Simplified Hydrolysis of Cellulose and Substituted Cellulose: Observations on Trifluoroacetic Acid Hydrolyses

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Synopsis

Hydrolyses of selected soluble polysaccharides (e.g., polyoses) and disordered and crystalline celluloses, each bearing 2-diethylaminoethyl (DEAE) substituents at low degree of substitution (DS < 0.05) were explored in 100% and 2*N* trifluoroacetic acid (TFA). Analyses were evaluated relative to hydrolysis with 72% sulfuric acid. DEAE–polyoses hydrolyzed readily in 100% and 2*N* TFA during extended reaction periods with complete liberation of DEAE–glucoses. Hydrolyses of cellulose and DEAE–celluloses in 100% TFA were critically dependent upon cautious incremental dilution of the TFA during stepwise reaction to avoid precipitation of incompletely hydrolyzed products. DEAE–celluloses were partially solubilized and hydrolyzed in heterogeneous reaction in 2*N* TFA at 121°C. Complete liberation of DEAE–glucoses in the solubilized components was achieved after supplementary hydrolysis at 121°C. In all hydrolyses, including those with 72% sulfuric acid, extended hydrolyses were essential for complete liberation of the 2-O- and 6-O-DEAE–glucoses. Under optimum conditions, results of hydrolyses in 100% TFA were comparable to those in 72% sulfuric acid. Ease of release of DEAE–glucopyranoses in hydrolyses of DEAE–cellulose in 2*N* TFA was in the same order (3-O- ≫ 6-O- > 2-O-) as in homogeneous hydrolyses in sulfuric acid and in 100% TFA. Differences in release of isomeric DEAE–glucoses were greater in the heterogenous system. Degradation of glucose and DEAE–glucopyranoses occurred in extended periods of reaction in all acidic media. Rates of degradation of glucose and DEAE–glucopyranoses in dilute acid at 100°C were similar for glucose and 6-O-DEAE–glucose. The rates were about half as fast for 3-O-DEAE–glucose and an order of magnitude lower for 2-O-DEAE–glucose.

INTRODUCTION

Hydrolysis of polysaccharides to component sugar units is a common and critical step in structural analyses. Soluble polysaccharides (e.g., polyoses) are broken down to component monosaccharides in 2*N* trifluoroacetic acid (TFA)[1] and 100% TFA,[2] but cellulose is less tractable. The ineffectiveness of 2*N* TFA for hydrolyzing cellulose has been aptly illustrated by Meinert and Delmer[3] whereas success with 100% TFA has been reported by Fengel and Wegener.[2] In examination of hydrolyses of cotton cellulose and derivatized cotton cellulose with 100% TFA and 2*N* TFA we encountered difficulties and discrepancies that were not evident to us in reports of those preceding studies.

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These problems may be small or nonexistent with some polysaccharides, but they are critical in our studies of substituted celluloses and likely important in studies involving substituted or branched chain polysaccharides. In a recent report on hydrolyses of carboxyethylcelluloses and carboxymethylcelluloses with sulfuric acid and with TFA, Kasulke et al.[4] assessed completion of hydrolyses by enzymatic determination of the glucose content of the hydrolyzate for comparisons to theoretical glucose yields based on Spurlin's model of substituent distribution. They touched on some of the problems we report here, e.g., effectiveness and completeness of hydrolysis, degradation of substituted glucoses, and retention of TFA.

Our studies involve 2-diethylaminoethyl (DEAE) celluloses at low degree of substitution (DS < 0.05) but include, in this report, explorations of DEAE–polyoses, also at low DS. These derivatized substrates hydrolyze partially with facility generally similar to that of the original polysaccharide. However, release of substituted glucoses occurs less readily than release of glucose. It is essential in our studies of derivatized celluloses that we realize total hydrolyses (or determine the deviation from total hydrolyses) of all substituted D-glucopyranosyl units in the cellulose chain.[5,6]

There is a general need in structural studies of cellulose and certainly our studies of substituted celluloses[5,6] for a method of hydrolysis of crystalline celluloses that is substantially simpler than that involving sulfuric acid[5] while retaining the reliability and accuracy of the sulfuric acid method. Additionally, we have sought a method in which the hydrolysis of cellulose or substituted celluloses may be carried out progressively in order to fully characterize acid-sensitive regions that are loci of molecular degradation[7] in the cotton fiber.

We consider here (a) a procedure for complete hydrolysis of cellulose and substituted celluloses with 100% TFA and (b) a method of partial progressive hydrolysis of cellulose derivatives in heterogeneous 2*N* TFA medium.

EXPERIMENTAL

Materials. The cotton was clean mature fiber harvested by hand at 104+ days post-anthesis in the course of preceding studies.[5,6] Disordered cellulose was prepared by ball milling purified cotton fibers in an air-cooled vibratory mill.[8] The mature fibers were 80% lattice I, degree of polymerization above 3000; disordered cellulose exhibited only amorphous scatter, degree of polymerization about 500. The amylose was from K & K Laboratories,* Plainview, NY 11803. Curdlan, which was free of other glucans, was a gift of Prof. Tokuya Harada, Osaka University, Japan. Pustulan (*Umbilicara papullosa*) from Calbiochem-Behring, LaJolla, CA 92037 was deacetylated by dissolving 2 g in 175 mL of hot water, adding 25 mL of 2*N* NaOH while the solution of pustulan was maintained at 100°C, and agitating without heat for 18 h. The solution was neutralized with 2*N* HCl, and pustulan was precipitated with 500 mL of 95% ethanol. The dried product (1.8 g) showed no evidence of a carbonyl peak in IR spectra. Technical grade 2-chloroethyldiethylamine hy-

*Names of companies or commercial products are given solely for the purpose of providing specific information; their mention does not imply recommendation or endorsement by the U.S. Department of Agriculture over others not mentioned.

drochloride from Hexagon Laboratories, Bronx, NY 10469, was recrystallized from 95% ethanol and dried under vacuum. *N,N*-Diethylaziridinium chloride (DAC) was prepared as needed from 2-chloroethyldiethylamine, which was liberated from the hydrochloride with an equimolar amount of 50% NaOH. The free amine was dried over anhydrous magnesium sulfate, filtered, weighted, and converted to DAC by dissolution in water at the specified molarity.[8,9]

Preparation of 2-Diethylaminoethyl (DEAE) Polysaccharides. A weighed sample of polysaccharide was contacted with 0.11*M* DAC (for soluble polyoses, amylose, pustulan, curdlan) in 0.5*N* NaOH or with 0.55*M* DAC (for substrates insoluble in water, e.g., cellulose) in 0.5*N* NaOH. Each reaction was maintained at 20°C for 10 min. The ratio of reagent to polysaccharide ranged from 40 to 100 mL/g. Reaction was terminated promptly at 10 min duration by neutralizing and flushing with an excess of 0.5*M* acetic acid. The acetic acid solution for polyoses contained ethanol or tertiary butanol to effect precipitation. Totally aqueous quenches were used for celluloses. Products were filtered and washed. Products from polyoses and disordered cellulose were washed four times with increasing concentrations of alcohol from 50 to 100% and finally with ethyl ether. Other products were washed four times with water. All were air-dried. The products were DEAE–polysaccharides with DS below 0.05 (based on nitrogen contents of products).

Hydrolysis of DEAE–Polysaccharide to Component Monosubstituted Glucoses with 72% Sulfuric Acid.[7] An accurately weighed sample of DEAE–polysaccharide (about 0.25 g if based on cotton fibers, 0.1–0.5 g if based on disordered cellulose or polyose) was swollen in 3.75 g of cold 72% sulfuric acid for 7–8 h in under argon; the flask initially immersed in ice bath and subject to mild agitation was allowed to warm to room temperature in about 3 h. The solution was diluted with 5 g of ice and water and stirred overnight, whereupon it was diluted with 30 mL deionized water (final concentration 1.48*N*) and refluxed for the period of time designated in the results. The solution was neutralized with 8.67 g $Ba(OH)_2 \cdot 8H_2O$. Accurately measured internal standards (0.5 mg of inositol and 0.8 mg of phenyl *beta*-D-glucoside) were added to the hydrolyzate before centrifugation and filtration. The glucose was removed from concentrated (Roto-Vac) hydrolyzates after adjustment of pH to 4–4.5 by rapid fermentation at 36–37°C for 2–3 h with 0.15 g of Fleishmann's active dry yeast (Nabisco Brands, East Hanover, NJ 07936) for each 0.25 g of original DEAE–polysaccharide. The filtered fermentate was concentrated under reduced pressure and freeze-dried. In many cases, the sample was divided into two accurately measured parts and analyses pursued with and without fermentation.

Hydrolysis of DEAE–Polysaccharide to Component Monosubstituted Glucose with 100% TFA.[2] An accurately weighed sample (see above) was covered with 20 mL of 100% TFA and swollen overnight at room temperature in the case of polyoses[2a] and disordered cellulose or at 36–37°C in the case of DEAE–polysaccharides that were insoluble in water.[2b] The resulting clear solution was refluxed for 1 h under argon and then gradually diluted with water to 85, 67, 46, and 30% (w/w) TFA with 15–25 m reflux periods for the first three concentrations and for periods specified in the results for the final 2.9*N* (30%) concentration. Fewer dilutions were required for polyoses and

disordered cellulose. To the cooled hydrolyzate were added accurately measured amounts 0.5 mg inositol and 0.8 mg phenyl *beta*-D-glucoside) of internal standards. The solution was evaporated to dryness (Roto-Vac). Three additional evaporations were conducted after additions of 15–20 mL of water. The final residue was redissolved again in water and freeze-dried or subjected to fermentation (see above) and then concentrated and freeze-dried.

Hydrolyses of DEAE–Polysaccharides with 2*N* TFA.[1] An accurately weighed sample of DEAE–polysaccharide (about 0.25 g) was covered with 25 mL of 2*N* TFA in a thick-walled serum bottle of 50 mL capacity. The tightly sealed bottle was shaken in an autoclave with a special apparatus[10] for 30 min at 121°C. Solubilization was complete for amylose, pustulan, and curdlan. In the case of celluloses the reaction mixture was centrifuged and the supernatent solution withdrawn to a round-bottom flask containing internal standards (see above). The undissolved solid was rinsed with 5 mL of 2*N* TFA. The supernatent solution, combined with rinsings, was reduced to dryness by evaporation under reduced pressure with the heating bath maintained below 40°C. The resulting residue was thrice dissolved in 15 mL of water and distilled to dryness under reduced pressure. The final residue was dissolved again in water and freeze-dried, or fermentation was conducted as described above. In selected cases, the solid that remained undissolved from hydrolysis in the autoclave was subjected to additional hydrolysis in 2*N* TFA in the manner described here.

Supplementary Hydrolysis. This treatment, applied to clear hydrolyzate solutions, was conducted in the tightly sealed thick-walled serum bottles of 50 mL capacity. Reactions were carried out in an autoclave at 121°C for stated durations. Hydrolyzate solutions were transferred quantitatively to round-bottom flasks containing internal standards and treated as the supernatant solution above.

Degradation of Glucose and DEAE–Glucoses in Acidic Media. Solutions of glucose and DEAE–glucoses were prepared from the hydrolyzate of 2 g of DEAE–cellulose with 72% sulfuric acid and a final reflux stage of 6 h. Following neutralization with barium hydroxide and centrifugation, supernatent solutions were reduced to dryness, dissolved in water, filtered (Millipore 0.45 μm) and dried. Abundance of 2-O-, 3-O-, and 6-O-DEAE–glucoses was in the ratio 2.20 : 0.24 : 1.00. Aliquots of this combination of glucose and DEAE-glucoses were subjected to 1.48*N* sulfuric acid, 2.9*N* TFA, and 2.0*N* TFA under conditions used in final stages of hydrolyses with 72% sulfuric acid and 100% TFA and throughout the hydrolysis of 2.0*N* TFA. Freeze-dried products were analyzed in the usual manner (below).

Analyses of Products. The freeze-dried product from hydrolysis of the DEAE–polysaccharide or the freeze-dried product after fermentation was silylated with trimethylsiylimidazole.[5,6] Gas–liquid chromatography was conducted on a 56 m × 0.25 mm DB-1 bonded fused silica capillary column (J & W Scientific, Inc., Rancho Cordova, CA 95670) with film thickness of 0.25 μm at 200°C with a He flow of 19 cm^3/s and flame ionization detection. Peaks of 2-O-, 3-O-, and 6-O-DEAE–glucoses were identified according to procedures described in preceding studies.[11] The peaks for 4-O-DEAE–glucose were delineated from analysis of reaction products of DAC with methyl *beta*-D-glucopyranoside and with curdlan (a 1,3-glucan).[5,12]

TABLE I
Hydrolyses of DEAE–Polyoses with TFA

Polyose	System	Yield[a, b] 2-O-DEAE–Glu	3-O-DEAE–Glu	4-O-DEAE–Glu	6-O-DEAE–Glu
Amylose	2*N* TFA	0.97	1.01	—	1.05
	100% TFA	0.86	0.94	—	0.98
Curdlan	2*N* TFA	0.90	—	0.97	0.95
	100% TFA	0.90	—	1.03	0.96
Pustulan	2*N* TFA	0.79	0.80	1.01	—
	100% TFA	0.86	0.93	0.98	—

[a] Relative to the yield from hydrolysis with 72% sulfuric acid involving fermentation and 6-h reflux at the final stage of dilution. DEAE–Glu = DEAE–glucose.

[b] In the absence of the fermentation step before chromatographic measurement of DEAE–Glu's from DEAE–amylose, yields of 2-O-, 3-O-, 4-O- and 6-O-DEAE–Glu were increased by 10, 11, 106, and 10%.

Reproducibility. The basic information obtained as mg of 2-O-, 3-O-, 4-O-, and 6-O-DEAE–glucoses per g of DEAE-polysaccharide with $n = 3$ was characterized by relative standard deviations of 1–4%.

RESULTS

Exploratory Hydrolyses. Results of hydrolyses of DEAE–polyoses (DS 0.01–0.05) with 2*N* TFA by the procedure of Albersheim et al.[1] (1 h reaction) and with 100% TFA by the procedure of Fengel and Wegener[2] for soluble polysaccharides but with double the final reflux period (2 h) are summarized in Table I. Differences in yields were small and some were within experimental variation. However, we observed consistently lower recoveries of 2-O-DEAE–glucose in comparison to recoveries of the other isomeric DEAE–glucoses.

Hydrolyses with 72% Sulfuric Acid. We employed hydrolysis with 72% sulfuric acid as the basis of comparison with other hydrolyses, expressing yields of DEAE–glucoses from hydrolyses with TFA as fractions of the corresponding yield measured from hydrolysis with 72% sulfuric acid. As illustrated in Figure 1, a 6-h final stage of hydrolysis proved adequate for mature cotton fibers and disordered (ball-milled) cellulose. The completeness of hydrolysis in the 6-h period depends on the effectiveness (i.e., duration, temperature) of each of the preceding stages. The 6-h period is that which was considered essential for complete hydrolysis[13] and which was employed in all our preceding hydrolyses of substituted celluloses,[14] although selective liberation of individual substituted glucoses in homogeneous sulfuric acid hydrolyses was not recognized at that time. In the absence of an absolute measure of DEAE–glucose in the hydrolyzate, 6 h represents a compromise between adequate time for liberation of individual substituted glucoses and minimum time for degradation of these products.

Progressive Recoveries of DEAE–Glucoses from Hydrolysis of DEAE–Amylose with 2*N* TFA. Results from DEAE–amylose, hydrolyzed by the general procedure of Albersheim et al.[1] for increasing periods of time, are summarized in Figure 2. Yields of DEAE–glucoses as fractions of those

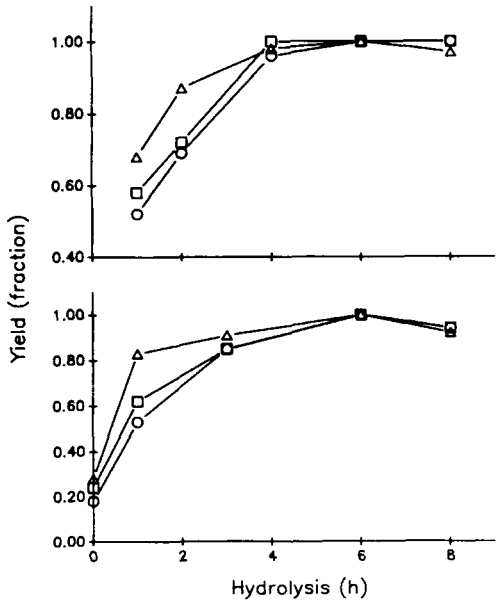

Fig. 1. Yields of DEAE–glucoses from DEAE–mature cotton (upper curves) and from DEAE-disordered cellulose (lower curves) by hydrolyses with 72% sulfuric acid. Data are presented as fractions of the individual yields measured at 6-h reflux at the final level of dilution: (○), 2-O-; (△), 3-O-; (□) 6-O-DEAE–glucose.

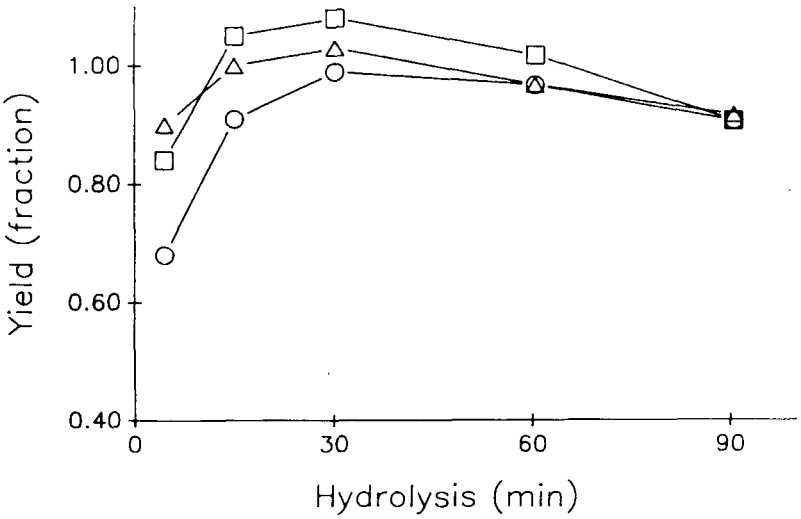

Fig. 2. Recoveries of DEAE–glucoses from hydrolysis of DEAE–amylose with 2*N* TFA. Yields are expressed as the fraction obtained for the same product from hydrolysis with 72% sulfuric acid. Symbols have the same meaning as in Figure 1.

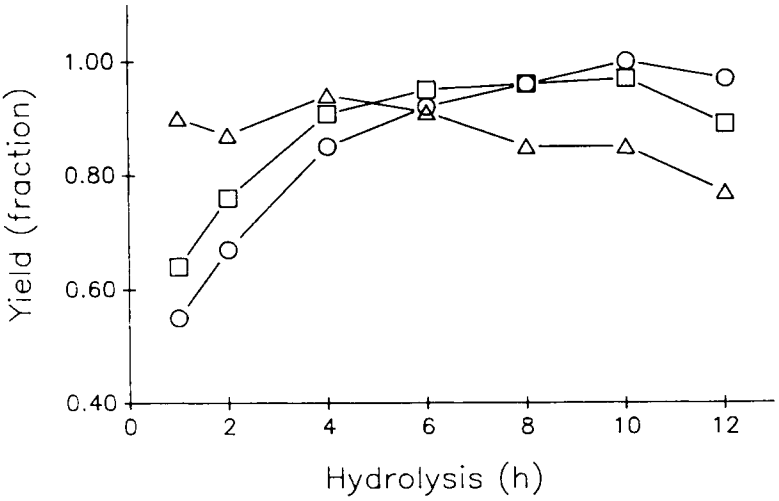

Fig. 3. Recoveries of DEAE-glucoses from hydrolyses of DEAE-mature cotton with 100% TFA. Yields are the fractions obtained for the same product from hydrolysis with 72% sulfuric acid. Symbols have the same meaning as in Figure 1.

measured with 72% sulfuric acid decreased after maxima at 30-min reaction. Albersheim et al.[1] reported that the hydrolysis components (except glucose) from cell walls of pinto-bean hypocotyls peaked in the range of 15–60 min.

Hydrolysis of DEAE-Cellulose with 100% TFA. For total hydrolysis with 100% TFA it is important that dissolution of cellulose and DEAE-celluloses be complete and that the initial solution and subsequent partial hydrolyzates be diluted only to the extent that sparkling clear solutions were maintained. A precipitate of cellulose is difficult to resolubilize. When lignin is present together with the cellulose, turbidity from a precipitate of lignin is inevitable. Frequent dilutions during hydrolysis with adequate hydrolysis periods are essential. Our hydrolysis procedure calls for three times the final reflux period stated by Fengel and Wegener[2] for the most resistant (high lignin content) cellulose. The hydrolysis of DEAE-mature cotton cellulose is illustrated in Figure 3. The yield of 3-O-DEAE-glucose decreased beyond 4 h hydrolysis whereas yields of 2-O- and 6-O- products increased to near unity at 10 h reflux at the final level of dilution. A similar pattern of maximum for the 3-O-glucose at 4 h and maxima for 2-O- and 6-O-DEAE-glucoses at 10 h was observed for corresponding analysis of DEAE disordered cellulose (more readily solubilized) with 100% TFA.

Hydrolyses of DEAE-Celluloses with 2*N* TFA. Treatment of DEAE-mature cotton with 2*N* TFA at 20°C for 24 h caused no measurable solubilization; 1 h at reflux released traces of DEAE-glucoses; 8.5 min at 121°C with agitation in a sealed container that was heated in a sterilization autoclave[10] released substantial amounts of DEAE-glucoses. Progressive recovery of solubilized materials from hydrolysis at 121°C is illustrated in upper curve of Figure 4. Yields of 2-O-, 3-O-, and 6-O-DEAE-glucoses in the soluble hydrolyzate are shown in the three lower curves. At this stage of hydrolysis, soluble components were partly in the form of DEAE-cellulose oligomers and cellulose oligomers. This was clarified by peaks observed in high-temperature

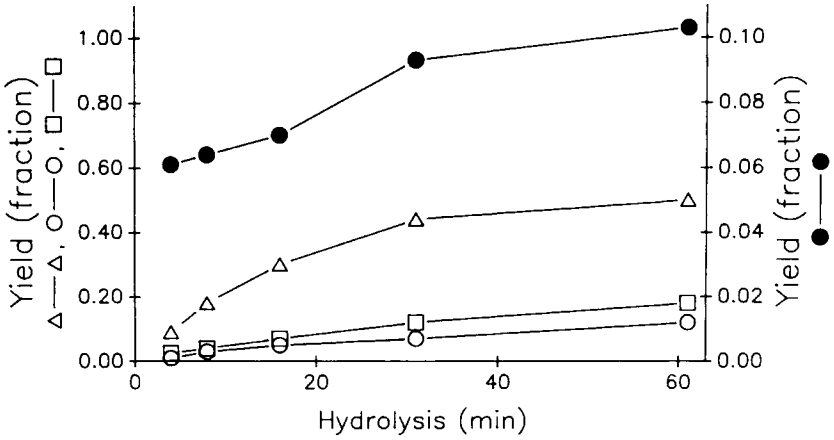

Fig. 4. Yields of products in soluble fractions from hydrolysis of DEAE-mature cotton with 2*N* TFA. Top curve, right scale: yield of products from solubilized DEAE-mature cotton (●) from hydrolysis with 2*N* TFA. Lower three curves, left scale: yields of 3-O-DEAE-glucose (△), 6-O-DEAE-glucose (□), and 2-O-DEAE-glucose (○) in the soluble phase as a fraction of the total 3-O-, 6-O-, or 2-O-DEAE-glucose measured in total hydrolysis of the original DEAE-mature cotton with 72% sulfuric acid.

chromatograms and by increases in yields of DEAE-glucoses upon supplementary hydrolysis of the soluble fraction. Data plotted in Figure 5 show increases in yields of DEAE-glucoses when the clear filtrate from hydrolysis of DEAE-disordered cellulose was subjected to various periods of supplementary hydrolysis in the autoclave at 121°C. Maximal increases in yields of the individual DEAE-glucoses from the supplementary treatment were 3% for

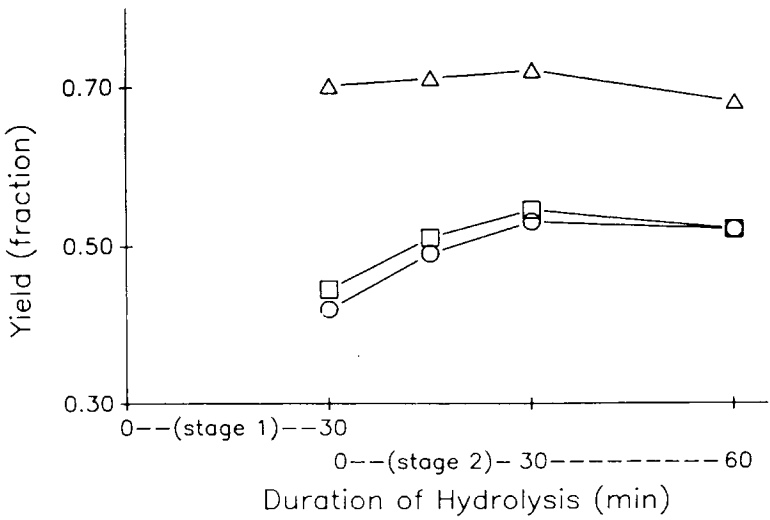

Fig. 5. Effect of supplementary hydrolysis for various periods of time on yields of DEAE-glucoses from DEAE-disordered cellulose with 2*N* TFA. Supplementary treatment was conducted at 121°C after removal of the insoluble component remaining after 30-min initial hydrolysis with 2*N* TFA. Symbols have the same meaning as in Figure 1.

TABLE II
First-Order Rates of Disappearance of Glucose and DEAE-Glucoses in Acidic Media

Substrate	Rates of degradation (10^{-6} s^{-1})		
	1.48*N* sulfuric	2.9*N* TFA	2*N* TFA
Glucose	1.0	1.1	15
2-O-DEAE-glucose	0.1 (0.77)[a]	0.1 (0.99)	5 (0.99)
3-O-DEAE-glucose	0.6 (0.62)	0.5 (0.96)	10 (0.68)
6-O-DEAE-glucose	1.1 (0.05)	0.9 (0.56)	8 (0.87)

[a] Confidence levels (1-*p* value) for significant differences in rates of degradation of the isomeric DEAE-glucose and glucose are shown in parentheses, i.e., there is a 77% probability that the rate of degradation of 2-O-DEAE-glucose in 1.48*N* sulfuric acid is different from that of glucose.

the 3-O- product, 26% for the 2-O- product, and 23% for the 6-O- product. This pattern of increase was characteristic of supplementary hydrolyzates from DEAE-celluloses in 2*N* TFA. The maxima were reached around 30–45 min of supplementary hydrolysis. The 3-O-DEAE-glucose was marginally ahead of the 2-O- and 6-O-DEAE-glucoses in undergoing degradation.

Three sequential 30-min hydrolyses of DEAE-mature cotton fibers, each with 30-min supplementary treatment of the clear hydrolyzate, resulted in increases in total hydrolyzate of 18 and 11% from the second and third treatments, respectively. The proportion of 2-O- and 6-O-DEAE-glucoses relative to 3-O-DEAE-glucose increased in the second treatment by 18% and in the third treatment by 50%, but actual increases in total yield of solubilized material was raised only from 10% in a single treatment to 13% in a triple treatment.

First-order rate constants from statistical treatment of decreasing concentrations of glucose and DEAE-glucoses during treatment in 1.48*N* sulfuric acid (100°C), 2.9*N* TFA (100°C), and 2.0*N* TFA (121°C) are summarized in Table II. For treatments at 100°C in 2.9*N* TFA significant differences in rates of degradation between individual DEAE-glucoses and glucose are indicated for 2-O- and 3-O-DEAE-glucoses. Results are similar for treatments in 1.48*N* sulfuric acid but with lower confidence levels. Rates in 2.0*N* TFA at 121°C are 14, 50, 20, and 9 times those of glucose and 2-O-, 3-O- and 6-O-DEAE-glucoses in 2.9*N* TFA and 100°C.

DISCUSSION

Hydrolysis of DEAE-polysaccharides with 72% sulfuric acid[5,14,15] has been employed in preceding studies without evidence of incomplete hydrolysis or uncontrolled destruction of products. With refinements in operations and procedures from the original[13] to the current operation,[5] precision and reliability have been improved. Although still lacking an absolute verification of the accuracy of the method, this report finds that the results from hydrolysis with 72% sulfuric acid stand up well as a basis for comparison of results obtained via 100% TFA and 2*N* TFA.

Current results confirm an earlier conclusion[13] that 6-h reflux in the final stage for dilution for the hydrolysis of DEAE–cellulose with 72% sulfuric acid constitutes a good balance for completion of hydrolysis with minimum degradation of products. This period of hydrolysis is now appreciated to be essential for complete hydrolyses of the slowest hydrolyzing isomeric monosubstituted DEAE–glucopyranosyl unit.[15]

The total analytical hydrolysis of cellulose with sulfuric acid, as we employed it over a period of many years, involves (a) cold dissolution, (b) stepwise hydrolysis, (c) neutralization, (d) fermentive concentration, (e) derivatization, and (f) gas-chromatographic analysis.[5,15] The operation may be simplified by employing 100% TFA in place of 72% sulfuric acid to eliminate step (c) which is particularly cumbersome. Frequently, we found it desirable with 100% TFA to retain step (d) since in our hands it proved to be more reliable with multiple distillation as a means of breaking up TFA–glucose adducts and realizing agreement in yields of substituted glucoses with those from 72% sulfuric acid. The original reason for introducing step (d) into our analytical hydrolysis was to remove the overwhelming excess of glucose over DEAE–glucoses and to eliminate column overloading. Improved columns have reduced the need for this step in hydrolyses with 72% sulfuric acid. In the absence of step (d) or an effective breakdown of the TFA adducts, yields of 2-O-, 3-O-, and 6-O-DEAE–glucoses were higher by 10% or more than those measured with the sulfuric acid process (with or without fermentation). The 4-O-DEAE–glucose, which was present in very small amounts, was subject to large variations in analyses conducted without fermentation. Under the best conditions, reliability of the 100% TFA analytical hydrolysis was equivalent to that of the 72% sulfuric acid analysis.

Yields of DEAE–glucoses reached peaks in the course of hydrolyses with 100% TFA (and also with 72% sulfuric acid). Where differences were noted among the isomeric DEAE–glucoses, the 3-O-DEAE–glucose exhibited most extensive degradation, probably because this product was liberated earliest and subject to degradation longest. The hydrolysis with 100% TFA generated the flattest peaks, suggesting mildest degradation among the processes described here.

Hydrolyses of DEAE-celluloses with 2*N* TFA occurred in two stages: (a) solubilization and partial hydrolysis of the more accessible segments of the cellulose and (b) completion of hydrolysis of soluble components to glucose and DEAE–glucoses. Although the absolute yield of 2-O-DEAE–glucose is greater than that of the 3-O-DEAE–glucose at any stage of all hydrolyses, the predominance in relative yield (see footnote a of Table III) of 3-O-DEAE–glucose in this heterogeneous hydrolyzate was substantially greater than those already noted for products from hydrolyses with 72% sulfuric acid or with 100% TFA. The dominance is indicated by the ratio of yield of 3-O-DEAE–glucose to yield of 2-O-DEAE–glucose (or 6-O-DEAE–glucose) for which values are summarized in Table III. We reported earlier[15] that sensitivity of the glucosidic linkage to cleavage in homogeneous solution was influenced by the location of a DEAE substituent. Stability toward acid hydrolysis increased in the order 3-O- $\ll$ 6-O- $<$ 2-O-DEAE– substitution. Data in Table III show that differences in resistance between the 3-O-substituted glucopyra-

TABLE III
Dominance in Yield[a] of 3-O-DEAE-Glucose over 2-O-DEAE-Glucose in Hydrolyzates of DEAE-Mature Cotton

Hydrolyzing agent	Type of hydrolysis	Ratio yield 3-O-DEAE-Glu/yield 2-O-DEAE-Glu	
		At early stage of hydrolysis	At completion of hydrolysis
72% sulfuric acid	Homogeneous solution	1.33 at 1 h[b] 1.30 at 2 h	1.0 (6 h)
100% TFA	Homogeneous solution	1.62 at 1 h[c] 1.33 at 2 h	1.0 (6 h)
2*N* TFA	Heterogeneous phase	3.92 at 15 min plus[d] 3.73 at 60 min plus 3.17 at 120 min plus	[e]

[a] Relative to the total yield with 72% sulfuric acid involving fermentation and 6 h reflux at the final stage of dilution.

[b] Hours of reflux in the final stage of hydrolysis. Refer to curves in Figure 1 for hydrolysis of DEAE-mature cotton.

[c] Hours of reflux in the final stage of hydrolysis. Refer to curves in Figure 3 for hydrolysis of DEAE-mature cotton.

[d] Minutes of reaction of 121°C, in each case followed by 30–45 min of supplementary hydrolysis of the clear solution.

[e] Complete dissolution of the cotton was not attained in these consecutive treatments of 30 min each or in extended treatment of 120 min at 121°C.

nosyl unit and the 2-O-substituted glucopyranosyl unit are increased in heterogeneous hydrolysis of DEAE-celluloses.

Rates of degradation of glucose and DEAE-glucoses in acidic media exhibited a specific dependency upon presence and location of the DEAE substituent. Rates varied from a high glucose (1×10^{-6} s^{-1}) in dilute acid at 100°C to a low for 2-O-DEAE-glucose (1×10^{-7} s^{-1}). The higher rates in 2*N* TFA at 121°C are attributable to the elevated temperature.

The abundance of 3-O-DEAE-glucose relative to 2-O-DEAE-glucose resulting from hydrolysis of DEAE-cellulose in 2*N* TFA is (a) the sequence of high availability of tagged O(3)H in strained accessible segments of elementary fibrils, which segments are especially sensitive to acid hydrolysis[7] and (b) the consequence of the relatively high resistance of 2-O- and 6-O-DEAE-glucopyranosyl units in cellulose to hydrolysis in acidic media.[15]

At this time it is not possible to compensate or correct the ratio of 2-O- : 3-O- : 6-O-DEAE-glucoses in hydrolysates on the basis of specific rates of degradation of the isomeric DEAE-glucoses without more information on the concentration of each of the isomeric DEAE-glucoses throughout the hydrolysis.

CONCLUSIONS

Hydrolysis of cellulose, including mature cotton fibers and DEAE-mature cotton fibers, into glucose and substituted glucoses in 100% TFA is readily accomplished with primary dependence upon complete dissolution, dilution

without precipitation, and adequate hydrolysis at each level of TFA concentration in the process.

Supplementary hydrolysis, essential for completion of hydrolysis of oligomers in the clear hydrolyzate from DEAE–mature cotton with 2*N* TFA occurs readily at 121°C in an autoclave.

Analyses of DEAE–glucoses from hydrolyses with 100% TFA conducted with the fermentation step or thorough hydrolytic dissociation of TFA adducts agreed closely with results from hydrolysis with 72% sulfuric acid (with or without the fermentation step). In the absence of complete dissociation of TFA adducts, analyses following hydrolysis with 100% TFA are generally about 10% higher than those from sulfuric acid hydrolysis.

Glucose and DEAE–glucoses undergo degradation during extended treatments in acidic media. The rate of degradation of 2-O-DEAE–glucose at 100°C is about 10% of that of glucose or 6-O-DEAE–glucose. The 3-O-DEAE–glucose degrades at a rate approximately midway between these extremes.

Differences in rates of liberation of 2-O- and 3-O-DEAE–glucoses are accentuated substantially in hydrolyses of DEAE–cellulose in heterogeneous media.

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