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Note

Iodine–maltosaccharide complexes: relation between chain-length and colour

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Amylose forms an intensely blue colour with iodine in the presence of iodide. Early spectral and X-ray diffraction studies of the starch–iodine complex revealed that the iodine atoms are surrounded by an amylose helix[1–3]. More-recent investigations have shown that the chromophore in the complex is not simply iodine, but involves[4] the tri-iodide ion, I_3^-, or alternating I_2 and I_3^- units[5]. Other workers provided evidence[6] that the preponderant polyiodide species within the amylose helix is I_5^-. Comparatively little is known about maltosaccharide–iodine complexes. Although the reaction of maltosaccharides with iodine is widely used for the determination of the degree of starch degradation, not many data exist on the relation between chain-length and colour. Maltosaccharides of degree of polymerisation (d.p.) 6–22 have been isolated and their iodine-staining properties examined[7].

We now report spectrophotometric data concerning the effect of chain-length of maltosaccharides in the range d.p. 3–70 on the colour of the carbohydrate–iodine complex. A gel-chromatographic method[8] for analytical and preparative separations of oligosaccharides up to d.p. 60 has been used to fractionate and isolate maltosaccharides up to d.p. 70 formed by partial, acid hydrolysis of amylose. Fig. 1 illustrates the fractionation of a series of homologous maltosaccharides on a column of Bio-Gel P-6. Due to the small increase in molecular mass, separation to the baseline was incomplete. The d.p. of the first 35 members of the series was obtained directly from the peaks of the elution profile. For larger oligosaccharides, the d.p. was determined by linear-regression analysis[8]. In order to minimise the overlap of the peaks, only one 2.78-mL fraction for each maltosaccharide peak was used for further analyses. The fractions containing the individual maltosaccharides

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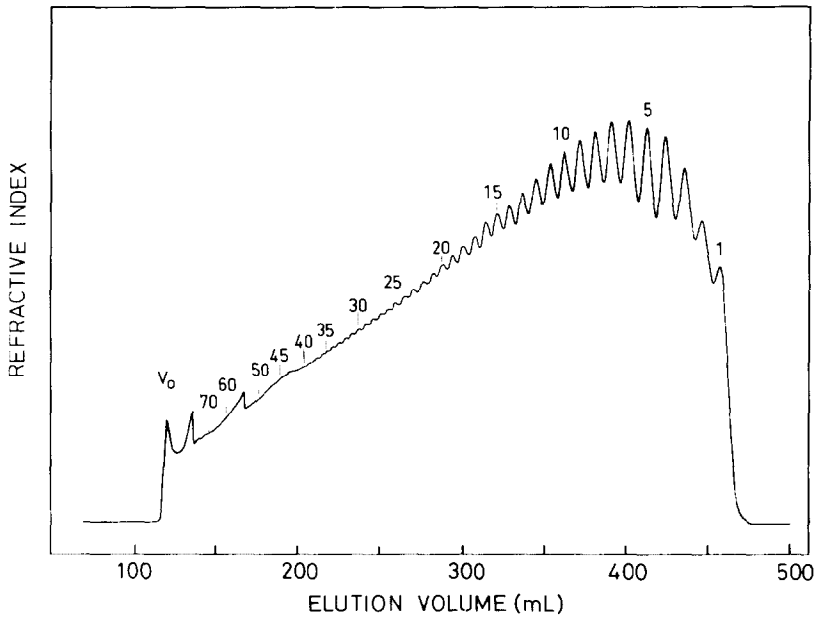

Fig. 1. Chromatography of maltosaccharides on Bio-Gel P-6 (see Experimental) The numbers over the peaks indicate the d.p.; d.p values ≤35 were taken directly from the elution diagram, whereas d.p. values >35 were calculated[8] by linear-regression analysis of the negative logarithm of the distribution coefficient ($-\log K_{av}$) *versus* d.p.

from d.p. 3 to 70 were concentrated by lyophilisation and stained with iodine. Iodine–iodide saturation was achieved by adding small increments of iodine–KI to the samples (see Experimental).

Absorbance spectra of the maltosaccharide–iodine complexes were measured between 300 and 800 nm, and the resulting λ_{max} values were plotted *versus* d.p. (Fig. 2). In a typical absorbance spectrum for a maltosaccharide–iodine complex, there is only one peak having λ_{max} in the range 440–590 nm. Furthermore, absorbances at 353 and 288 nm, which are the absorbance maxima of the I_3^- ion[9], were observed. With d.p. <20, the absorbance peak due to complex formation was not separated from the I_3^- peak at 353 nm. Our experimental data show that the d.p. where no more colour formation occurs ("achroic limit") is ~20, which is in contrast to findings of other workers who suggested[10] an "achroic limit" of d.p. 7. As shown in Fig. 2, each increment of one glucosyl group in this homologous series of maltosaccharide–iodine complexes yields an increase in λ_{max}. With increase in chain-length, the colour of the maltosaccharide–iodine complexes changes from brown (d.p. 21–24) to red (d.p. 25–29), red-violet (d.p. 30–38), blue-violet (d.p. 39–46), and finally blue (d.p. >47).

A plot of $1/\lambda_{max}$ *versus* 1/d.p. yields two straight lines (A and B) having different slopes, as shown in the inset of Fig. 2. The results of linear-regression

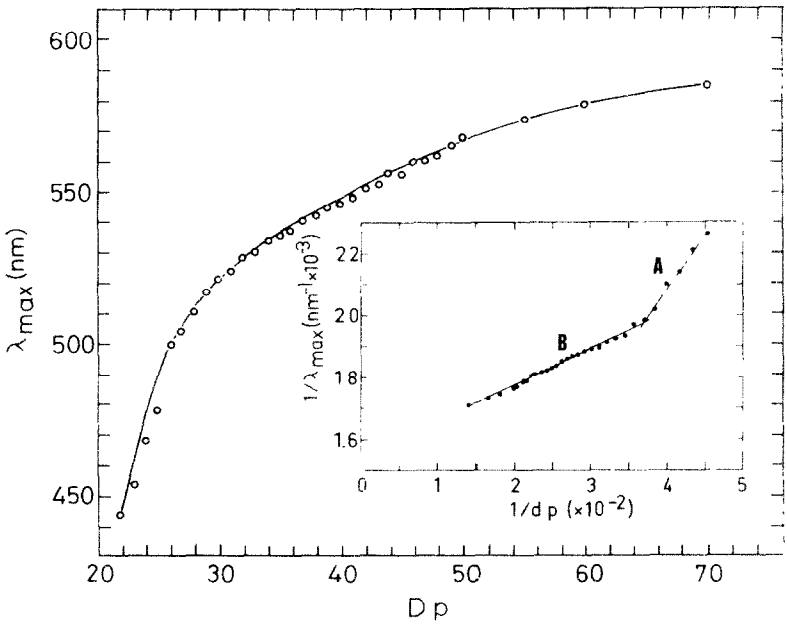

Fig. 2. Relationship between λ_{max} of maltosaccharide–iodine complexes and d.p. In the double-reciprocal plot shown in the inset, $1/\lambda_{max}$ is plotted against 1/d p. Linear-regression analysis: line A, slope 0.0348, intersection with the ordinate 0.686×10^{-3}, and correlation coefficient 0.9778; line B, slope 0.0116, intersection with the ordinate 1.537×10^{-3} and correlation coefficient 0.9916.

analysis of this plot are given in the legend of Fig. 2. The intersection of the two lines is at d.p. 27, corresponding to λ_{max} 506 nm. Using double-reciprocal plots of λ_{max} and d.p. in the range of d.p. 6–22, three separate straight-line sections, corresponding to d.p. 6–11, 12–18, and 18–22, were observed by other workers[7]. On extension of line B (Fig. 2) beyond d.p. 70, the intercept on the ordinate would yield a λ_{max} of ~650 nm for amylose having infinite d.p. This double-reciprocal plot and linear-regression analysis enables calculation of the d.p. of (1→4)-α-D-glucans merely by measuring λ_{max} of the carbohydrate–iodine complex.

EXPERIMENTAL

Partial, acid hydrolysis of amylose (potato type III, Sigma Chemical Co.) was carried out as described[8]. For the preparation of maltosaccharides, the amylose hydrolysate (30 mg dissolved in 0.4 mL of hot water) was fractionated on a column (197.6 × 1.8 cm) of Bio-Gel P-6 (particle diameter, 47 ± 4 μm) by elution at 60° with water at a flow rate of 16.7 mL/h. The column effluent was monitored with a differential refractometer, and fractions of 2.78 mL were collected and lyophilised. The carbohydrates were dissolved in 2.0 mL of water at 80°, aliquots (0.8 mL) of the solution were placed in a glass cuvet of 1-cm pathlength, and iodine solution (10mM I_2 in 20mM KI) was added in increments of 0.01–0.02 mL until a constant

value of maximal absorbance was reached for the peak in the 440–590-nm region. Absorbance spectra were recorded at 25° between 300 and 800 nm with a Kontron Uvikon Model 820 spectrophotometer at a spectral band-width of 0.5 nm.

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