Specific Hydroxide Ion Catalysis of the Endwise Depolymerization of Cellulose*

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Synopsis

The endwise depolymerization (unzipping reaction) of hydrolyzed cotton cellulose ($x = 200$) in water under a nitrogen atmosphere was followed at 98°C at several alkalinities in the pH range of 8.0–10.5. The observed apparent first-order rate constant k_1 was invariable at low alkalinity ($k_1 = k_0$), while above pH 8.5, k_1 increased with pH. The data conform with the expression

$$\text{rate} = (k_0 + k_{OH}\text{-}[OH^-])[SH] = (0.3 + 2.1 \times 10^3\,[OH^-])[SH]$$

where [SH] denotes substrate concentration. The specific hydroxide ion catalysis is considered to involve ionization of the anomeric hydroxyl group at the reducing chain end that leads to elimination of the glucosidic oxygen atom bearing the polymer chain from C4 of the terminal D-glucose residue. In this initiation process, the glucosidic oxygen is eliminated as an anion so that rapid propagation of the unzipping along the polymer chain may occur. Thus, entire chains will depolymerize. The kinetic chain length ν is defined as the ratio $k_1{:}k_t$, where k_t is the pseudofirst-order rate constant for chain terminations, and a value of $\nu \sim 100$ D-glucose residues was found at all the alkalinities investigated.

INTRODUCTION

The 1,4-glucosidic linkage in cellobiose is ruptured anaerobically in aqueous alkali by the beta-alkoxycarbonyl elimination mechanism. In this way, too, the homologous polysaccharide cellulose is depolymerized from the reducing chain ends yielding saccharinate monomers (the stepwise depolymerization, "unzipping" process),[2] whereby some molecular chains are completely unzipped along their entire length.[3] This scheme implies that there is an initiation step at the reducing chain terminus involving liberation of the terminal D-glucose unit and that subsequent propagation of the depolymerization occurs at an enhanced rate along the entire chain.

In the present paper, we describe the influence of changing hydroxide ion concentration on the kinetics of unzipping. The rate equation obtained indicates a mechanism for the beta-alkoxycarbonyl elimination reaction that is in keeping with this hypothesis.

RESULTS AND DISCUSSION

The endwise depolymerization of "leveling-off degree of polymerization" (LODP) cellulose[4] in boiling water was measured at constant pH values in the range of 8.0–10.5. The pH of reaction mixtures at the elevated reaction tem-

* A preliminary report was presented at the 41st Meeting of the Israel Chemical Society held in Tel Aviv, Israel, 1971.[1]

0021-8995/78/0022-0711$01.00

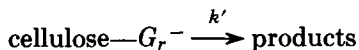perature was determined by extrapolation from the measured variation of pH with temperature.
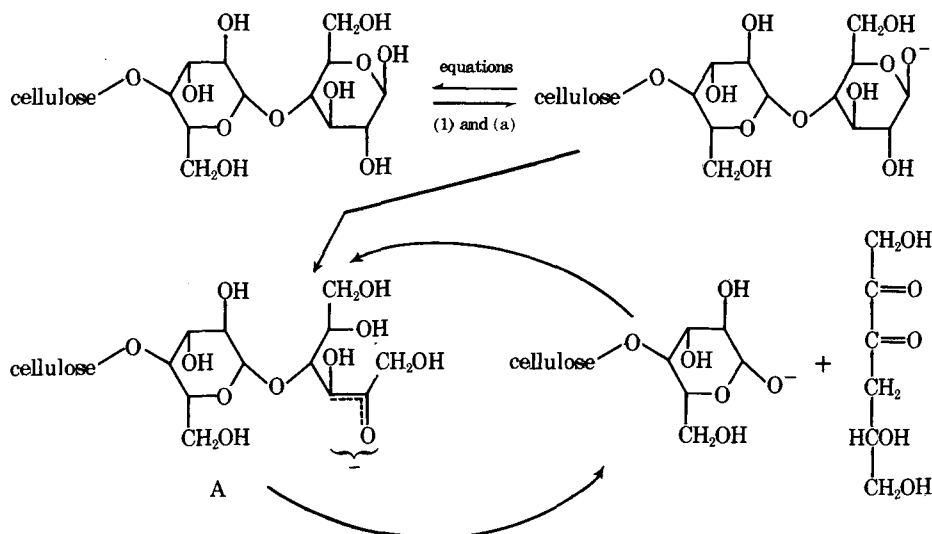
The apparent first-order rate constants for unzipping ($k_{1,obs}$) were calculated from the degradation data (Tables I and II) in accordance with the theory of Haas, Hrutfiord, and Sarkanen[5]:

$$\text{rate} = k_{1,obs}[G_r]$$

where $[G_r]$ is the mole fraction of the reducing end groups that are available for reaction. The value of $k_{1,obs}$ is invariably 0.3 hr^{-1} at lower pH (Table I) and is taken to represent the rate constant for the uncatalyzed reaction (k_0):

$$\text{cellulose---}G_r\text{H} \xrightarrow{k_0} \text{products.}$$

At elevated pH, base catalysis is observed (Table II), and the plot of log $k_{1,obs}$ against pH is rectilinear with unity slope (Fig. 1). This relationship indicates a mechanism involving specific hydroxide-ion catalysis, as is evident from the following considerations. A prior ionization of the substrate which comes to equilibrium (equilibrium constant K) is followed by a slower reaction of the resulting anion with a first-order rate constant k':

$$\text{cellulose---}G_r\text{H} + \text{OH}^- \overset{K}{\rightleftharpoons} \text{cellulose---}G_r^- + \text{H}_2\text{O} \qquad (1)$$

$$\text{cellulose---}G_r^- \xrightarrow{k'} \text{products}$$

$$\text{rate} = k_{1,obs}[G_r] = k_{1,obs}[G_r\text{H} + G_r^-]$$

$$= k_0[G_r\text{H}] + k'[G_r^-]$$

$$= (k_0 + k'KK_w[\text{H}^+]^{-1})[G_r\text{H}]$$

where K_w is the ionization constant of water. When $[G_r\text{H}] > [G_r^-]$, then

$$k_{1,obs} = k_0 + k'KK_w[\text{H}^+]^{-1} \qquad (2)$$

TABLE I
Uncatalyzed Degradation of Hydrocellulose at 98°C[a]

| Expt. no. | Ionic strength | pH at | | $L_\infty{}^b$ | $[G_r]_0{}^b$ $\times 10^5$ | ν^c | Data[b] | $k_t,{}^d$ $hr^{-1} \times 10^4$ | $k_{1,obs},$ $hr^{-1} \times 10^2$ |
		25°C	98°C						
1	1.20	7.9	8.0	0.355	462	77	L	47 ± 5	36
							A	42 ± 7	32
2	0.61	8.4	8.2	0.377	510	74	L	41 ± 5	30
							A	42 ± 3	31
3	1.20	8.4	8.2	0.573	510	112	L	28 ± 9	31
							A	31 ± 12	35

[a] Composition of buffers: at pH 7.9, 10% sodium hydrogen carbonate; at pH 8.4, 5% sodium hydrogen carbonate.

[b] See experimental section.

[c] Alkali-degradable chain length $\nu = k_1/k_t = L_\infty/[G_r]_0$ (see ref. 5).

[d] Calculated from degradation data of Table III.

and

$$d(\log k_{1,obs})/d(pH) = 1$$

as is found in Figure 1.

The data obtained are also amenable to the following alternative treatment. Equation (2) may be written as

$$k_{1,obs} = k_0 + k'K_h{}^{-1}[OH^-] \tag{3}$$

or as

$$k_{1,obs} = k_0 + k_{OH^-}[OH^-] \tag{4}$$

where K_h is the hydrolysis constant of G_rH, given by $K_h = [G_rH][OH^-]/[G_r{}^-]$, and k_{OH^-} is the catalytic coefficient. Values of k_{OH^-} were calculated in accordance with eq. (4) for the experimental data in Table II, and they did not change significantly when the alkalinity was raised, even though reaction rates increased sixfold. This provides additional support for specific hydroxide-ion catalysis. Data at ionic strength 1.2 in Table II yield an average value of 2.1×10^3 hr^{-1} $mole^{-1}$ for k_{OH^-}. The results obtained are thus in keeping with the expression

$$\text{rate} = (k_0 + k_{OH^-}[OH^-])[SH] = (0.3 + 2.1 \times 10^3 [OH^-])[SH].$$

It follows from eqs. (3) and (4) that the specific rate constant for the catalyzed reaction k' can be obtained using the equation

$$k' = k_{OH^-} \cdot K_h \tag{5}$$

The values of K_h for α-D-glucopyranose and β-D-glucopyranose at 98°C were calculated from the relationships given by Los and Simpson[7] as 0.158 and 0.0485, respectively. Using the value for the more strongly acidic anomer, eq. (5) yields a value of 102 for k'. It follows that the uncatalyzed reaction is accelerated by a factor of 340 (k'/k_0) due to the base catalysis.

The demonstration of specific hydroxide-ion catalysis of the depolymerization provides direct support for the reaction scheme proposed by Lai and Sarkanen[8] for the analogous case of amylose depolymerization, whereby the initial ionization equilibrium, eq. (1), occurs at the anomeric hydroxyl group and the unzipping

TABLE II
Catalyzed Degradation of Hydrocellulose at 98°C[a]

Expt. no.	Ionic strength	pH at 25°	pH at 98°	L_∞[b]	$[G_r]_0$[b] $\times 10^5$	v[c]	k_t,[e] $hr^{-1} \times 10^2$	$k_{1,obs}$, hr^{-1}	k_{OH^-},[d] $hr^{-1} mol^{-1} \times 10^{-2}$
4	0.60	9.8	9.6	0.420	462	91	5.0 ± 1.3	4.6	21
5	1.20	9.8	9.6	0.462	510	91	5.3 ± 0.3	4.8	20
6	1.05	11.0	10.0	0.476	462	100	15 ± 2	16	27
7	1.20	11.0	10.0	0.495	510	97	12 ± 4	12	23
8	0.10	13.0	10.6	0.400	462	87	34 ± 5	30	13
9	1.20	13.0	10.5	0.520	510	100	30 ± 7	31	19

[a] Composition of buffers: at pH 9.8, 5% sodium hydrogen carbonate (4 vol) + 4% sodium hydroxide (1 vol); at pH 11.0, 5% sodium hydrogen carbonate (44 vol) + 4% sodium hydroxide (21 vol); at pH 13, 0.4% sodium hydroxide.

[b,c] As in Table I.

[d] Calculated with eq. (4): pH at 98° converted to pOH using $pK_w = 12.3$ at 98° (see ref. 13).

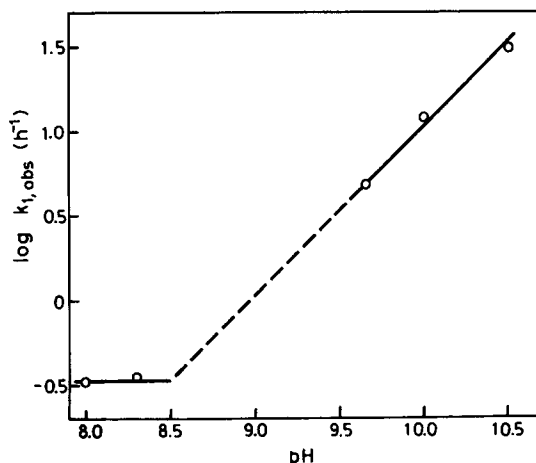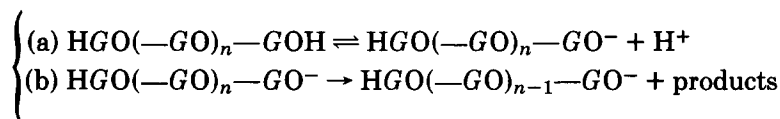[e] Calculated from degradation data of Table IV.

Fig. 1. Alkaline depolymerization of hydrocellulose. Observed rate constant $k_{1,obs}$ at ionic strength 1.2 plotted against pH at 98°C.

rate is directly proportional to the concentration of ionized endgroups. Scission of the terminal glucosidic linkage is achieved in an intermediate anionic enediol A by elimination of the glucosidic oxygen as an anion from C4 of the terminal unit. This anionic oxygen atom is now a new terminal ionized anomeric hydroxyl group, which permits propagation of the depolymerization at an enhanced rate until the complete molecular chain is degraded. This unzipping process is depicted in scheme 1 (where $n \sim 200$). Propagation may be prevented by formation of alkali-stable *meta*-saccharinic acid (MSA) residues, scheme 1, reaction (e), at the reducing termini of cellulose chains that are initially within the crystalline core of the elementary fibril.
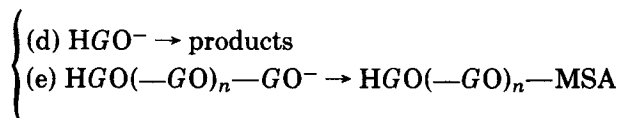
<div align="center">Scheme 1 (G = glucose moiety)</div>

Initiation

$$
\begin{cases}
\text{(a) } HGO(-GO)_n-GOH \rightleftharpoons HGO(-GO)_n-GO^- + H^+ \\
\text{(b) } HGO(-GO)_n-GO^- \rightarrow HGO(-GO)_{n-1}-GO^- + \text{products}
\end{cases}
$$

Propagation

$$\text{(c) } HGO(-GO)_{n-1}-GO^- \rightarrow HGO(-GO)_{n-2}-GO^- + \text{products}$$

Termination

$$
\begin{cases}
\text{(d) } HGO^- \rightarrow \text{products} \\
\text{(e) } HGO(-GO)_n-GO^- \rightarrow HGO(-GO)_n-MSA
\end{cases}
$$

The alkali-degradable chain length ν is defined as the ratio $k_1:k_t$, where k_t is the pseudofirst-order rate constant for chain terminations (see experimental section). The parameter ν denotes the average number of D-glucose residues that are eliminated for each reducing chain end initially present in the polymer. A value of ~ 100 is retained throughout the alkalinity range studied (Tables I and

II), and even in 1.25M sodium hydroxide it does not increase.[5] Thus, no alkali swelling is indicated.

Related studies of the alkali catalyzed depolymerization of amyloses are published separately.[14,15]

EXPERIMENTAL

Delta Pine cotton fiber was purified[9] by boiling with ethanol followed by sodium hydroxide (1%), and then cut into pieces 2–3 mm long. Samples of the cotton (6 g) were shaken with hydrochloric acid (5N, 300 ml) at 25°C in the dark for 12 days. The hydrocellulose product was washed repeatedly with deionized water to remove acid and dried over phosphorus pentoxide *in vacuo*.

Aldehyde group content $[G_r]_0$ was determined by chlorite oxidation.[10] Buffer solutions were prepared in boiled-out deionized water.

Alkaline degradation was performed with ~100 mg hydrocellulose in ~10 ml buffer solution under nitrogen in a sealed test tube that contained a magnetic stirring bar encased in Teflon. In some experiments, the ionic strength was adjusted to 1.2 with sodium sulfate. For controls, the substrate was suspended in boiled-out deionized water (pH 6.8). The sealed test tubes were immersed in a boiling water bath (98°C) over a magnetic stirrer, and single tubes were removed and placed in ice water to stop the reaction at suitable time intervals. The solid residue in the test tube was quantitatively collected by filtration and weighed after desiccation *in vacuo* over phosphorus pentoxide. The filtrate was adjusted to pH 10.4 and the absorbance measured at the 290-nm peak[11] in a 1-cm optical path cell using a Perkin-Elmer spectrophotometer Model 124.

The absorbance values A measure the concentration of the β-diketonic chromogen responsible for the yellowing of 1,4 glucans during alkaline degradation.

TABLE III
Kinetic Data[a] Used for Calculating k_t in Table I

Time t, hr	Expt. 1 L	Expt. 1 A	Expt. 2 L	Expt. 2 A	Expt. 3 L	Expt. 3 A
2	—	—	0.032	0.4	—	—
4	—	—	0.054	0.94	—	—
6	—	—	0.049	1.04	—	—
24	0.066[c]	3.1[c]	0.076[d]	4.05[d]	0.080	3.7
30	—	—	0.070	4.25	—	—
48	0.087[e]	5.4[e]	0.101	6.60	—	—
72	0.117[f]	7.1[f]	0.173	9.50	—	—
96	0.126[g]	7.9[g]	0.175	10.2	0.184	10.8
170	0.223[h]	13.6[h]	0.211	13.2	0.241	15.2
240	0.247	18.6	0.262	17.6	—	—
336	0.285	18.8	0.285	20.2	—	—
456	—	—	0.332	23.8	—	—
744[b]	0.355[k]	26.0[h]	0.377[i,k]	26.5[i,l]	0.573[j,k]	35.0[j,l]

[a] A, spectrophotometric absorbance at 290 nm; L, fractional weight-loss.
[b] Control (744 hr), $L = 0$, $A = 0.14$ (no peak).
[c] $t = 23$.
[d] $t = 25$.
[e] $t = 47$.
[f] $t = 71$.
[g] $t = 93$.
[h] $t = 144$.
[i] $t = 696$.
[j] $t = 528$.
[k] L_∞.
[l] A_∞.

TABLE IV
Kinetic Data Used for Calculating k_t in Table II

Time t, hr	Absorbance[a] measured at 290 nm, A					
	Expt. 4	Expt. 5	Expt. 6	Expt. 7	Expt. 8	Expt. 9
0.25	—	—	2.04	—	3.45	4.90
0.50	1.36	1.88	3.40	3.40	6.35	8.20
0.75	—	—	4.97	—	8.50	—
1.0	2.00	2.40	5.90	6.05	9.70	12.9
1.5	2.50	3.10	8.00	9.70[b]	—	17.8[b]
2.5	3.80	5.05	10.2	—	15.2	—
3.5	4.20	5.20	13.0[c]	13.4[c]	—	—
6.0	4.60[d]	7.80	15.6	—	20.4[d]	23.6
6.5	6.20	12.6[e]	—	17.0	22.0[f]	27.6[l]
24	—	14.8[g]	24.5	—	—	26.2
48	—	20.8	—	28.5	—	26.5
96	—	23.2[k]	27.0[h,k]	28.8[k]	25.0[i,k]	27.2[k]
144	21.0[k]	23.2[j,k]	27.0[k]	—	—	—

[a] Control (744 hr), A = 0.14 (no peak).   [g] t = 18.
[b] t = 2.   [h] t = 72.
[c] t = 4.   [i] t = 101.
[d] t = 5.   [j] t = 168.
[e] t = 13.5.   [k] A_∞.
[f] t = 7.   [l] nt = 15.5.

They provide a quantitative assay of the amount of polymer that has undergone stepwise depolymerization.[11,12] The experimental data are given in Tables III and IV.

The rate constant for propagation $k_{1,obs}$ was obtained by the method of Haas, Hrutfiord, and Sarkanen[5] in accordance with their equation

$$k_{1,obs} = k_t \cdot L_\infty / [G_r]_0$$

where k_t is the total apparent first-order rate constant for the chain termination; L_∞ is the fractional weight loss in alkali, L, at infinite time; and $[G_r]_0$ is the mole fraction of reducing endgroups that are available for reaction at zero time. Two values for k_t were obtained in each experiment: (a) from weight loss data (L) and (b) from absorbance (A), the accordance with the equations

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

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

L_∞ and A_∞ were determined as the constant values of L and A after long reaction times. The k_t values and 95% confidence limits given in the tables were calculated by the method of least squares. No significant difference was found between the two values (Table I). The spectrometric data gave better rectilinear plots, and they were used in Table II.

The temperature dependence of the pH of the media used was determined from room temperature to 90°C, except for the room-temperature pH 9.8 (μ 0.6) and pH 7.9, which were measured only up to 60°. The data were extrapolated graphically to 98°. BDH-pH 11 buffer dropped in pH on heating exactly as did the pH 11 medium used in the kinetics. A Radiometer Titrator Type TTT 1b was used for measuring pH with a combined glass-Calomel electrode. The pH

diminutions with heating corresponded to the published data for standard buffers.[13]

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