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INFLUENCE OF THE LOCATION OF SUBSTITUENTS IN SUBSTITUTED CELLULOSES ON SOLUTION HYDROLYSIS OF THE D-GLUCOSIDIC LINKAGES

STANLEY P. ROWLAND* AND PHYLLIS S. HOWLEY**
*Southern Regional Research Center***, New Orleans, Louisiana 70179 (U.S.A.)*
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ABSTRACT

Sensitivity of the D-glucosidic linkages in cellulose to hydrolysis in homogeneous acidic media was found to be directly related to the location of a substituent in the D-glucoyranosyl unit. The 2-diethylaminoethyl (DEAE) substituent caused sensitivity toward hydrolysis to decrease in the order D-glucose > 3-*O* >6-*O*- ≅ 2-*O*-DEAE-D-glucopyranosyl-unit in hydrolyses beginning in 72% sulfuric acid and in 100% trifluoroacetic acid (TFA). Differences in the substituent effects were larger in TFA than in sulfuric acid. The effects reported for acid-catalyzed hydrolyses in homogeneous media are discussed relative to enzymic hydrolysis of a water-soluble, *O*-substituted cellulose.

INTRODUCTION

Much definitive information concerning the microstructure of cellulose has been obtained over a period of many years through heterogeneous hydrolysis of cellulose in acidic media[1]. This approach was extended in the recent decade to substituted celluloses[2]. In contrast, homogeneous hydrolysis of cellulose and cellulose derivatives has been generally limited to cleaving the cellulose chain into component D-glucose and substituted D-glucose units for subsequent analyses. For this purpose, dissolution in 72% sulfuric acid followed by progressive reflux and dilution has been generally employed[3,4]. More recently, a hydrolysis procedure starting in 100% trifluoroacetic acid (TFA) has been recommended for its brevity and the simplicity of its operations[5].

From examination of homogeneous hydrolyses of a substituted cellulose, we have found that a substituent in the cellulose chain affects the rate at which D-glucosidic linkages are cleaved. The location of the substituent in the D-glucopyranosyl

*Present address: 16343 Old Hwy 99 SE, Tenino, WA 98589, U.S.A.
**To whom communications should be addressed.
***One of the facilities of the Southern Region, Agricultural Research Service, U.S. Department of Agriculture.

unit has a specific bearing on the resistance to hydrolysis of the D-glucosidic linkage. The dependence of resistance of the D-glucosidic linkage in acid-catalyzed hydrolysis upon location of a substituent has escaped recognition until now. The relationship appears pertinent to resistance of water-soluble, substituted celluloses to enzymic hydrolysis and to potential generation of cotton fibers having superior resistance to degradation during and following acid-catalyzed, chemical-finishing reactions. We report here the homogeneous hydrolyses of (2-diethylaminoethyl) (DEAE)-cellulose at very low degrees of substitution (*i.e.*, consisting essentially of mono-DEAE-substituted D-glucosyl units) in processes beginning in 72% sulfuric acid and in 100% TFA. The DEAE substituent is especially interesting in this regard, because it can be introduced into cellulose with little or no alteration of the microstructural features of the cellulose[2].

EXPERIMENTAL

Materials. — The cotton was clean, mature fiber harvested by hand at 104+ days post anthesis[6,7]. This cotton is typically 80% crystalline (X-ray diffraction) with degree of polymerization (d.p.) >3000. Disordered cellulose was prepared by ball-milling purified cotton fibers in an air-cooled, vibratory mill[7,8]. This cellulose shows none of the X-ray diffraction peaks characteristic of crystalline celluloses, and has a d.p. of ~500. Technical grade 2-(2-chloroethyl)diethylamine hydrochloride (Hexagon Laboratories, Bronx, N.Y., U.S.A.*) was recrystallized from 95% ethanol and dried under vacuum. *N,N*-Diethylaziridinium chloride (DAC) was prepared, as needed from 2-(2-chloroethyl)diethylamine which was liberated from the hydrochloride with an equimolar amount of 50% NaOH. The free amine was dried (anhydrous magnesium sulfate), filtered, weighed, and converted into DAC by dissolution in water at the specified molarity[8,9]. The wetting agent was Protowet-100 (Proctor Chemical Co., Salisbary, N.C., U.S.A.).

Preparation of DEAE-celluloses. — A weighed sample of cellulose was mixed with 0.55M DAC in 0.5M NaOH, and maintained for 10 min at 20°. The ratio of reagent solution to cellulose was 500 mL/16 g for cotton fibers, and 50 mL/1.5 g for disordered cellulose. Two drops of wetting agent were added. Reaction was terminated by diluting the mixture, and neutralizing the base with 0.5M acetic acid in water for fibers, or in tertiary butyl alcohol (TBA) for disordered cellulose. The product was separated by filtration, and washed 3–4 times with an excess of water or 50% TBA.

Homogeneous hydrolysis of DEAE-celluloses with sulfuric acid. — A weighed sample of DEAE-mature cotton (0.2500 g) or of DEAE-disordered cellulose (0.0500–0.1000 g) was dissolved in cold, 72% (w/w) sulfuric acid, and hydrolyzed to D-glucose and DEAE-D-glucoses during stepwise dilution[10]. The final

*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.

reflux stage of this hydrolysis, commonly 6 h, was conducted for various periods of time in separate experiments, as reported in the results. To each sample were added inositol (0.5000 mg) and phenyl β-D-glucoside (0.8000 mg) immediately after neutralization of the sulfuric acid with barium hydroxide. The D-glucose was removed by rapid fermentation for 3 h at 36–37° with 0.15 g of Fleischmann's active dry yeast for each 0.25 g of original DEAE-cellulose. The filtered fermentate was evaporated under diminished pressure, and freeze-dried. In experiments for measurement of release of D-glucose from cellulose, the fermentation step was not used.

Homogeneous hydrolysis of DEAE-cellulose with TFA. — A weighed sample of DEAE-mature cotton (0.2000 g) or of DEAE-disordered cellulose (0.0500–0.1000 g) was covered with 100% TFA (20 mL) and kept overnight at 36–37° for DEAE-mature cotton or at room temperature for DEAE-disordered cellulose. The clear solution resulting was refluxed for 1 h under argon, and then gradually diluted with water to 85, 67, 46, and 30% (w/w) of TFA, with 25-min reflux periods at the first two or three concentrations, and for periods indicated in the results at the final (30%) concentration. The second and third stages of dilution could be combined in the case of DEAE-disordered cellulose. To the cooled hydrolyzate was added inositol (0.5000 mg) and phenyl β-D-glucoside (0.8000 mg), and the solution was evaporated to dryness in a rotary vacuum evaporator. Three additional evaporations were conducted after additions of 15–20 mL of water. The dry hydrolyzate was analyzed as such, or redissolved and subjected to fermentation (as already described) before freeze-drying to a solid product.

Analysis of products. — The freeze-dried product from hydrolysis of DEAE-cellulose, or the freeze-dried product after fermentation for removal of D-glucose, was silylated with (trimethylsilyl)imidazole[6,7]. Gas–liquid chromatography was conducted on a DB-1-bonded, fused-silica, capillary column (60 m × 0.25 mm; J & W Scientific, Rancho Cordova, CA, U.S.A.) with film thickness of 0.25 μm, at 200° with a He flow of 19 cm/s and flame ionization detection. Peaks of 2-*O*-, 3-*O*-, and 6-*O*-DEAE-glucose were identified as described in a preceding report[11]. The products studied herein were DEAE-celluloses having[2] d.s. <0.05, monosubstitution in the D-glucopyranosyl units being predominant, and an estimated d.s. at 0.01 for DEAE-mature cotton and 0.02 for DEAE-decrystallized cellulose. Relative abundances of 2-*O*-, 3-*O*-, and 6-*O*-DEAE substituents in the DEAE-celluloses (or the isomeric DEAE-glucoses in the final hydrolyzates) were in the ratios of 2.40:0.20:1.00 for DEAE-mature cellulose and of 1.83:0.52:1.00 for DEAE-disordered cellulose.

Reproducibility of yields of DEAE-glucoses. — Triplicate analyses gave relative standard deviations of measurements of 2-*O*-, 3-*O*-, and 6-*O*-DEAE-glucoses of 1–3.5% for products of hydrolysis in sulfuric acid and in TFA.

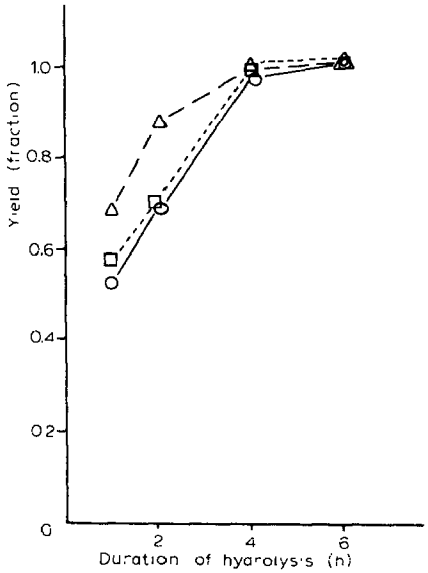

Fig. 1. Yields of individual 2-*O*-, 3-*O*-, and 6-*O*-DEAE-D-glucoses liberated during the final, dilute, reflux stage of the hydrolysis of DEAE-cellulose derived from mature cotton fibers in sulfuric acid. [Symbols denote: O = 2-*O*-, △ = 3-*O*- and □ = 6-*O*-DEAE-D-glucose. Data are presented as fractions of the inidividual yields at 6 h, the period generally accepted for completion of this hydrolysis and that employed in our preceding studies.]

RESULTS

Homogeneous hydrolysis of DEAE-cellulose beginning in 72% sulfuric acid. — Progressive liberation of mono-DEAE-glucoses from DEAE-cellulose during the final step of the hydrolysis that begins with dissolution in 72% sulfuric acid and ends with reflux for 6 h in 0.75M acid is shown in Fig. 1. The DEAE-cellulose was prepared from mature-cotton fibers. The D-glucopyranosyl unit mono-substituted at O-3 was the most sensitive to glucosidic hydrolysis. The 6-*O*- and 2-*O*-DEAE-D-glucoses were less sensitive, and very close in their ease of cleavage of glucosidic linkages.

Major liberation of glucose was found to be complete at 2 h reflux, as shown by the ratio of glucose to DEAE-glucose in the hydrolyzate. In hydrolysis of this DEAE-cellulose, the ratio reached a maximum of 127/1 at 1 h reflux, and decreased progressively to 82/1 at 8 h reflux.

Hydrolysis of DEAE-cellulose prepared from disordered cellulose (*i.e.*, ball-milled cotton fibers, d.p. ~500) is illustrated in Fig. 2. The results are generally similar to those for DEAE-mature-cellulose, substantiating that the effect is essentially independent of the physical state (subdivision) of the cellulose, and of such factors as crystallinity, accessibility, d.p. of the original cellulose, and even the potential for clustered distributions *versus* uniform distributions of DEAE-sub-

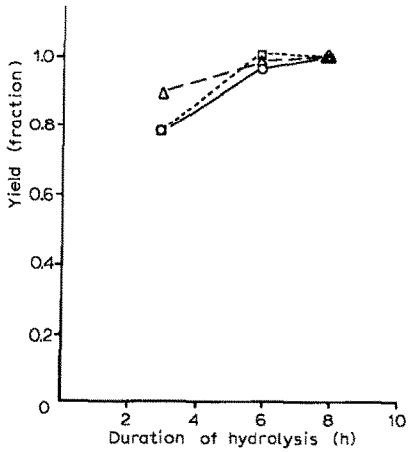

Fig. 2. Yields of 2-*O*-, 3-*O*-, and 6-*O*-DEAE-D-glucoses from DEAE-cellulose derived from disordered (ball-milled) cotton cellulose in sulfuric acid, in accord with the designations for Fig. 1. [Yields of individual DEAE-glucoses are expressed as fractions of the yield of the corresponding product measured at 8-h reflux.]

stituents along the cellulose chains (in DEAE-mature-cellulose and DEAE-disordered cellulose, respectively).

Homogeneous hydrolysis of DEAE-cellulose beginning in 100% TFA. — Releases of DEAE-glucoses in homogeneous hydrolyses of DEAE-mature-cellulose and DEAE-disordered-cellulose as functions of duration of the final reflux period are summarized in Figs. 3 and 4. The final stage for this hydrolysis is in 2.96M

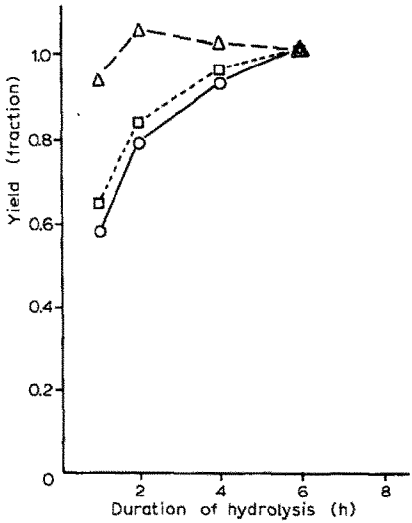

Fig. 3. Yields of 2-*O*-, 3-*O*-, and 6-*O*-DEAE-D-glucoses from hydrolysis of DEAE-mature cotton cellulose in TFA as a function of the duration of hydrolysis in the final, dilute, reflux stage. [The yield is expressed as the fraction of the corresponding DEAE-glucose measured at 6-h hydrolysis. Symbols have the same meaning as in Fig. 1.]

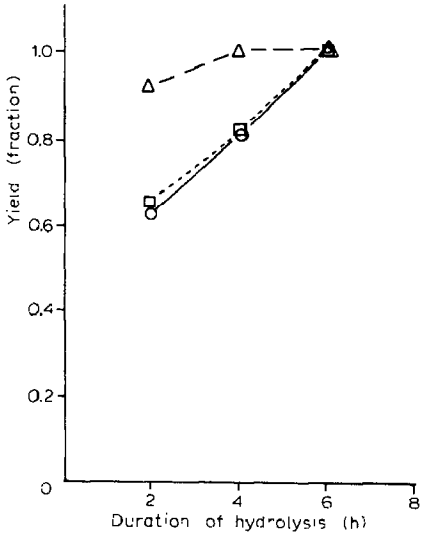

Fig. 4. Yields of individual DEAE-D-glucoses from hydrolysis of DEAE-disordered cellulose in TFA. [Designations are the same as in Fig. 3.]

(30%) TFA. The yields of 2-*O*-, 3-*O*-, and 6-*O*-DEAE-glucose are expressed as fractions of the yields measured at 6-h reflux (three times that proposed by Fengel and Wegener[5] for their most strenuous conditions of hydrolysis). The patterns of hydrolysis show the same relationship of 2-*O*-, 3-*O*-, and 6-*O*-DEAE-glucose when the yields are expressed as fractions of the yield from 6-h hydrolysis in the procedure based on sulfuric acid. The patterns of liberation of individual DEAE-glucoses in TFA are similar to those shown in Figs. 1 and 2 for hydrolysis in sulfuric acid.

The major liberation of D-glucose from hydrolysis of DEAE-mature-cellulose in TFA was complete at 1 h at reflux, at which point the ratio of glucose to DEAE-glucose in the product was 146/1. The ratio decreased progressively to 89/1 for the product from 8-h reflux.

DISCUSSION

Distinct differences in rates of liberation of D-glucose and 2-*O*-, 3-*O*-, and 6-*O*-DEAE-D-glucoses are apparent during homogeneous hydrolyses of DEAE-celluloses individually in sulfuric acid and in TFA. The order of decreasing rates is D-glucose >3-*O*- >6-*O*- ≅ 2-*O*-DEAE-D-glucose. Differences among the DEAE-D-glucoses are notably larger in TFA than in sulfuric acid, probably because the attacking species of the TFA is larger than that of the sulfuric acid. Dominant retardations of hydrolysis from substitution at O-2 and O-6 may be attributed to effective steric blocking by these substituents at adjacent glucosidic linkages, O-1 and O-1′, respectively. The less effective 3-*O*-substituent is remote from the 1-*O*-

glucosidic linkage, and lacks extension to reach the 1′-*O*-glucosidic linkage that is provided by $-CH_2-$ to the substituent at O-6. Retardation from a 6-*O*-DEAE substituent may well be exercised at both the O-1 and O-1′ linkages, but, overall, the effectiveness is slightly less than that of a 2-*O*-substituent.

It is proposed that a primary effect of the DEAE substituent in homogeneous hydrolysis is steric restriction of the attacking species at the D-glucosidic linkage. A secondary effect may lie in a moderating contribution from proximity of the oxyethyldiethylammonium form of the substituent. Inductive effects from the substituents through ring-carbon atoms may make some contribution to the ease of hydrolysis of the glucosidic bonds.

We have found no reports concerning the effect of substituent location on the ease of homogeneous, acid hydrolysis of cellulose. It appears to have been generally assumed that acid hydrolysis is insensitive to differences that might be contributed by substituents at O-2, O-3, or O-6 in the D-glucopyranosyl unit. It has, however, been reported that enzymic hydrolysis of cellulose takes place only at sites of unsubstituted D-glucopyranosyl units[12], and that, for O-(2-hydroxyethyl)celluloses, there exists a linear relationship between the concentration of unsubstituted D-glucopyranosyl units and the number of chain breaks resulting from enzymic hydrolysis[13].

In spite of large differences in size, accessibilities, and complexities of attacking species of acids, and especially, of enzymes, intriguing similarities between acid-catalyzed and enzymic hydrolyses have been reported[14,15]. However, acid and enzymic hydrolyses of cellulose occur in very different time intervals, such as 2 h at reflux for 2.5M hydrochloric acid being equivalent to 24 h at 50° for fungal-culture filtrate *(Trichoderma viride)*. It appears likely that the effect of a substituent in a D-glucopyranosyl unit of cellulose is similar in nature, but different in degree, for acid-catalyzed and enzymic hydrolyses in homogeneous media.

Klop and Kooiman[16] reported that products of enzymic hydrolysis of O-(2-hydroxyethyl)cellulose were consistent with the proposal that a glucosidic linkage of an unsubstituted (U) D-glucopyranosyl unit is susceptible to enzymic attack provided that the aglucon is either U or a 6-*O*-substituted D-glucopyranosyl unit (Su-6). In their study, 2-*O*- and 3-*O*-substituted D-glucopyranosyl units were not specifically identified. Substantial, substituted D-glucopyranosyl units, in addition to Su-6 were retained in hydrolysis-resistant tri- and tetra-saccharidic chains. These would most likely have consisted of the 2-*O*-substituted D-glucosyl residues (Su-2), which are the components second in abundance to Su-6 in (2-hydroxyethyl)celluloses prepared by the O-(2-hydroxyethyl)ation process of that time. The 3-*O*-substituted D-glucopyranosyl units (Su-3) would have amounted to only 5–10% of the total substituted components. Thus, a conclusion unstated by Klop and Kooiman, but suggested by their data[17,18], is that a glucosidic linkage of U to Su-6 is less resistant to enzymic attack than a glucosidic linkage of U to Su-2. Thus, the order of decreasing ease of enzymic hydrolysis of D-glucosidic linkages, or of liberation of components, would be D-glucose $\cong$ 6-*O*- > 2-*O*-DEAE-D-glucose.

Our observations are pertinent to the increased resistance to enzymic degradation realized by Glass *et al.*[17] for *O*-(2-hydroxyethyl)cellulose prepared under special conditions. In this case, the cellulose was swollen and decrystallized in 6.8M NaOH, and it reacted partially [to a molar substitution (m.s.) of 0.8–1.2] before the concentration of NaOH was lowered to M for completion of the *O*-(2-hydroxyethyl)ation (to m.s. 4–5). Substitution reactions conducted in a low normality of base become more predominant at O-2, and less at O-6, as the concentration of NaOH is lessened[18–21], insuring that a greater proportion of the substituted D-glucopyranosyl units is highly resistant to hydrolytic degradation, as well as providing a more uniform distribution of substituents among the D-glucopyranosyl units of the cellulose chain.

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