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Enzymatic Degradation of Water-soluble Cellulose Ethers

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Submitted in honour of the sixtieth birthday of my teacher, Professor *Arne Fredga*

The degradation of some water-soluble cellulose ethers and one cellulose ester under the influence of cellulase derived from *Aspergillus niger* has been investigated. The influence of enzyme concentration and of the degree of polymerization (D.P.) are discussed. Some factors determining the resistance to degradation are pointed out.

Among the water-soluble high polymers the cellulose ethers are finding an increasing utilisation as suspending, thickening, stabilizing, and film-forming agents. In the solid state the ethers are quite stable in storage; in solution, however, they can undergo deterioration.

The enzyme responsible for the hydrolysis of cellulose and also cellulose derivatives is known as cellulase. Miller *et al.*[1] have recently shown the presence of twenty-four or more electrophoretically distinct, cellulolytically active components in a cellulase preparation from the mould *Myrothecium verrucaria*.

The cellulase used in this investigation (Takamine cellulase 4000 *) is derived from *Aspergillus niger*. It is stated by the manufacturer that it contains appreciable quantities of pectinase and hemicellulase but negligible amounts of protease, amylase, glucose oxidase, and catalase.

The depolymerizing effect of the enzyme on ethyl-hydroxyethyl cellulose in water is seen in Fig. 1. Many other cellulose derivatives are degraded in the same way.

The relation between the quantity of lichenase and the rate of hydrolysis of lichenin (a carbohydrate which resembles cellulose and may be obtained from Iceland moss) has been discussed by Fåhræus [2]. No direct proportion was found to exist. If in this case the remaining viscosity in percent of the original is plotted against the cellulase concentration in percent (calculated on pure and dry cellulose ether) in a semilogarithmic diagram, with time as a parameter, a straight line is obtained, Fig. 2. By changing the enzyme concentration from,

* Manufactured by the Miles Chemical Company, U.S.A.

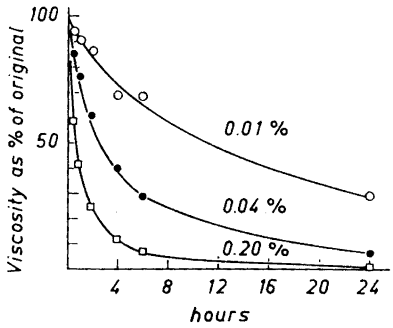

Fig. 1. The decrease in viscosity with time of ethyl-hydroxyethyl cellulose (Modocoll E 600, see experimental part) at different cellulase concentrations (calculated on pure and dry ether). 2.0 % ether in water. Starting viscosity 2 020 cp. pH = 5.8. 20.0°C.

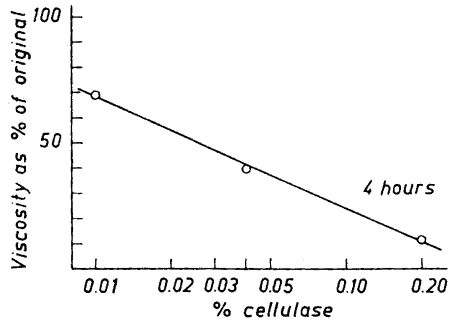

Fig. 2. The relationship between the remaining viscosity and the cellulase concentration (calculated on pure and dry ether) for Modocoll E 600. Reaction time 4 h. 2.0 % ether in water. Starting viscosity 2 020 cp. pH = 5.8. 20.0°C.

for instance, 0.01 % to 0.02 % the remaining viscosity is only decreased by a factor 1.3.

Both temperature and pH have a large influence on the cellulase activity. From Fig. 3 can be seen that the pH optimum for the cellulase used lies between 4 and 5. When comparing the biological resistance of different cellulose derivatives in solution, this fact must be kept in mind. All comparisons between different cellulose derivatives in this investigation are made at constant pH.

Another factor, which must be taken into account when comparing the enzymatic degradation of different cellulose derivatives by following the per-

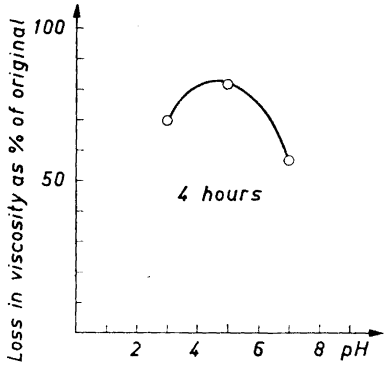

Fig. 3. The loss in viscosity as % of original as a function of pH. 0.20 % cellulase. Temperature 20.0°C. Reaction time 4 h.

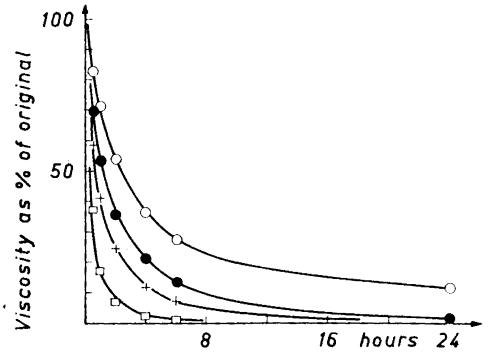

Fig. 4. The decrease in viscosity with time for some ethyl-hydroxyethyl celluloses with different starting viscosities (in all cases the same concentration, 2.0 %). 0.20 % cellulase. 20.0°C. O E 20 (76.0 cp, pH = 6.2); ● E 100 (334 cp, pH = 5.8); + E 600 (1 350 cp, pH = 5.8); □ E 1 200 (8 500 cp, pH = 5.2).

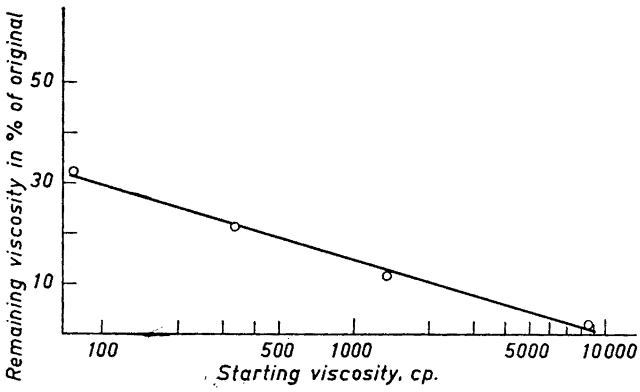

Fig. 5. The remaining viscosity in % of original, as a function of starting viscosity. 0.20 % cellulase. pH = 5.8. Reaction time 4 h. 20.0°C. 2.0 % ether solution in water.

cent loss in viscosity, is the original viscosity of the solution (measured at the same concentration). In Fig. 4 is shown the rate of loss in viscosity for four grades of ethyl-hydroxyethyl cellulose with different starting viscosity (in all cases 2.0 % ether solution in water). The viscosity values at different times are not corrected with respect to the pH influence. The fact that the loss in viscosity, in percent of the original, under identical conditions is less for a low viscosity cellulose ether than for a high one has also been found by Fåhræus and Göransson [3] when degrading the ethers mentioned above with pure cultures of different bacteria and fungi.

This can be expected if it is assumed that the same number of fissions are brought about by the enzyme in a certain period. If in a hypothetic ethyl-hydroxyethyl cellulose, homogeneous with respect to molecular weight (200 000), each chain could be split into three chains of the same length the molecular weight would be 66 700. If in the same amount of a hypothetic ethyl-hydroxyethyl cellulose, with the molecular weight 100 000 each chain is split into two chains of the same length (the same number of fissions in every case) the molecular weight would be 50 000. From eqn. (1) can be calculated that the remaining viscosity in percent of original is higher for the low viscosity than for the high viscosity derivative (14.6 and 1.0 % respectively).

$$M_v^{0.8} = 5113 \cdot \log\eta - 1\,658 \qquad (1)$$

M_v = viscosity average molecular weight
η = Brookfield viscosity in cp

If the remaining viscosity, in percent of the original, is plotted against the starting viscosity (2.0 % solution) at a constant cellulase concentration in a semilogarithmic diagram, with time as a parameter, a straight line is obtained, Fig. 5. The values are corrected with the aid of Fig. 3 to a pH of 5.8 in the solution.

By using the diagrams in Figs. 3 and 5, comparison can be made between the resistance to cellulase degradation of ethyl-hydroxyethyl cellulose and any

cellulose derivative investigated under the conditions, valid for diagram 5 (pH = 5.8, 0.20 % cellulase calculated on pure and dry ether, reaction time 4 h, 20.0°C, 2.0 % ether solution in water). If for instance a cellulose ether with a starting viscosity of 1 000 cp has been found to retain 30 % of the original viscosity under the conditions given above, its biological resistance in comparison to ethyl-hydroxyethyl cellulose can be obtained by dividing 30 by 15 (see Fig. 5 for a starting viscosity of 1 000 cp).

Manley [4] has given the expression below (2) for the relation between the intrinsic viscosity and the viscosity average molecular weight for ethyl-hydroxyethyl cellulose.

$$[\eta] = 3.98 \times 10^{-4} \, M_v^{0.8} \qquad (2)$$

The relation between the intrinsic viscosity and the Brookfield viscosity for a 2.0 % solution of ethyl-hydroxyethyl cellulose in water is also known [5], see eqn. (3).

$$[\eta] = 2.035 \cdot \log\eta \; -0.66 \qquad (3)$$

From eqns. (2) and (3) eqn. (1) is obtained. The decrease in viscosity molecular weights has been calculated for the situations given in Fig. 4. If the remaining viscosity average molecular weight, in percent of the original, is plotted against time the curves for the three products with the lowest starting viscosity (E 20, E 100 and E 600) are very close to each other. The curve for the fourth product (E 1200) lies below the other curves. This product, however, should not, according to the manufacturer, be compared to the others in this situation. This indicates, as Greathouse [6] has pointed out, that the attack of the enzyme is random in which case the degree of polymerisation should have little effect on hydrolysis.

Some data obtained in the way discussed above are given in Table 1 for nine different water-soluble cellulose derivatives. All experiments have been made at the pH which the derivative gives when dissolved in water. Buffer-solutions (sodium hydroxide and potassium biphthalate, pH = 5.0) were first tried in this investigation, but as the influence of electrolytes on different cellulose derivatives varies considerably, the results obtained were not considered representative for the practical situations which are of primary interest in this investigation. The method of using Fig. 3 as a correction curve is in itself not so correct, but it gives, the author believes, a better picture of what happens under practical circumstances.

Although much work has been done to determine the functional group or groups in the cellulose molecule which must be blocked in order to prevent microbiological utilisation the exact mechanism of microbiological attack on cellulose is not known. The maximum possible substitution in cellulose is three groups per anhydroglucose unit. Siu *et al.*[7] investigated the susceptibility of 9 glucose, 3 mannose and 3 cellobiose derivatives to 9 different fungi, and of 11 cellulose derivatives to *Myrothecium verrucaria*. They conclude: "As long as there is at least one firmly bound substituent in every anhydroglucose unit, the resulting derivative is not susceptible to microbiological attack."

From Table 1 is seen that four derivatives have a value in the last column of 0.3—0.4. That means that they are less resistant than ethyl-hydroxyethyl

cellulose (Modocoll, see experimental part) to cellulase attack. This is in agreement with practical experience. All these derivatives contain alkylene oxide groups. With, for instance, hydroxyethyl cellulose substitution occurs on the primary hydroxyls of the introduced hydroxyethyl groups leading to polyethylene oxide side chains. Even if the total substitution as moles of ethylene oxide per anhydroglucose unit (M.S.) is rather high the substitution directly on the three hydroxyl groups of each anhydroglucose unit (D.S.) can be less than one. For the hydroxyethyl cellulose used D.S. can be expected to have a value of about 0.8 [8].

Modocoll has a D.S. of about 1.3. The value of 2.2 in the last column for the methyl cellulose used (D.S. = 1.83) is thus in agreement with the results obtained by Reese *et al.*[9], for carboxymethyl cellulose, which shows that an increase in D.S. from 0.5 to 1.2 increases the resistance to enzymatic attack. The fact that products with D.S.-values over one are degraded is explained by the nonuniform substitution which exists in most technical products and which results in the cleavage of the glycosidic linkages adjacent to the unsubstituted anhydroglucose units. The rule that a D.S. of more than one is required to give protection does not, however, apply to surface modification of fibers [10]. The minimum D.S. for protection of methylated cotton has been found to be 0.7 when diazomethane was used. An equivalent methylation with dimethyl sulfate did not give protection which indicates that the synthetic method used is also of importance.

The relatively high microbiological resistance of the three last derivatives in Table 1 was not expected because of the low D.S.-values. They are all ionic derivatives. Reese [10] has pointed out that the nature of the substituent and its location may be of importance. It is possible that both steric and electronic properties of the substituent play a role together with the D.S. when dealing with microbiological attack on cellulose derivatives.

From what has been said it is clear that there are many factors which are difficult to treat quantitatively but which are of great importance for the resistance to microbiological attack. For testing cellulose derivatives against enzymatic attack the use of a standardised enzyme, like the one used, which is commercially available can simplify the investigation and yet give enough informations.

EXPERIMENTAL

Hydroxyethyl cellulose. Bleached sulphite pulp with an alpha content of 89.5 % was soaked in 19.0 % sodium hydroxide solution for 45 min at 22°C. Excess alkali was removed by pressing to a press factor of 2.42. 121 g of shredded alkali cellulose together with 181 g of ethyl chloride and 35.2 g of ethylene oxide were heated in an autoclave, without stirring, at 55°C for 4 h. The crude product was washed three times in methanol and the last amount of sodium hydroxide was neutralized in the second wash with acetic acid. Total substitution as moles of ethylene oxide per anhydroglucose unit (M.S.) = 1.50 corresponding to a degree of substitution (D.S.) of 0.80 [8].

Ethyl-hydroxyethyl cellulose. This water-soluble cellulose ether is manufactured by Mo och Domsjö AB, Örnsköldsvik, Sweden, and is sold under the name of Modocoll. The different products used, E 20, E 100, E 600 and E 1200 differ mainly in viscosity. The total degree of substitution is, for E 20 1.4, and for the other products 1.7. The ethoxyl and hydroxyethyl substitution is about the same.

Table 1.

Derivative	Schematic formula	D.S.	Starting visc., cp 2.0 % solution	pH	Remaining visc. in % of original after 4 h[b]	Remaining visc. corrected to pH 5.8	Remaining visc. in % of original divided by the corresponding value for Modocoll[c] with the same starting visc.
Hydroxyethyl cellulose	$-O-CH_2-CH_2-OH$	1.50[a]	3 740	6.8	2.1	1.7	0.3
Hydroxypropyl cellulose	$-O-CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle H}{\underset{\displaystyle |}{\overset{\displaystyle |}{C}}}}-OH$	1.2–1.5	2 150	6.1	4.5	4.4	0.4
Ethyl-hydroxypropyl cellulose	$-O-C_2H_5$ $-O-CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle H}{\underset{\displaystyle |}{\overset{\displaystyle |}{C}}}}-OH$	1.5–1.7[a]	1 455	5.1	4.7	5.0	0.4
Methyl cellulose	$-O-CH_3$	1.83	6 300	6.2	7.3	6.9	2.2
Methyl-hydroxyethyl cellulose	$-O-CH_3$ $-O-CH_2-CH_2-OH$	1.66	2 450	3.5	3.8	3.8	0.4
Methyl-hydroxypropyl cellulose	$-O-CH_3$ $-O-CH_2-\underset{\displaystyle CH_3}{\overset{\displaystyle H}{\underset{\displaystyle |}{\overset{\displaystyle |}{C}}}}-OH$	–	4 030	6.8	13.2	10.7	1.8
Carboxymethyl cellulose	$-O-CH_2-COONa$	0.70	3 540	7.1	17.8	12.6	1.9
Sulfomethyl cellulose	$-O-CH_2-SO_3Na$	0.28	449	4.4	58.9	55.0	2.8
Cellulose sulfate	$-O-\underset{\displaystyle O}{\overset{\displaystyle O}{\underset{\displaystyle \|\|}{\overset{\displaystyle \|\|}{S}}}}-ONa$	0.40	43.4	3.0	30.4	27.6	0.8

a) M.S. values (total substitution as moles of alkylene oxide per anhydroglucose unit).
b) 0.20 % cellulase calculated on pure and dry derivative. 20.0°C.
c) Ethyl-hydroxyethyl cellulose (see experimental part).

Hydroxypropyl cellulose. Bleached sulphite pulp with an alpha content of 89.5 % was soaked in 19.5 % sodium hydroxide solution for 45 min at 20°C. Excess alkali was removed by pressing to a press factor of 2.39. 95.6 g of shredded alkali cellulose together with 181 g of ethyl chloride and 40.1 g of propylene oxide were heated in an autoclave, without stirring, at 70°C for 4.5 h. The crude product was washed twice in methanol and the last amount of sodium hydroxide was neutralized with acetic acid. The total substitution as moles of propylene oxide per anhydroglucose unit has not been determined. M.S. can be expected to have a value between 1.2 and 1.4.

Ethyl-hydroxypropyl cellulose. Bleached sulphite pulp with an alpha content of 89.5 % was soaked in 19.5 % sodium hydroxide solution for 45 min at 20°C. Excess alkali was removed by pressing to a press factor of 2.40. 96.0 g of shredded alkali cellulose together with 52.3 g of ethyl chloride and 21.5 g of propylene oxide were heated in an autoclave, without stirring, first at 70°C for 60 min, and then at 100°C for 125 min. The crude product was washed three times in methanol and the last amount of sodium hydroxide was neutralized in the second wash with acetic acid. M.S. is not exactly known but can be expected to have a value between 1.5 and 1.7.

Methyl cellulose. The product used, Methocel MC 4 000, is manufactured by the Dow Chemical Company, U.S.A. D.S. = 1.83.

Methyl-hydroxyethyl cellulose. The product used, Tylose MH 1 000, is manufactured by Kalle AG, Germany, D.S.: methoxyl = 1.53; hydroxyethyl = 0.13.

Methyl-hydroxypropyl cellulose. The product used, Methocel 65 HG, NF-grade, is manufactured by the Dow Chemical Company, U.S.A. M.S. or D.S. are not known.

Carboxymethyl cellulose. The product used, Cekol HVB, is manufactured by Uddeholms AB, Skoghall, Sweden. D.S. = 0.70.

Sulfomethyl cellulose. This product was first described by Porath [11,12]. – Bleached sulphite pulp with an alpha content of 89.5 % was soaked in 19.0 % sodium hydroxide solution for 45 min at 20°C. Excess alkali was removed by pressing and the press factor was determined. Alkali cellulose corresponding to 50 g of pulp was shredded, and enough 19.0 % sodium hydroxide solution was added to give a press factor of 4.0. A water solution (120 ml), containing 23.1 g of Na-monochloromethane sulfonate and an equimolar amount of sodium chloride (obtained by reacting dichloromethane with sodium sulphite) was added during a period of 25 min with continuous stirring. The reaction mixture was put into an autoclave and the water was driven off under vacuum and heating. The temperature increased almost continuously during the reaction to about 110°C at the end of the reaction. The crude product was washed three times in 75 % ethanol and the last amount of sodium hydroxide was neutralized with acetic acid. D.S. = 0.28.

Cellulose sulfate. This product differs from the other derivatives used as it is an ester of cellulose. It has been obtained from Eastman Kodak Company, U.S.A. D.S. = 0.40.

2.0 % solutions have been used throughout this investigation. The products have been dissolved at 20°C. The water content in the solid product has been determined by heating at 105°C to constant weight.

The cellulase has been weighed on a precision balance and directly dissolved in the solution under investigation. In most cases the cellulase concentration has been 0.20 % calculated on dry and pure cellulose derivative.

The temperature has in all cases been 20.0°C.

The decrease in viscosity has been followed with a Brookfield viscometer, type LVF. Different spindles and rotation speeds can be used for different viscosity ranges. The combinations of spindles and speeds shown in Table 2 have been used.

Table 2.

Measuring range, cp	Spindle	Rpm
0 — 100	1	30
100 — 500	1	12
500 — 2 500	2	12
2 500 — 10 000	3	12

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