(V_{ai}-V_a)$ *vs.* C plots for amylose-iodine systems.
Sample solution: Concn of KI 0.004 mol/l
Concn. of amylose 0.08%
Gel column: Concn of KI 0.004 mol/l

such an iodide free solution, gel filtration of 0.08% amylose was carried out under the same conditions as in system II. The result is shown in Fig. 7 (system I). The value a was calculated to be 19×10^{-6} mol/l. We further made the gel filtration of 0.08% amylose which is similar to system II except that the gel column was filled with aqueous potassium iodide solution of the same concentration as that for system II. The results are shown in Fig. 8 (system III). The value a was also calculated from the $1/(V_{ai}-V_a)$ *vs.* C plots to be 1.1×10^{-6} mol/l. As seen in Figs. 7 and 8, the plots scatter about a straight line and the value a calculated are not accurate. However, it might be certain from V_{ai} of system II that the value a of system III is smaller than that of system II.

Potentiometric determination of a was also carried out for system III, giving 1.6×10^{-6} mol/l which is in agreement with the result of gel filtration. This may be expected since the titration system resembles the gel filtration system for system III more closely than the other two systems as regards the addition of iodide ions. The results obtained from Figs. 5, 7 and 8 are summarized in Table 1. The effect of iodide ions in the solution and in the gel matrix on gel filtration of the amylose-iodide system is compared. It is seen that the values a and V_{ai} decrease as the iodide ions added becomes distributed over the solution and gel matrix. In the region of constant a, mol of iodine bound per g of amylose at a given concentration C is equal to $(C-a)/0.8$ for $C=1.0\times10^{-4}$ mol/l. We see from Table 1 that in order to obtain a nearly equal amount of iodine bound to amylose, 19×10^{-6} mol/l free iodine is

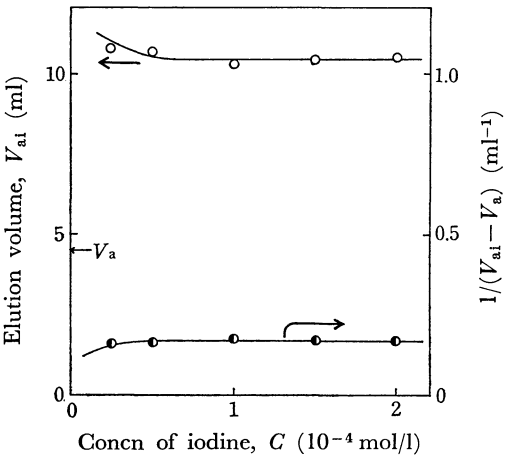

Fig. 9. V_{ai} and $1/(V_{ai}-V_a)$ *vs.* C plots for amylopectin-iodine systems
Sample solution: Concn of KI 0.004 mol/l
Concn. of amylopectin 0.08%
Gel column: Concn of KI 0 mol/l

necessary for system I and 9.1×10^{-6} mol/l for system II, but only 1.1×10^{-6} mol/l is sufficient for system III. Thus, iodide ions seem to favor the complex formation.

Iodide ions are bound to amylose in addition to iodine molecules. If amylose-iodine complexes of system II (Table 1) do not bind iodide ions, its elution front, which is in advance of the free iodide ions and is therefore exposed to the iodide free medium, is in the same condition as that elution front of the system I, and the value a should be equal to that of system I,

This, however, is not the case. Incorporation of iodide ions into amylose molecule increases iodide ions at the front and gives a lower a value compared with that of system I. Thus, iodide ions can be explained as bound to to amylose molecule.

(3) Amylopectin. Amylopectin, another component of starch, was further used for gel filtration. The result is shown in Fig. 9. For a wide range of iodine concentration, elution volume of amylopectin–iodine complexes, V_{ai} is nearly constant and is very large compared with the elution volume of amylopectin, V_a and amylose–iodine complexes V_{ai} for the same concentration of iodine. Plots $1/(V_{ai}-V_a)$ *vs.* C show a straight line. However, the nearly zero inclination of this straight line as well as the large V_{ai} value might give a large a value, and this in turn indicates the instability of amylopectin–iodine binding. The potentiometric titration curve (not shown), showing no plateau, is also in accordance with this. All these facts are in agreement with the general view that amylopectin–iodine binding is not so strong as soluble starch- and amylose–iodine binding.[6]

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