Effect of branching of amylopectin on complexation with iodine as steric hindrance

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The structure of an amylopectin–iodine coloured complex has been investigated by small-angle X-ray scattering. The amylopectin shows the scattering profile of a branched polymer with persistent chain and curvature. The complexation of amylopectin with iodine induces different structural changes compared with those found for linear polymers, such as amylose and partially formalized poly(vinyl alcohol). This suggests that the presence of branch points in the amylopectin molecule contribute to steric hindrance in suppressing the structural change induced by the formation of polyiodine in the polymer chains.

(Keywords: amylopectin; branching; complexation)

Introduction

Amylopectin is a major component of starch as well as amylose. Amylopectin has many branched chains just as glycogen does, while amylose does not[1–4]. The structural differences affect the coloured complex formation with iodine; in particular, the length of the branched chain has a critical effect on the formation of an iodine complex[5]. It is known that when the external chain-length is less than about 15 glucose units (i.e. degree of polymerization (DP) = 15), virtually no iodine is bound at either low or high temperature. When $DP = 20$–25, only a small amount of iodine is bound at 20°C but an appreciable amount at 1.5°C, although there is evidence of some kind of surface effect as well as helix formation. At $DP \geqslant 30$, iodine is bound in considerable amounts at both 1.5°C and 20°C. In these experiments only the external chain-length was varied, and the core of the branched macromolecule was the same in all cases. However, as the branch points may be regarded as a source of interference in the formation of the regular structure necessary to yield a stable complex, it is highly probably that the changes in degree of branching, involving variation of both outer and inner chain-lengths, would also be reflected in changes in iodine binding capacity.

The iodine complex with amylose has been investigated extensively and has been shown to be an inclusion compound, in which polyiodine chain is occluded in the helical cavity of the amylose and shows blue coloration[6–9]. In the iodine complex with amylopectin, however, the polyiodine, which is the colouring matter, formed in the complex shows only reddish coloration, suggesting that the polyiodine chain is not long enough[10–13]. The reddish colour is attributable to the formation of I_3^-, and the blue colour to I_5^- as the major polyiodine component[14,15]. In our previous studies using small-angle X-ray scattering (SAXS) we revealed the structural changes of amylose and partially formalized poly(vinyl alcohol) induced by the complexation with iodine. In the case of amylose of 2.9 kDa (18 DP), the complexation induces the formation of an asymmetrically stacked structure, from a small sphere to a long cylinder, whereas in the case of amylose of 16 kDa (98 DP) the whole structure of the amylose is slightly contracted by the complexation[16]. In the case of the partially formalized poly(vinyl alcohol) of 26 kDa (560 DP), the whole structural change, which shows the same tendency as the amylose of 16 kDa, accompanies the critical internal conformational change in the polymer chain from a persistent chain to a Gaussian chain[17].

To clarify whether the complexation of amylopectin with iodine accompanies the particular conformational change, we have used SAXS with a synchrotron radiation source, using an X-ray beam strong enough for the extraction of useful information from the strong background scattering caused by heavy atoms such as iodine.

Experimental

Amylopectin was guaranteed grade purchased from the Nakarai Chemical Industries Ltd. The amylopectin was repurified according to the procedure reported by Witnauer *et al.*[18]. The amylopectin obtained was subjected to molecular weight determination. Measurements of molecular weight were carried out using high performance liquid chromatography (Tohsoh CO-8000). The column used (GS-710, Asahi-Kasei) had an i.d. of 7.6 mm and a length of 500 mm. Amylopectin in the eluate was detected by a refractive index meter (Eruma ERC-7515A). Amylopectin was dissolved in the eluting solution using a sonicator. The amount injected into the column was

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100 μl. The eluting solution was 50 mM of aqueous sodium nitrate solution with a pH of 8.0 adjusted by NaOH. The flow rate was 1.0 ml min^{-1}, and the column was at ambient temperature. The calibration curve used for the estimation of molecular weight was determined by using 13 standard poly(ethylene oxide) samples with molecular weights ranging from 62 to 3 750 000 Da. The amylopectin was estimated to have $M_w = 43\,000$, with $M_w/M_n = 2.5$.

For the X-ray scattering measurement, the samples were prepared according to the following procedure. Amylopectin was dissolved in 0.5 M of KOH aqueous solution. The stock solution of amylopectin (0.032 g ml^{-1}) was neutralized by titration with acetic acid immediately before complexation. In basic aqueous solution, iodine is easily reduced to iodide, but this is not desirable for the complexation. Complexation of amylopectin with iodine was carried out by adding an aliquot of aqueous iodine solution to the neutralized amylopectin aqueous solution. The final concentration of the amylopectin was adjusted to 0.02 g ml^{-1}. The ionic strength of the complex solution thus prepared was adjusted to 0.48 M. The effect of addition of iodine and iodide on the ionic strength of the complex solution was negligible compared to that of KOH (0.48 M) under the experimental conditions. Complex solutions with the composition ratio of $[I_2]/[KI] = 0.10$ were prepared by adding an aliquot of the stock solution whose concentration was $[I_2]/[KI] = 0.020$ M/0.20 M. The reddish coloured solutions of the complex were used for X-ray scattering measurements.

X-ray scattering experiments were carried out with the small-angle scattering spectrometer for enzymes installed at BL10C line of the 2.5 GeV storage ring in the Photon Factory of the National Laboratory for High Energy Physics, Ttsukuba, Japan. The wavelength used was 1.49 Å and the sample-to-detector distance was 87 cm. Details of the instruments are given elsewhere[19]. Samples were contained in a quartz cell with 1 mm path length, and the temperature of the sample holder was maintained at 25.0 ± 0.1°C by circulating water. The exposure time was 600 s for each sample. The electron current in the storage ring was 140–250 mA.

The following scattering data analyses was carried out. The Guinier analysis was done for the beginning of the scattering curve $I(q)$ (where q is the magnitude of the scattering vector, defined by $q = (4\pi/\lambda)\sin(\theta/2)$; θ is the scattering angle) by using the Guinier equation in the form:

$$I(q) = I(0)\exp(-q^2R_g^2/3) \quad (1)$$

where R_g is the radius of gyration, and $I(0)$ designates the zero-angle scattering intensity[20]. To determine the apparent value of the radius of gyration, denoted as R_g^*, we use the least-squares method for the Guinier plot ($\ln I(q)$ *versus* q^2) on the data sets of this q range. For the middle scattering angle region, which is large compared to the particle size ($qR_g \gg 1$) but small compared to typical chemical bond distances a ($qa \ll 1$), the scattering curve is known to depend on the simple power law given by:

$$\log I(q) = \text{const} - (6 - D_f)\log q \quad (2)$$

where $(6 - D_f)$ is the Porod exponent and D_f the fractal dimension. By evaluating the Porod exponent we can assume the internal conformation of polymer chains[21–25]. The analysis using the distance distribution function, $p(r)$, was done by calculating the Fourier inversion of the scattering intensity $I(q)$ as:

$$p(r) = \frac{2}{\pi}\int_0^\infty rqI(q)\sin(rq)\,dq \quad (3)$$

It depends both on the particle geometry, expressing numerically the set of distances joining the volume elements within the particle, and on the inner inhomogeneity of scattering density distribution in the particle. To calculate the function $p(r)$, the extrapolation method was undertaken by using the least-squares method on the Guinier plot for the small-angle data sets, and the modified intensity

$$I'(q) = I(q)\exp(-kq^2) \quad (4)$$

(k is the artificial damping factor) was used to remove the Fourier truncation effect. The maximum diameter D_{max} of the particle can be estimated from the $p(r)$ function satisfying the condition $p(r) = 0$ for $r \geqslant D_{max}$.

Results and discussion

The observed scattering profiles of the amylopectin at various iodine concentrations are shown by the double-logarithmic plot in *Figure 1*. To take into account both the molecular size and typical chemical bond distance of the amylopectin, we defined the region of 0.06–0.3 $Å^{-1}$ as the Porod regime. The Porod slope gradually decreases from −2.12 to −2.22. This indicates that the complexation of amylopectin with iodine is attended by local structural change in the polymer chain. It is also supported by the Kratky plots, described below. The Porod slope is known to be −5/3 for a swollen linear polymer (self-avoiding walk), −1 for a linear ideal polymer (random walk), −2 for a swollen branched polymer, and

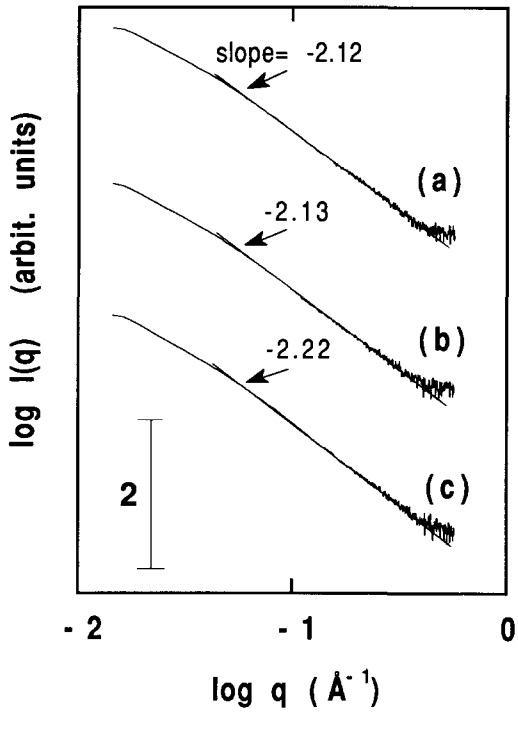

Figure 1 Observed small-angle X-ray scattering curves of amylopectin–iodine complexes at a series of iodine concentrations (mol l^{-1}): (a) $[I_2] = 0$; (b) $[I_2] = 4.0 \times 10^{-4}$; (c) $[I_2] = 9.3 \times 10^{-4}$. The concentration of solute, amylopectin, is 0.02 g ml^{-1}. The straight lines show the Porod slopes

$-16/7$ for a randomly branched ideal polymer[22]. Therefore, the above change of the Porod slopes suggests that during the complexation process the polymer chain of the amylopectin undergoes a slight change in conformation from a swollen branched to a randomly branched polymer.

To avoid an artifact caused by difference in the range used for the Guinier analysis, we chose the q range of 0.020–0.035 Å^{-1} and determined the apparent value of the radius of gyration R_g^* by using the least-squares method for the Guinier plot on the data sets of this q range. Although the gradual increase of R_g^* from 56.4 to 58.7 Å is comparable to the experimental error, it is assumed that the structure of the amylopectin is slightly expanded and this slight change is attributable to the change of the electron density distribution of amylopectin, as will be shown by the distance distribution analysis below.

Figure 2 shows the distance distribution functions $p(r)$ obtained by Fourier inversion of the scattering curves in *Figure 1*. The $p(r)$ function represents the distribution of intramolecular distances joining the volume elements within a particle and also corresponds to the molecular geometry. The maximum diameter D_{max} of the amylopectin molecule, which was estimated from the zero cross point at longer distance, increases slightly from 198 to 202 Å. The peak position of the $p(r)$ function shifts to a longer distance, from 51.9 to 58.0 Å; this indicates that the complexation with iodine causes the interaction between segments in the polymer chains of amylopectin to change slightly to enhance the presence probability at longer distance. The structural parameters obtained are summarized in *Table 1*.

Figure 3 shows the Kratky plots ($q^2I(q)$ as a function of q) of the scattering functions in *Figure 1*. On the assumption that there are no long-range interactions in the macromolecule, the Kratky plot is usually applied to examine the characteristic of polymer chains governed by a certain rigidity called persistence[26–28]. The presence of the hump at around 0.05 Å^{-1} reflects the folded structure of amylopectin in solution. The profile in the q range of 0.4–0.6 Å^{-1} indicates that amylopectin has the structure of a chain with persistence of direction and curvature[29–31], and that with increasing iodine concentration the internal conformation in the amylopectin

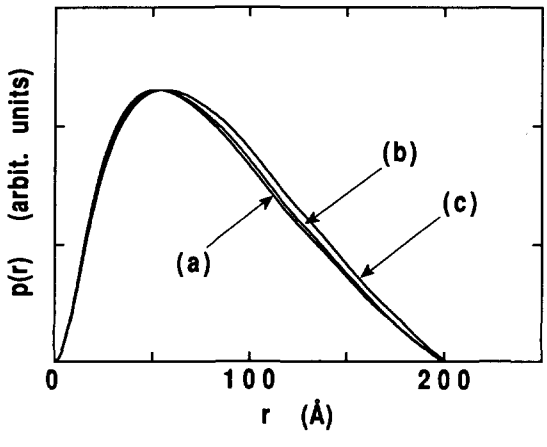

Figure 2 Distance distribution functions, $p(r)$, obtained by applying the extrapolation method and the artificial damping factor to the scattering curves. (a), (b) and (c) as in *Figure 1*

Table 1 Characteristic parameters of amylopectin samples at different iodine concentrations: amylopectin, 0.02 g ml^{-1}

$[I_2]/[KI]$ (10^{-3} mol l^{-1})	Porod slope[a]	R_g^{*b} (Å)	D_{max} (Å)	Peak position of $p(r)$ (Å)
0	−2.12	56.4 ± 1.6	198	51.9
0.40/4.0	−2.13	57.1 ± 1.7	200	54.1
0.93/9.3	−2.22	58.7 ± 1.8	202	58.0

[a] Porod slopes are obtained at the q region of 0.06–0.3 Å^{-1}
[b] Apparent radius of gyration R_g^* is determined at the q range of 0.020–0.035 Å^{-1} using the least-squares method

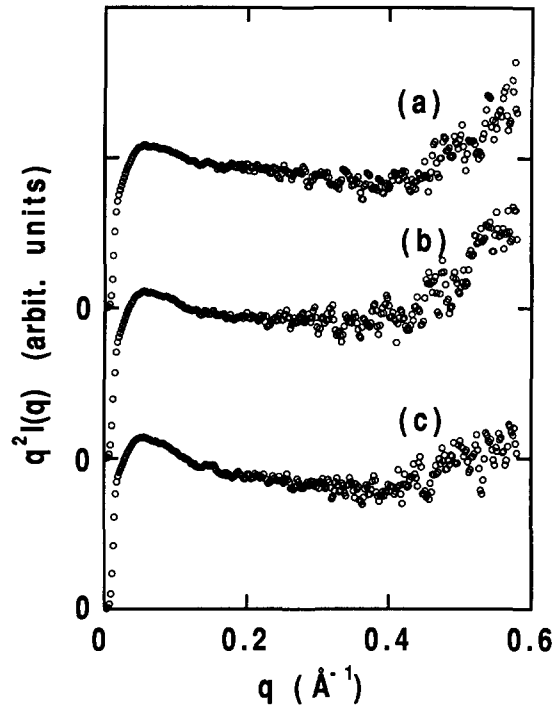

Figure 3 Kratky plots of the scattering curves in *Figure 1*. (a), (b) and (c) as in *Figure 1*

polymer chains changes only slightly to reduce its persistence. This suggests that owing to the characteristics of the amylopectin as a branched polymer, the branch points contribute to steric hindrance to the formation of polyiodine in the polymer chains, and that conformational change by a helical structure formation is suppressed. This result agrees well with that obtained from the Porod slopes.

In conclusion, as the effect of ionic strength does not seriously affect the conformation of the amylopectin under the present experimental conditions, the structural changes observed can be assumed to originate from the complexation with iodine. The profile of the $p(r)$ function and values of both D_{max} and R_g^* indicate that the whole structure of the amylopectin is rather compact and a little ellipsoidal. The Porod slope and the Kratky plot suggest that the internal polymer chain structure of amylopectin is characterized as a branched chain with persistence of direction and curvature. The above characteristics clearly result from branching of amylopectin. As indicated so far, the complexation of long linear polymers with iodine induces contraction of the whole structure of the solute polymer with the shift of gravity of scattering density distribution to a short distance, and also induces a drastic conformational change of the internal polymer chain

structure[16,17]. On the contrary, in the case of amylopectin, complexation with iodine induces a slight expansion of the whole structure with the shift of gravity of scattering density distribution to a long distance. In addition, the conformational change of the internal polymer chain is rather minor and its persistence is mostly retained in the complexation process. Thus the structural changes of the amylopectin differ critically from those of the linear polymers. Taking into account the previous results from spectroscopic studies, it is suggested that the formation of a helical structure by the complexation of amylopectin with iodine is seriously suppressed by the presence of branch points in polymer chains. The present study serves as direct evidence for the structure of the amylopectin–iodine complex as a branched polymer, which is in good agreement with previous results obtained by other investigators.

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