*The Alkali-Catalyzed Depolymerization of Amyloses in Relation to Iodine-Complexing Properties**

When hydrolyzed in random fashion by acid or by α-amylase, amylose gradually loses the capacity to stain with iodine. As the degradation advances, the hyperchromic effect exhibited by the absorption spectra of the iodine complex is accompanied by a hypsochromic shift.[1] A quantitative measure of the specific absorption coefficient of the complex is provided by the peak value (P.V.), which is defined[2] as the absorbance of a 10^{-3}% solution of polysaccharide in a standard iodine–iodide medium measured at the wavelength of peak absorption, λ_{max}, in a 4-cm cell.

Precise data on the relationship between the number-average degree of polymerization, $\overline{D.P.}_n$, and the spectral properties of the iodine complex have been obtained using phosphorylase-synthesized amyloses.[2,3] The decrease in P.V. from a maximum value of 1.35 occurs steeply only below a $\overline{D.P.}_n$ of 30. The achroic point is reached[2,4] at $\overline{D.P.}_n$ 12 and at a monodisperse D.P. of 18. The hypsochromic shift from $\lambda_{max} \sim 640$ nm commences below $\overline{D.P.}_n \sim 350$, with the major drop occurring below $\overline{D.P.}_n$ 80 to λ_{max} 490 nm at the achroic point.[2] The $\overline{D.P.}_n$–λ_{max} relationship takes the form of a Langmuir absorption isotherm.[3]

The phosphorylase-catalyzed synthesis of amylose, occurring as it does by a unidirectional, stepwise addition of D-glucose residues to the chain termini of substrate molecules, may be regarded essentially as a reversal of the alkali-catalyzed depolymerization process since the latter proceeds by a unidirectional, endwise peeling off of the monosaccharide residues. This similarity would suggest that it may also be possible to detect changes of iodine-complexing properties when amylose is degraded in aqueous alkali.

Our previous experience with amylose of $\overline{D.P.}_n \sim 1000$, however, revealed that λ_{max} of the iodine stain (and consequently the P.V.) remains constant when the polysaccharide degrades to form an alkali-stable residue of ~50% yield.[5] This is not surprising since in this range of D.P., λ_{max} and P.V. are insensitive indicators of molecular size. We therefore prepared low molecular weight amyloses so as to permit an examination of the iodine-complexing properties during alkali-catalyzed peeling in that range of $\overline{D.P.}_n$ where major spectral changes occur with synthetic amyloses.

Thus, retrograded potato amylose was suspended in 5*N* hydrochloric acid for 2 hr. As a result, the $\overline{D.P.}_n$ dropped from ~1000 to ~130, and the iodine complex showed a spectral shift of λ_{max} from 625 to 570 nm and a decrease in P.V. from 1.35 to 1.18. A second sample of the amylose was treated with acid for 18 hr: neither the viscosity nor the iodine stain data obtained for this product revealed any further change in molecular dimensions (Table I).† This observation recalled the phenomenon of "leveling-off degree of polymerization" of hydrocellulose,[6] which is also prepared by an acid hydrolysis of the solid polysaccharide. It appears that the supramolecular organization (including crystallinity) of the *B*-amylose renders it stable to further major degradation in dilute acid.

The alkali-catalyzed depolymerization of the "hydroamyloses" was performed in a 0.1*N* sodium hydroxide solution at 100°C under nitrogen. The products were not isolated, but serial aliquots were directly examined for iodine-staining properties. The absorption spectra showed that λ_{max} retains a constant value of 570 nm throughout the reaction sequence (Fig. 1). The extent of reaction, L, was calculated from the absorbance values and reached a maximum limiting value, L_∞, of 0.92 for both substrates after 1–2 hr of reaction time.

Evidence for a constant P.V. was also obtained by monitoring the in situ appearance of yellowing chromogen[5] during the alkaline depolymerization. The absorption spectral data for this chromogen (Table I) are indistinguishable from those found for the alkaline aqueous extracts of the parent amylose,[5] hydrocellulose,[7] and the reducing moieties of maltose[5] and cellobiose.[7] This chromogen is a low-yield, β-diketonic product in the β-elimination reaction of 1,4-glucans (peeling), and its absorbance value at 288 nm provides a quantitative assay of the extent of depolymerization by the peeling process.[5] A graphic plot of yellowing absorbance against the extent of reaction, L, gives a straight line (Fig. 2). Since L values are calculated from the iodine stain absorbance at 570 nm, the

* A preliminary report was presented to the 43rd Annual Meeting, Israel Chemical Society, Beersheba, Oct. 1975, Proceedings, p. 130.

† A similar result has been reported by us[17] for the solid residue (34%) that remains after hydrolysis of amylose with 1*N* sulfuric acid at 98°C for 2.5 hr.

Journal of Applied Polymer Science, Vol. 23, 1883–1887 (1979)

0021-8995/79/0023-1883$01.00

TABLE I
Properties of Acid-Degraded Amyloses[a]

	Hydrolysis time, hr		
	0	2	18
$[\eta]$, ml/g	142	18.5 ± 1.9	17.5 ± 0.6
Iodine complex			
Peak value (P.V.)	1.35	1.18	1.00
λ_{max}, nm	625	570	570
Yellowing chromophore			
Isosbestic point, nm	278	n.d.	271
λ_{max} (pH 13), nm	290	288	288
ϵ (pH 13)	830	811	840
λ_{max} (pH 2.5), nm	255	n.d.	254
pK'_a	8.5	n.d.	8.6

[a] n.d. = Not determined.

absence of any deviation from a rectilinear slope in Figure 2 indicates that a constant P.V. (per mg) is maintained even when 92% of the hydroamylose has degraded.

The invariability found for the $\overline{D.P.}_n$-dependent properties, λ_{max} and P.V., provides a possible indication that $\overline{D.P.}_n$ does not decrease appreciably during alkali-catalyzed depolymerization. (Accordingly, the limiting viscosity number $[\eta]$ of hydroamylose has been found[18] to be unchanged after 43% degradation.) Here, we may note again an apparent similarity to LODP-hydrocellulose, which undergoes peeling with no decrease in limiting viscosity number $[\eta]$. In the case of cellulose, this phenomenon has been taken as evidence for an unchanged $\overline{D.P.}$, the considerable decomposition being ascribed to the complete end-to-end peeling of some molecular chains.[8] Kinetic evidence has been found for a suitable mechanism whereby an initial rate-limiting scission of the glucosidic linkage at the reducing terminal of the cellulose molecule is followed by a rapid chain-propagated depolymerization along the entire chain length.[9] Hydroamyloses in solution thus appear to decompose in a similar fashion.

The kinetic data recorded for yellowing absorbance and for L permitted calculation of the pseudofirst-order rate constants for the three processes that occur[10] during alkali-catalyzed chain depolymerization, namely, (i) k_1, for stepwise chain propagation (the peeling off); (ii) k_2, for chain

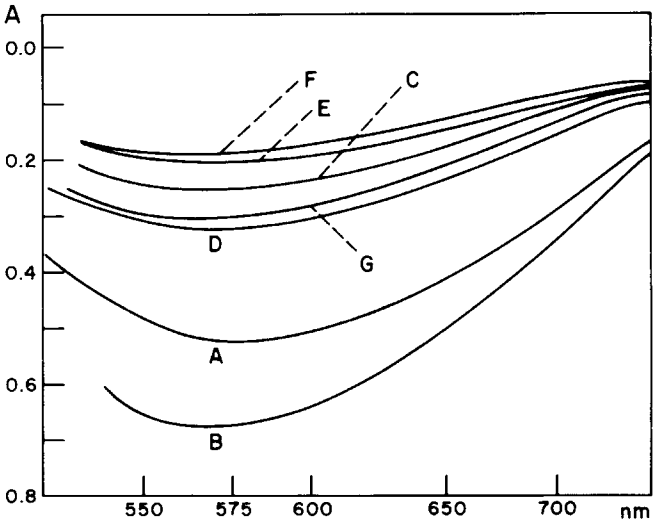

Fig. 1. Absorbance spectra of iodine complexes formed with hydroamylose 18 hr sampled at various time intervals during alkaline decomposition: curve A, 0 min, 0.5 ml sample, L = 0; curve B, 5, 1, 0.34; curve C, 20, 1, 0.76; curve D, 30, 2, 0.84; curve E, 35, 1.5, 0.87; curve F, 40, 1.5, 0.88; curve G, 60, 4, 0.92.

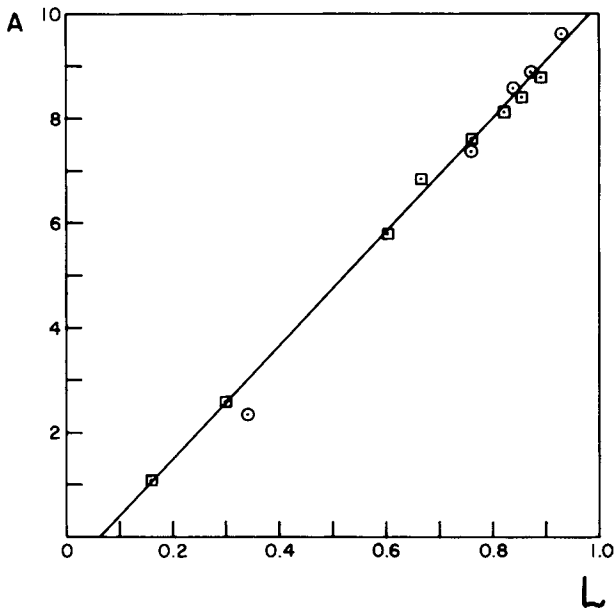

Fig. 2. Graph of yellowing absorbance A vs. extent of reaction L (see text) in the course of alkaline peeling of hydroamylose 2 hr (⊡) and of hydroamylose 18 hr (⊙).

termination by the formation of alkali-stable endgroups; (iii) k_3, for chain termination by the complete end-to-end peeling off of entire polymeric molecules. The values obtained are given in Table II and may be compared with those reported for the degradation of disaccharides,[10] amylose,[10] and hydrocellulose.[9] Since k_2 is known[10] to increase with raised substrate concentration, the finite values of k_2 exhibited by the hydroamyloses (in contrast to the zero value for the parent amylose) are probably related to the higher molar concentration of hydroamyloses used. The rate constants for chain terminations (k_t, k_2, and k_3) in hydroamylose and maltose have a similar value and are one order of magnitude greater than those for the unhydrolyzed amylose. Scission of the glucosidic bond by peeling off is two orders of magnitude faster in the polymers than in the disaccharides. This difference is in keeping with the hypothesis[9,10] that the glucosidic linkage at the reducing terminal of the substrate 1,4-glucan is broken at a slower rate than the analogous linkages along the polymer chain, which are subsequently ruptured in the chain-propagated depolymerization.

In our previous paper[10] it was proposed that the enhanced intermolecular interaction in the solid state would cause hydrocellulose particles to undergo peeling off at a slower rate than dissolved amyloses. This suggestion is confirmed by the values of k_1 obtained for hydrocellulose[9] (4.8 hr^{-1} at pH 9.8 and 31 hr^{-1} at pH 13) and for amylose[10] and hydroamylose (34 hr^{-1} at pH 9.8 and ~300 hr^{-1} at pH 13).

TABLE II
Pseudofirst-Order Rate Constants

	Hydrolysis time, hr			
	0[a]	2	18	Maltose[a,b]
Maximum extent of reaction L_∞	1.0	0.92	0.93	0.48
Rate constants, hr^{-1}				
k_t	0.28	3.0	4.7	3.4
k_1	294	378	565	3.2
k_2	0	0.24	0.31	0.13
k_3	0.28	2.8	4.3	3.2

[a] Values from ref. 10.

[b] Maltose (0.7mM) degraded in 5mM sodium hydroxide at 74°C.

EXPERIMENTAL

AVEBE potato amylose-retrograded starch fraction (10 g) was shaken in hydrochloric acid (5*N*, 500 ml) at ambient temperature. After 2 or 18 hr, the solid residue was separated by centrifugation and washed with water until neutral and then five times with methanol. The produce was ground to a homogeneous powder and dried over phosphorus pentaoxide in vacuo.

A solution of the hydrolyzed amylose (200 mg) in aqueous potassium hydroxide (1*N*, 20 ml) was prepared with magnetic stirring under nitrogen in an ice bath and was filtered through a no. 4 sinter. Flow times were determined in a Cannon–Ubbelhode viscometer using filtered potassium hydroxide solution for dilutions. The limiting viscosity number $[\eta]$ and the standard error were determined by the method of least squares, using the equations

$$\ln (\eta_{sp}/c) = \ln [\eta] + k'[\eta]c$$

and

$$\eta_{sp}/c = [\eta] + k'[\eta]^2 c$$

Arithmetic means of the two values for $[\eta]$ are reported in Table I, in accordance with the procedure recommended by Sakai[11] (cf. ref. 12). The $\overline{\text{D.P.}}_n$ was calculated using the relationship[13]

$$\overline{\text{D.P.}}_n = 7.4 \times [\eta]$$

Anaerobic alkaline degradations were performed on solutions of the hydrolyzed amylose (20 mg) in aqueous sodium hydroxide (0.1*N*, 10 ml) sealed under nitrogen in test tubes that were immersed in a boiling water bath for the required time period. After rapid cooling of the tube in iced water, the UV absorption spectra of the yellow-brown tinted reaction mixture were recorded at various pH values, and an isosbestic point was found at 271 nm. Absorption spectra were recorded with a Perkin-Elmer 450 spectrophotometer using a 1-cm optical path cell. The apparent pK_a of the chromophore was calculated from the absorbance values A at λ_{max} for each pH using the equation[14]

$$pK_a' = \text{pH} - \log \frac{A - A_{AH}}{A_{A^-} - A}$$

where A_{AH}, A_{A^-}, and A are the respective absorbances of the acid AH (measured at pH 2.5), the ion A^- (measured at pH 13), and the solution at the pH being investigated.

Aliquots of the reaction mixture were mixed with iodine reagent as prescribed[15] for the Blue Value assay, viz., 0.5 mg substrate, 1 mg iodine, 10 mg potassium iodide, and 100 mg potassium hydrogen tartrate (pH 3.5) in a volume of 50 ml. The visible spectrum of the resulting blue complex was recorded (Fig. 1). The untreated hydroamyloses obeyed the Beer law in the Blue Value assay. The extent of reaction, L_t, after time t was calculated using the expression

$$L_t = 1 - \frac{\text{iodine complex absorbance at 570 nm at time } t}{\text{iodine complex absorbance at 570 nm of untreated hydroamylose}}$$

The data thus obtained for L_t and A were used to calculate the pseudofirst-order rate constant for the termination of stepwise depolymerization, k_t, in accordance with the expression derived by Haas, Hrutfiord, and Sarkanen[16]:

$$k_t = \frac{\ln [L_\infty/(L_\infty - L_t)]}{t}$$

In this equation, L_∞ denotes the asymptotic maximum value for L at infinite time. The values used for L_∞ were chosen so as to give the best rectilinear plot.[16] In this equation for k_t, the values obtained for A may be substituted for L_t values, yielding the equation

$$k_t = \frac{\ln [A_\infty/(A_\infty - A_t)]}{t}$$

A combined plot of A and L data was used to obtain k_t.

The pseudofirst-order rate constants for depolymerization (k_1), for formation of stable endgroups (k_2), and for total lengthwise unzipping (k_3) were obtained using the following equations, which were derived in a separate communication[10]:

$$k_1 = L_\infty \times k_t \times \text{degree of polymerization}$$

$$k_2 = (1 - L_\infty)k_t$$

$$k_3 = L_\infty \times k_t$$

Professor M. Lewin, Director of the Institute, is thanked for interest and encouragement and useful discussions.

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Received April 25, 1977
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