Amylose-Iodine Complex. II[1]. *Effects of Inorganic Salts*

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(Received March 21, 1960)

The starch-iodine reaction in solution is markedly affected by some conditions (i. e. temperature, history of amylose solution and concentrations of reactants, etc.) under which the reaction takes place. However, the effect of salts on starch-iodine reaction has scarcely been investigated except in the case of studies[2] made at the beginning of this century at which time starch chemistry had not yet been established. The authors have found that certain salts strongly aid the formation of the amylose-iodine complex in a solution containing small amount of iodide ion. Referring these results to the helical theory[3], the authors intend to elucidate the structure of the amylose-iodine complex in solution.

Experimental

Materials.—Amylose was obtained from potato starch by a selective precipitation method with *n*-butyl alcohol and isoamyl alcohol[4]. This amylose was recrystallized twice from boiling water in the presence of excess butanol. All other reagents were of the purest grade commercially available and used without further purification.

Procedure.—Hot water was poured on the recrystallized dry amylose of known amount and the suspension was heated on a water bath, being stirred for one hour in order to complete the dispersion of amylose. After being cooled, the solution was filtered with a glass filter and the filtrate was diluted to a desired volume with water. The above prepared solution, aqueous iodine solution, iodide solution and salt solution were used to make the final solutions which were composed of $0.005\sim0.015\%$ amylose, $10^{-5}\sim2\times10^{-5}$ M iodine, $10^{-6}\sim10^{-5}$ M potassium iodide and $10^{-5}\sim10^{-1}$ M salt. In these compositions the amount of iodine was less than one third of that sufficient to saturate the amylose, and the ratio of iodide to iodine was unusually small, compared with that in a usual case. After being stood for more than one hour, the final colored solutions were used for spectrophotometric measurements.

A nearly saturated solution of iodine which was used to prepare the final solutions was made by shaking iodine crystals in water at room temperature. The concentrations of iodine and iodide ion in this solution were determined immediately before use by measuring their absorbances at wavelengths 288, 350 and 460 mμ[5], since iodide ion is formed by the hydrolysis of iodine.

The spectrophotometric measurements were carried out at room temperature by a Shimadzu type QB-50 spectrophotometer with 1.00 cm. thick silica cells.

Results and Discussion

Iodide ion plays an important role in the amylose-iodine reaction but it remains uncertain whether or not amylose can form an iodide-free iodine complex in solution[6]. To solve this question, an aqueous iodine solution, in which iodic acid, perchloric acid or sulfuric acid was contained to repress any iodide ion due to the hydrolysis of iodine, was added to the amylose solution. Absorption at 350 mμ

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[1] S. Ono, S. Tsuchihashi and T. Kuge, *J. Am. Chem. Soc.*, **75**, 3601 (1953) is the Part I of this series.

[2] For a review, see "Chemistry and Industry of Starch", edited by R. W. Kerr, Academic Press, Inc., New York, (1950), Chapter XVII, p. 455.

[3] R. E. Rundle and D. French, *J. Am. Chem. Soc.*, **65**, 554 (1943); R. E. Rundle and R. R. Baldwin, ibid., **65**, 558 (1943); R. E. Rundle, ibid., **69**, 1769 (1957); R. S. Stein and R. E. Rundle, *J. Chem. Phys.*, **16**, 195 (1948).

[4] E. J. Wilson, T. J. Schoch and C. S. Hadson, *J. Am. Chem. Soc.*, **65**, 1380 (1943).

[5] A. D. Awtrey and R. E. Connick, ibid., **73**, 1842 (1951).

[6] E. O. Forster, Ph. D. Thesis, Columbia University, (1951), through J. A. Thoma and D. French, *J. Am. Chem. Soc.*, **80**, 6142 (1958).

due to triiodide ion was not observed in 0.2 M iodic acid solution and the blue coloration due to complex formation did not occur with the amylose solution. On the contrary, when the other acid solutions were used, the absorption due to a small amount of triiodide ion was always observed and the coloration took place. Therefore, the blue amylose-iodine complex seems to be formed only when iodide is in solution.

Gilbert and Marriot[7] have suggested that the constitution of the complex is $(3I_2\cdot 2I^-)$ or (I_8^{2-}) and this resonating ion is thought to be responsible for the blue color. However, as mentioned in the previous paper[1], the amount of iodide ions necessary for the coloration may be very small compared with that concluded by the above authors. Rundle and French[8] have demonstrated the formation of colored complex by absorption of iodine vapors by helical amylose in solid state[9]. It was, hence, inferred that a large amount of iodide ions is not necessary for the coloration. Recently, Holló and Szejtli[10] have agreed with our opinion concerning the constitution of the complex from amperometric studies. Although there are some evidences[7,11,12] indicating that iodide ions are adsorbed as well as iodine molecules by amylose in solution, it is still in doubt whether they all take part in the resonating polyiodine chains which are responsible for the blue color. The authors are inclined to think that the iodide ions indispensable for the resonating chain are only small fractions of adsorbed iodide ions. The triiodide ion adsorbed on the surface of amylose will be discussed in the following paper[13].

From Fig. 1 it is seen that the amylose-iodine complex has a spectrum having two absorption maxima, each of which is in the visible and ultraviolet region, respectively. The absorption is small in the low concentration of potassium iodide and it increases considerably with increasing iodide concentration until 5×10^{-3} M iodide is reached, after which the blue shift of the spectrum takes place, accompanying the decrease of the visible absorption.

Fig. 2 shows the changes of the spectra with the increase of potassium chloride at constant iodide concentration (10^{-5} M). It is seen that the increase in the concentration of potassium chloride causes the shift of the wavelength of maximum absorption $\lambda_{\max}$, to a longer wavelength in the visible region, accompanying the considerable increase of absorption.

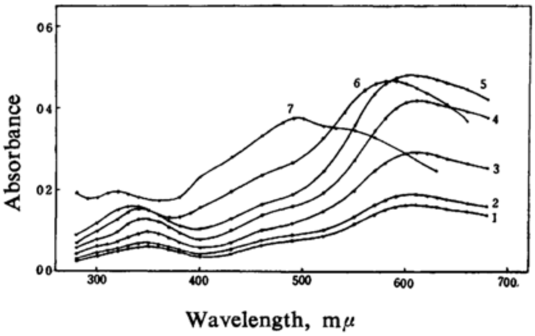

Fig. 1. The absorption spectra of amylose-iodine complexes formed under the various concentrations of potassium iodide: amylose 0.005%, total iodine 1.5×10^{-5} M, potassium iodide (1) 1.3×10^{-5} M, (2) 1.8×10^{-5} M, (3) 6.3×10^{-5} M, (4) 5×10^{-4} M, (5) 5×10^{-3} M, (6) 5×10^{-2} M, (7) 5×10^{-1} M.

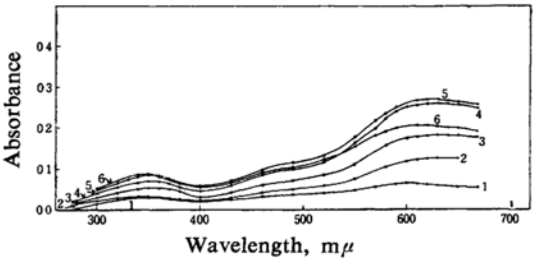

Fig. 2. The absorption spectra of amylose-iodine complexes formed under the various concentrations of potassium chloride: amylose 0.008%, total iodine 1.3×10^{-5} M, potassium iodide 1×10^{-5} M, potassium chloride (1) zero, (2) 5×10^{-4} M, (3) 2.5×10^{-3} M, (4) 1.3×10^{-2} M, (5) 6×10^{-2} M, (6) 1.2×10^{-1} M.

Certain other salts[14] also aid the complex formation in a manner similar to that of potassium chloride. These results are collected in Fig. 3. Fig. 3 (a) shows the relationship between $\lambda_{\max}$ and the salt concentration and (b) the dependence of absorbance at $\lambda_{\max}$ on the salt concentration, in the visible region. The amylose-iodine complexes formed in the solution, which contains 0.005~0.015% amylose, $10^{-5}\sim2\times10^{-5}$ M iodine and $10^{-6}\sim10^{-5}$ M iodide but no any other salt, has $\lambda_{\max}$ at 600 mμ, and its absorbance $E_{\max}$, is very small. It is seen

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[7] G. A. Gilbert and J. V. R. Marriott, *Trans. Faraday Soc.*, **44**, 84 (1948).

[8] R. E. Rundle and D. French, *J. Am. Chem. Soc.*, **65**, 1707 (1943).

[9] Even with dry amylose, traces of iodide ions appeared to be produced by reduction of iodine by impurities (remaining water etc.).

[10] J. Holló and J. Szenjtli, *Die Stärke*, **10**, 248 (1958).

[11] R. S. Higginbotham, *Shirley Institute Memoris*, **23**, 159 (1949).

[12] D. L. Molud, *Biochem. J.*, **58**, 593 (1954).

[13] T. Kuge and S. Ono, This Bulletin, **33**, 1273 (1960).

[14] Salts, whose aqueous solutions show alkaline pH, for example, sodium carbonate, potassium carbonate and disodium hydrogen phosphate, etc., inhibited the coloration. It can be ascribed to the reduction of iodine by hydroxyl ions.

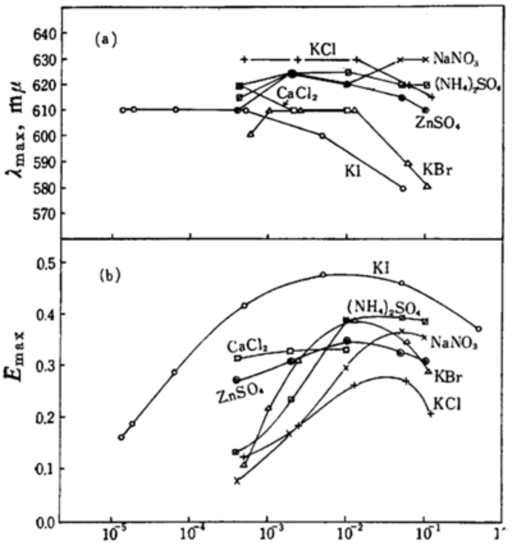

Fig. 3. The effects of various salts on the formation of amylose-iodine complex.

that E_{max} shows a maximum value at 5×10^{-3} M iodide. The maximum value seems to be determined by the iodine concentration in the solution, while the maximum values caused by the additions of the other salts seem to be mainly determined by the iodide concentration. The addition of more iodide than 10^{-3} M causes the large shift of λ_{max} to a shorter wavelength as described in the previous paper$^{1)}$. The blue shifts due to additions of an excess of the other salts are small and λ_{max} are always more than 600 mμ. Only bromide is exceptionally similar to iodide in respect of the blue shift. This seems to be caused by the formation of the iodine-bromide ion$^{15)}$ that would enter the polyiodine chain and shorten the resonating length as iodide ions do$^{1)}$, but it may not be the case with other ions.

As described above, the complex formation seems to be influenced to a great extent by iodide and the other salts. Iodide, however, has a different role in the complex formation from the other salts; that is, iodide is essential for the reaction, while the other salts aid strongly the formation of the complex from amylose, iodine and iodide ion when the concentration of iodide is as low as 10^{-5} M. Since the decrease of E_{max} observed with the addition of salts over 0.01 M seems to be partly owing to the precipitation of the complexes, this phenomenon will not be further discussed.

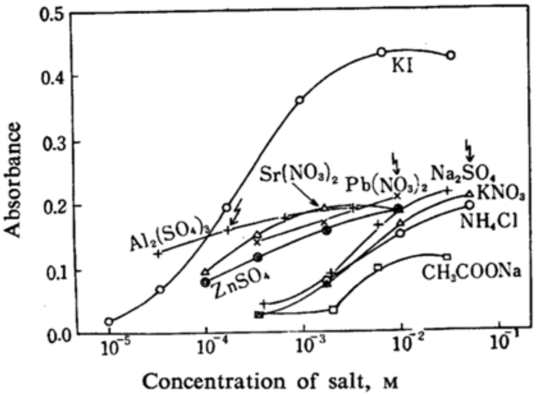

Fig. 4. The changes of absorbance at 610 mμ with increasing concentrations of various salts: amylose 0.007%, total iodine 1.25×10^{-5} M, potassium iodide 1×10^{-5} M.

Fig. 4 shows another series of experiments carried out. It can be seen that the investigated salts are divided into three groups according to their valency. The higher the valency which the cation has, the lower becomes the concentration of the salt efficient for the completion of the amylose-iodine reaction. The arrows in Fig. 4 indicate the concentrations at which the precipitant of the complex becomes apparent to the naked eye. This concentration also changes in a regular fashion with the valency of cation. These facts may be connected with the Schulze-Hardy rule. Thus it seems quite natural to correlate the increase in absorbance of the complex by added salts with the increase of the aggregation of amylose through the neutralization of the charge on amylose. It is, hence, plausible that the aggregation of amylose caused by the addition of salts would play an important role in the formation of the stable amylose-iodine complex.

Since amylose has been known to have few ionized groups (about one phosphate group per three thousand glucose residues, calculated from the data obtained by Kerr et al.$^{16)}$), adsorbed iodide ions would mainly be the source of the charges that prevent the aggregation of amylose.

Although the nature of the amylose-iodine interaction remains controversial$^{17)}$, Stein and Rundle$^{3)}$ have presented a theory for the dipolar attraction between amylose and iodine in solid state on the basis of the helical model of amylose. It should be noted that, according to them, the dipolar field provided from the helical amylose in aqueous solution may be

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15) A marked change of the absorption spectrum of aqueous iodine solution was observed by the addition of bromide or chloride, indicating the strong interaction between iodine and bromide or chloride ion. The other salts hardly altered the spectrum.

16) R. W. Kerr and G. M. Severson, *J. Am. Chem. Soc.*, **65**, 193 (1943).

17) H. Murakami, *J. Chem. Phys.*, **22**, 367 (1954); C. T. Greenwood and H. Rossotti, *J. Polymer Sci.*, **27**, 481 (1958).

materially reduced by polarization of water, and no strong interaction between amylose and iodine may occur if the helical amylose is surrounded by water molecules with a higher macroscopic dielectric constant.

It is not unreasonable to thnik that since the complexes consisting of amylose, iodine and iodide ion are negatively charged, they can be kept separated without aggregation owing to their Coulombic repulsive force, if the concentration of cation is largely insufficient to repress the force. The complex thus formed may be supposed to be meta-stable since amylose-iodine interaction may not be strong for the reason described above, and may be called intermediate complex. The intermediate complex may not have well-shaped helical structure because of the charges due to adsorbed iodide ion.

By addition of salts these intermediate complexes may tend to gather to aggregates through which water may be excluded from the interstices among them. Moreover, if the aggregates contain helical amylose molecules in close packing, it may be expected that the dipolar field in a given helix would be enhanced by surrounding amylose chains. Then the amylose-iodine complexes formed in aqueous solution would be stabilized by the formation of the aggregates in which the helical complexes would probably be refined on shape and oriented parallel to one another.

Foster and Paschall[18)] found that the disaggregation of amylose aggregates results in a decrease in iodine binding capacity and a decrease in the wavelength of maximum absorption of the complex. They also suggested the significance of crystalline order in the complex formation. This seems to support the authors' opinion concerning the nature of the complex as above described. Mayer and Bernfeld[19)] considered that the adsorption of iodine occurs on colloidal micells of amylose molecules. Although their conclusion may not be accepted without modification from the helical theory generally accepted at present, their experimental results also indicate that the state of the aggregate of amylose may be closely related to the formation of the blue complex.

In conclusion, it may be said that the blue complex can hardly be formed in solution where ionic strength is so low that the aggregation of the complex takes place with difficulty. The stable state of the blue amylose-iodine complexes formed in solution may be parallel-oriented aggregates composed of helical complexes.

Summary

The relations between the light absorptions of the amylose-iodine complex and the concentrations of various added salts have been investigated. The iodide ion appears to be indispensable for the formation of the blue complex. The complex formation can be greatly aided by certain salts when the concentration of iodide is low. This salt effect suggests that the parallel-oriented aggregation of amylose-iodine helical complex is essential for the formation of the stable blue complex.

The authors wish to thank Miss Setsuko Sugawara for her technical assistance in this study.

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[18)] J. F. Foster and E. F. Paschall, *J. Am. Chem. Soc.*, **75**, 1181 (1953).

[19)] K. H. Meyer and P. Bernfeld, *Helv. Chim. Acta*, **24**, 389 (1941).