Using Disaccharides as a Kinetic Model for Alkaline Degradation of Celluloses and Starches[1]

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Synopsis

Degradation kinetics of cellobiose and maltose in hot aqueous solution was determined at pH 9.8 and 13. The positive concentration-dependence found for the depropagation rate constant of cellobiose indicated that disaccharides decompose more slowly than amyloses under the same reaction conditions, in keeping with a mechanism whereby terminal glucosidic linkages in amylose are also ruptured slowly in an initiation step that is followed by a faster depropagation. Cellobiose termination has a lower activation energy than its depropagation, as does amylose: The opposite result that has been paradoxically ascribed to hydrocellulose is shown to be erroneous. The view that cellobiose does not undergo a chemical stopping reaction appears to be unjustified. The status of a "single-chain" mechanism for 1,4-glucan depropagation is discussed, and is considered to occur with cellobiose, maltose, hydrocellulose, and hydroamylose.

INTRODUCTION

At temperatures below 100°C, alkali-catalyzed scission of the 1,4-glucosidic bond in cellobiose and maltose occurs by *beta*-alkoxy-carbonyl elimination. In this reaction, the reducing moiety of the disaccharide is released as 4-deoxy-D-*glycero*-hexo-2,3-diulose, which forms various saccharinic acids. The nonreducing moiety is liberated as free D-glucose, which is subsequently converted into saccharinates. Chain-propagated endwise depolymerization of the homologous polysaccharides cellulose and amylose by this reaction is called "peeling" or "unzipping." In competition with this degradative elimination, terminal reducing D-glucose residues may alternatively be stabilized to elimination by their transformation to saccharinate residues, such as 3-deoxyhexonate, in the so-called "stopping" reaction.

The rate of glucosidic scission of cellobiose and maltose in alkali is considerably slower than amylose peeling, and we previously ascribed[2] this anomalous difference to a possible concentration dependence of the disaccharide kinetics. In the present report[1], this hypothesis is tested experimentally by measuring the decomposition of dilute solutions of the disaccharides. The results obtained also clarify controversial issues[3,4] relating to the macromolecular mechanism of 1,4-glucan degradation in alkali.

EXPERIMENTAL

Alkaline degradations were performed in a pH 9.84 buffer (20 mL 1*N* sodium hydroxide + 80 mL 5% sodium hydrogen carbonate); and in $10^{-1}N$ sodium hydroxide. Alkaline solutions of disaccharide (5 mL) were spurged with nitrogen gas, sealed in testtubes at room temperature, and immersed

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in a thermostated or boiling water bath. At appropriate time intervals, individual tubes were transferred to ice water, and an aliquot (2 mL) neutralized with hydrochloric acid (1 mL). The concentration of unbroken glucosidic bonds was measured by borohydride reduction followed by determination of the color given with the phenol-sulfuric acid reagent, as described by Painter,[5] using a Perkin-Elmer 124 Spectrophotometer with 10 mm optical-path cells.

RESULTS

Degradation kinetics of hot aqueous cellobiose and maltose were determined at several concentrations at pH 9.8 and 13. The experimental conditions were identical to those used in our previous studies of amylose[2] and hydroamylose.[6] Two rate constants were calculated from the measured data, as previously described[2]: k_1 is the coefficient for glucosidic scission and k_2 for the stopping reaction.

The results in Table I show, as expected, an acceleration of the reactions at the higher alkalinity. Furthermore, there was no significant difference between the behavior of cellobiose and maltose, in keeping with the findings of Painter.[5] When the cellobiose concentration was raised through 2 orders of magnitude, a small *acceleration* of the degradation rates was observed.

The temperature dependence of the reaction kinetics of cellobiose degradation in 10^{-4}M sodium hydroxide permitted calculation of Arrhenius activation energies. Table II shows values of approximately 30 kcal/mol for both glucosidic cleavage and stopping, the latter exhibiting lower values.

DISCUSSION

Concentration Dependence

The hypothetical explanation previously offered[2] for relatively slow disaccharide *beta*-elimination (relative to amylose) required that the reaction rate *decrease* considerably when the substrate concentration is raised. However, the present results reveal a small *positive* dependence of degradation rate on concentration, thereby invalidating concentration dependence as a possible explanation.

Accordingly, slow reaction rates should perhaps be regarded as a characteristic feature of *beta*-eliminative cleavage of the disaccharides, which occurs an order of magnitude more slowly than with amylose[2] and hydroamylose[5] under identical reaction conditions. This difference is in keeping with the hypothesis[2,7] that the first glucosidic linkage at the reducing end of 1,4-glucans is broken in an initiation reaction at a slower rate than the other glucosidic bonds that are subsequently severed as unzipping advances along the polymer chain. In kinetic terms, such polymeric behavior may be denoted by equating the experimentally determined k_1 with a rate coefficient for depropagation (k_P), while the preceeding end group initiation be represented by a separate rate coefficient k_E,[8] which in this case will be smaller than k_P and would correspond in value to k_1 of the disaccharides.

TABLE I
Kinetics of Disaccharide Decomposition

Expt. no.	Initial alkalinity (N)	Initial substrate (mM)	Temp (°C)	0.5 × extent of glucosidic scissions = L_∞ (%)		Rate constants (h^{-1})			
						k_t[a]			
				Mean	σ	Mean	σ	$k_1 = k_3$[a]	k_2
				Cellobiose					
1	10^{-4}	0.53	97	52	5.9	4.0	1.2	4.1	0.0
11	10^{-4}	1.0	67	43	—	0.25	—	0.21	0.04
6	10^{-4}	1.0	82	44	1.0	1.7	0.16	1.5	0.20
2	10^{-4}	1.0	97	49	5.5	6.1	1.6	6.0	0.09
3	10^{-4}	10	97	46	1.9	9.9	1.5	9.1	0.75
7	10^{-4}	100	82	40	1.2	1.5	0.13	1.2	0.30
4	10^{-4}	100	97	42	4.0	6.1	1.7	5.2	0.93
5b	10^{-1}	1.0	97	50[b]	—	n.d.[d]	—	—	—
5a	10^{-1}	100	97	37[c]	—	64[c]	—	47	17
				Maltose					
8	10^{-4}	1.0	97	41	3.1	4.5	0.69	3.7	0.82
9	10^{-4}	1.0	97	43	2.9	4.6	0.71	3.9	0.65
10	10^{-4}	100	97	38	0.4	13	0.46	10	3.0

[a] k_t and k_3 are not reaction constants. They are calculation aids (Ref. 2), and are recorded solely for comparison with polysaccharide data.
[b] After 3 min reaction.
[c] After 2 min reaction.
[d] n.d. = not determined.

TABLE II
Arrhenius Activation Energies for Cellobiose Decomposition[a]

Initial concn (mM)	Temperature range (°C)	Activation energy (kcal mol^{-1})	
		Glucosidic scission (k_1)	Stopping reaction (k_2)
1.0	67–82–97[b]	27.7	n.d.[c]
1.0	67–82	32.5	26.6
100	82–97	24.4	18.9

[a] In $10^{-4}M$ sodium hydroxide, from data in Table I.
[b] Least squares calculation.
[c] n.d. = not determined.

Green et al.[9] have stated that cellobiose degrades at a similar rate to "polysaccharides." This apparent contradiction to our present findings is actually a misnomer, since it refers solely to a comparison with *solid* celluloses, which are found[6] to degrade 10 times more slowly than *dissolved* amyloses.

Temperature Dependence

The positive dependence of cellobiose degradation (L_∞) on temperature (Table I) means that termination reactions (k_t) are relatively less important than glucosidic cleavage (k_1) at higher temperatures. The same behavior is exhibited by commercial amylose[10] and purified potato amylose.[4] Accordingly, the activation energy of stopping should be lower than that of depropagation, as has indeed been found for cellobiose (Table II) and for amylose.[7] The corresponding results reported[11] for hydrocellulose are, however, anomalous: while L_∞ increased with reaction temperature, the calculated activation energy for termination was paradoxically greater than for depropagation. Arbin et al.[4] consider that this contradiction cannot yet be resolved.

In fact, it has been recognized[2,10,12] that the original treatment of the data[11] was unsatisfactory in that it engendered erroneous values for k_2. More acceptable values may be obtained if termination due to putative inaccessibility is disregarded,[7,10,12] and if due weight is given to termination by endwise unzipping of entire polymeric chains.[2] Using the appropriate expression[2] to recalculate k_2 from the original data[11] for hydrocellulose, the activation energy was evaluated to be *lower* than the value for k_1, as required (Table III). This result provides impressive evidence for the validity of the generalized kinetic theory for unidirectional, endwise degradations of chain polymers and dimers, as developed in our previous paper,[2] and also indicates its applicability to heterogeneous reactions.

Stopping Reaction of Cellobiose

It has been concluded[13] that cellobiose does not undergo a stopping reaction and consequently is not a good model for cellulose, because disaccharide acidic products were not identified in chromatographic separations after degradation of cellobiose in dilute alkali ($< 0.1M$) at low temperatures

TABLE III
Recalculation of k_2 and Activation Energies for Alkaline Degradation of Hydrocellulose[a]

Temperature			Rate constants (h^{-1})			
(°C)	(10^3/K)	L_∞[a]	k_t[a]	k_1[a]	k_2[a] ($= k_t$-k_{cr})	k_2[b] [$= k_t$ (1-L_∞)]
65	2.96	0.15	0.061	4.0	0.0041	0.0518
78	2.85	0.162	0.294	17.8	0.031	0.246
78	2.85	0.40	0.294	20.8	0.056	0.176
87	2.78	0.167	0.74	46.3	0.097	0.616
100	2.68	0.174	2.1	147	0.50	1.73
100	2.68	0.421	2.1	151	0.518	1.22
132	2.47	0.187	20	1500	9.86	16.3
			Least-squares computation[c]			
			ln A	37	40.68	31.5
			E_a (kcal)	23.8	30.7	23.0
			r	−0.998	−0.994	−0.995

[a] Data from Haas et al.,[11] Tables II and IV.

[b] Calculated from data of Haas et al.,[11] using equation $k_2 = k_t$ (1-L_∞) from Ziderman and Belayche.[2]

[c] Computation of $y = \ln k$ and $x = T^{-1}$, according to $\ln k = \ln A$-E_a/RT, where R = 1.987 cal/mol, T = K, E_a is Arrhenius activation energy, and A is the frequency factor.

(< 51°C). However, it is known that the occurrence of 1,4-glucan stabilization is kinetically controlled, requiring sufficiently high alkalinity, substrate concentration and/or degree of polymerization (DP),[2,10,14] a suitable base cation valency,[14] or a requisitely low temperature (see above). Accordingly, under suitable reaction conditions, experimental evidence for a cellobiose stopping reaction has been forthcoming, namely, at 22°C in 1N sodium hydroxide,[15], at 60–90°C in 0.02N sodium hydroxide,[16] at 50°C in 0.15N sodium hydroxide,[16] at 74°C in 0.05N sodium hydroxide[2], and in Table I.

It would appear therefore that experimental characteristics of the stopping reaction do not detract from the validity of studies of a homologous series of 1,4-glucans, in accordance with the concept[17] of the reactivity of functional groups being independent of DP. This principle only applies, however, when reactants can be supplied to the sites of reaction.[17] Accordingly, solid celluloses undergo depropagation more slowly[6] than dissolved substrates.

The "Single-Chain" Mechanism

Hydrocelluloses[18] and hydroamyloses[3] undergo peeling *without* a decrease in average molecular size (viscosity). Three hypotheses have been offered to rationalized this apparent inconsistency. Richards[18] has stated that this phenomenon is accounted for in an end-attack mechanism that is derived[19,20] mathematically from a polymer model with the following properties:

i. The crystallite length parameter is nonuniform and is exponentially distributed.

ii. Crystallites of different lengths contain the same number of chain molecules in cross section.

iii. The end surfaces of the crystallites are uniformly eroded in alkali so that the relative distribution of crystallite lengths is preserved despite the decrease in number of crystallites.

iv. Crystallite length and chain length parameters are equivalent.

v. The rate of weight loss is proportional to the area eroded.

No experimental evidence has been presented for this model, in which assumption (i) is, in fact, inconsistent with the narrow molecular weight distribution of hydrocellulose, which is essentially monodisperse.[21-24] Furthermore, assumption (iii) is not in keeping with the kinetics of peeling.[7] In another explanation, it was suggested[12] that hydrocellulose possesses initially a high polymolecularity, which diminishes significantly by a loss of short polymers that is compensated by shortening of the longer chains. This view too is inconsistent with the narrow MWD of LODP–hydrocellulose.

We have consequently proposed[7,24] that entire molecular chains unzip in alkali, so that chain length distribution in the residual polymer remains unchanged. This model accords well with the degradative behavior of cellulose,[25] poly(methyl methacrylate), and poly(α-methylstyrene)[26] during pyrolysis. A sufficient condition for this "single-chain" mechanism would be that $k_E >> k_2$. Incidentally, it should be pointed out that a *sufficient* condition is not found in the necessary feature that the initial average DP be less than or the same as the peeling chain length ($\nu = k_P/(k_2 + k_3)$), as erroneously stated by Arbin et al.[4] Hydroamyloses in solution may undergo alkaline peeling by this same mechanism, since they too exhibit an LODP, as is found with hydrocelluloses.[2,3,6] Conclusive proof of the validity of a "single-chain" hypothesis would require[3] determination of MWD during peeling of the polymer.

Unified Theory for 1,4-Glucan Unzipping

In the case of an unhydrolysed potato amylose, we have shown[3] that peeling cannot proceed by a "single-chain" process. On independently corroborating this finding, Arbin et al.[4] have contended that it invalidates our theoretical kinetic treatment[2] of unidirectional, endwise degradation of polymers and dimers.

We take issue with this view, and wish to clarify here that the kinetic model itself[2] is actually independent of the "single-chain" assumption. The model accounts for polymer shortening in two competitive modes, one of which is terminated by a chemical stopping reaction (described by coefficient k_2), and the other by complete lengthwise depropagation (described by coefficient k_3). This concept should be generally applicable with a *limiting* case of "single-chain" degradation, when $k_3 >> k_2$. In our treatment,[2] the "single-chain" assumption was only invoked in order to derive an equation for the empirical evaluation of k_3. Although the relationship thereby obtained evidently does not apply to the case of unhydrolysed potato starch, it may—in principle—still be valid for amyloses having a different MWD function, and it appears to be correct for maltose, cellobiose, hydrocellulose, and hydroamylose, including the assumption[2] that k_3 is independent of the time variable.

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