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Studies on the Enzymatic Hydrolysis of Dextran

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SUMMARY

The enzymatic hydrolysis of well-defined dextran fractions is studied. The results show that the rate constants of degradation are proportional to the molecular weight for molecular weights higher than 5000; for lower molecular weights the rate constants decrease gradually. Enzymatic hydrolysis of dextran is not a statistical reaction, since the individual rate constants of bond cleavage increase from the midpoint of the chain towards both ends. The results are discussed in comparison with acid hydrolysis of dextran, and the characteristic differences between both reactions are pointed out.

INTRODUCTION

In a previuos paper it was shown that during the hydrolytic depolymerization of dextran by the action of strong acids the rate constants of cleavage of the individual polymer bonds increase from the midpoint of the chain towards both ends (BASEDOW et al. 1978). Furthermore, the rate constants were found to be proportional to the molecular weight (MW) of the polymer raised to the power of 2/3. The question, if similar considerations are also applicable to other hydrolytic depolymerization reactions, e.g. enzymatic hydrolysis of dextran, has not yet been fully answered.

In the present study the enzymatic hydrolysis of dextran is investigated. Comparatively little is known about the size distribution of the degradation products during the hydrolysis of dextran by endo-dextranases. From the action of the enzymes and the partially branched structure of all bacteriologically produced dextrans it can be concluded a priori that the cleavage of polymer bonds cannot be random, since the α-(1→6)-D-glucosidic linkages in the vicinity of branch linkages and at the ends of the chain are more stable than the other linkages. The final products of enzymatic hydro-

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lysis of dextran will thus be branched oligosaccharides, isomaltotriose, isomaltose and glucose (BOURNE et al. 1962, HUTSON and WEIGEL 1963). The Penicillium funiculosum dextranases hydrolyse dextran B-512 producing a distribution of fragments according to this scheme (JEANES et al. 1953, WALKER and PULKOWNIK 1974). In the present analysis the alteration of the molecular weight distribution (MWD) of dextran is investigated in the polymer range as a function of degradation time; the size distribution of the final degradation products is not taken into consideration. The aim of this paper is to establish the comparison between acid hydrolysis and enzymatic hydrolysis of dextran polymers.

EXPERIMENTAL

Two dextran fractions (from Leuconostoc mesenteroides, strain NRRL B-512) with narrow MWD's were used for the degradation experiments. The number average MW's were $\overline{M}_n$ = 76900 ($\overline{M}_w/\overline{M}_n$ = 1.14, $\overline{M}_z/\overline{M}_w$ = 1.14) for sample A and $\overline{M}_n$ = 6990 ($\overline{M}_w/\overline{M}_n$ = 1.27, $\overline{M}_z/\overline{M}_w$ = 1.26) for sample B. They were prepared from commercially available products (Pharmacia Fine Chemicals, Uppsala, Sweden) by preparative GPC on Sephadex. In the present study dextran is considered to be a linear polymer, since there are only relatively few and mostly short side chains (LARM et al. 1971, GARG and STIVALA 1978).

The hydrolytic depolymerization reaction of dextran was carried out in closed, thermostated glass vessels of 100 ml capacity, at 25 ± 0.05 °C in acetate buffer of pH = 5.2 . The enzyme used was an endo-dextranase from Penicillium funiculosum (Dextranase grade I from Sigma Chemical Co., St. Louis, USA) with a MW of 55000; the enzyme concentration was 0.05 units/ml (JANSON and PORATH 1966). The dextran concentration was 1%. After definite time intervals samples of 10 ml were taken, the reaction was stopped with KOH (pH ≈ 11), and the solution was poured into 100 ml of cold (≈-30 °C) methanol for precipitation of the dextran. The dextran was washed with methanol and dried under vacuum until constant weight. Oligosaccharides with degree of polymerization lower than 5 were not precipitated and therefore were disregarded.

MW averages and MWD's of the dextran samples were determined by GPC on controlled pore glass CPG-10 (Electro Nucleonics, Fairfield, USA) as described in detail previously (BASEDOW et al. 1976, BASEDOW 1977). Reproducibility in the MWD's and MW averages is ±0.5%; deviations in the absolute values are ± 2%.

RESULTS and DISCUSSION

The experimental results are summarized in Table 1. A combined polydispersity ratio (CPR), defined as $\overline{M}_w^2/\overline{M}_n\overline{M}_z$ (BASEDOW et al. 1978), and which constitutes an adequate parameter to characterize the type of the degradation reaction, is included. The kinetics of degradation was followed by the alteration of the number average MW ($\overline{M}_n$) with degradation time, because $\overline{M}_n$ permits to calculate directly the number of polymer bonds broken per polymer molecule. As shown recently, the kinetics of degradation can be expressed by the following equation (BASEDOW et al. 1978):

$$\frac{1}{\overline{M}_n(t)^a} = \frac{1}{\overline{M}_n(o)^a} + a\left(\frac{k}{162^a}\right)t \qquad (1)$$

in which $\overline{M}_n(o)$ and $\overline{M}_n(t)$ are the MW's at the beginning of the reaction and after the reaction time t, k is the first order rate constant, 162 is the MW of the monomer unit and a is a constant which represents the dependence of the rate constant of degradation of a definite molecule on its MW. The exponent a was found to be unity in both cases; it was evaluated by applying equation (1) to the experimental points and performing a computer regression analysis (Fig. 1). In the case of sample B deviations from linearity were found for MW's below 5000, indicating that for low MW's the reaction rate decreases. The reaction rate constants calculated from the slopes of the lines in Fig. 1 were $k = 4.46\times10^{-6}$ s^{-1} for high MW's and $k = 2.86\times10^{-6}$ s^{-1} for MW's around 4000. A decrease of the rate constants for very low MW's is expected, since enzymatic action is less pronounced in the oligomer range (WALKER 1978). It is interesting to notice that for acid hydrolysis of dextran the value of the exponent a is 2/3, and linearity holds down to MW's of about 3000 (BASEDOW et al. 1978).

For the general discussion of the enzymatic depolymerization it is assumed that each bond of the polymer chain has an individual rate constant of degradation, which depends on the degree of polymerization and the location of the considered bond in the polymer chain. The best method of investigation is to simulate the degradation reaction mathematically and to compare the results with experiment. This has been done in detail for acid hydrolysis of dextran in a previous paper. For computational simplicity it was assumed there, that the rate constants of the bonds increase from the midpoint of the chain towards both ends according to a parabolic function; the depth of the parabola was defined by a parameter b (BASEDOW et al. 1978).

TABLE 1

Kinetic data of enzymatic hydrolysis of dextran.

Time t (min)	$\frac{10^5}{\overline{M}_n}$	$\overline{M}_n$	$\overline{M}_w$	$\overline{M}_z$	CPR
		Sample A			
0	1.30	76900	87700	99800	1.00
10	3.07	32600	63300	84200	1.46
20	4.61	21700	50000	76300	1.51
30	6.33	15800	39800	65500	1.53
40	8.06	12400	31900	53600	1.53
50	9.62	10400	26800	46000	1.50
60	11.2	8970	23300	42600	1.42
80	14.4	6940	17000	32400	1.29
100	17.5	5710	13700	27200	1.21
Rate constant: $k = 4.46 \times 10^{-6}$ s^{-1}					
		Sample B			
0	14.3	6990	8870	11200	1.00
10	16.1	6200	8310	10800	1.03
20	17.6	5680	7780	10000	1.07
30	18.7	5340	7580	9950	1.08
40	20.4	4900	7300	9880	1.10
50	21.7	4610	7100	9760	1.12
60	22.3	4480	6940	9600	1.12
80	24.9	4020	6610	9390	1.16
100	27.0	3710	6360	9220	1.18
Rate constant: $k = 4.41 \times 10^{-6}$ s^{-1} for t = 0 min $k = 2.86 \times 10^{-6}$ s^{-1} for t = 80 min					

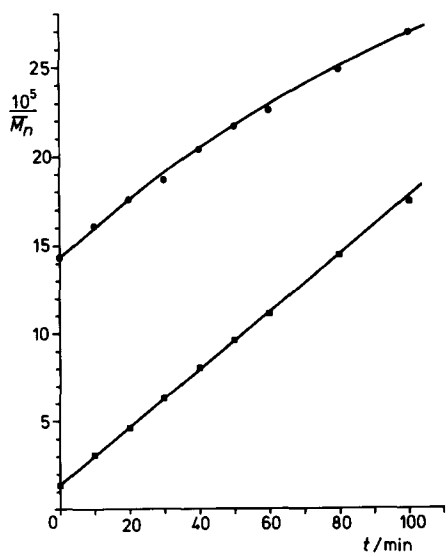

FIGURE 1:

Plot of the reciprocal number average MW $\overline{M}_n$ versus reaction time t

■ : Sample A

● : Sample B

The enzymatic hydrolysis of dextran was analyzed in the same way. Numerical values of the parameter *b*, obtained by incorporating the experimental values of the exponent *a* and the maximum CPR-value into the computer simulation of hydrolytic degradation reactions, are compiled in Table 2.

TABLE 2

Parameter *b* calculated for hydrolytic degradation of dextran by using the experimental values of *a* and CPR.

Reaction	*a*	CPR	*b*
Acid hydrolysis	2/3	1.80	0.4
Enzymatic hydrolysis	1	1.53	0.6

In the case of statistical degradation, i.e. all polymer bonds have the same probability of scission, the value of *b* in Table 2 should be unity; for $b = 0$ there should be no cleavage of the central bond of the chain. The value of $b = 0.6$ obtained for enzymatic hydrolysis of dextran means that less small MW fragments are produced, compared to acid hydrolysis ($b = 0.4$). This shows that the alteration of the rate constants of bond cleavage along the polymer chain is not as pronounced for enzymatic hydrolysis than it is in the case of acid hydrolysis. This can be attributed to a specific action of the enzyme. During scission of the polymer chain, conformational changes of the enzyme molecule contribute certainly to the separation of the fragments produced. For acid hydrolysis, on the other hand, it was concluded that the rate of degradation is partly controlled by disentanglement diffusion of the fragments (BASEDOW et al. 1978). The action of the enzyme in separating the degradation products is much greater than diffusion processes.

The considerations above are based on the assumption that dextran molecules are strictly linear. Chain branching, which is always present in bacteriological dextrans, affects *b* in direction to smaller values, thus complicating further the interpretation of the results. The effect of branches on the degradation kinetics was discussed recently (EBERT et al. 1979). Furthermore, the assumption that the rate constants of bond cleavage vary along the polymer chain according to a parabola, and which was introduced only as a first approximation because of computational reasons, has no experimental proof. In order to find out this function, the MWD (and not only CPR-values) of the degradation products must be simulated and matched to

the experimental MWD. The full understanding of the physico-chemical phenomena which govern enzymatic hydrolysis can only be given after the detailed knowledge of disentanglement diffusion of the polymer fragments, and the availability of kinetic data of degradation of perfectly linear dextran molecules. Investigations on these subjects are currently in progress.

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