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Distribution of Compounds in Polysaccharide Syntheses Using Dextransucrase

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Quantitative results for the synthesis of methyl α-*iso*maltoside and its homologues from methyl α-D-glucoside and sucrose using dextransucrase were recently reported. The distribution of the saccharides can be accounted for if it is assumed that the synthesis proceeds by means of a stepwise transfer of glucosyl groups from sucrose to the primary hydroxyl group of the growing polymer. The rate of the first step is lower than that of the following ones, which seem to be kinetically identical. The reaction thus formally is analogous to the polyaddition of ethylene oxide to water and alcohols. The consequences of these results for the synthesis of dextran are discussed.

Some years ago Hehre [1] put forward the hypothesis that there exists a formal analogy between enzymic polysaccharide syntheses and the formation of polyethylene glycol by polyaddition of ethylene oxide to ethylene glycol. Reactions of the latter type have been studied extensively. The reaction proceeds by means of a stepwise addition of ethylene oxide, the reacting hydroxyl group being regenerated at each step. The distribution of compounds formed was recently studied theoretically and experimentally by Weibull and Nycander [2], and by Weibull [3]. The reaction steps were found to be kinetically identical with the exception of the first one, which generally proceeds at a rate different from the following ones. This accords with the fact that the reacting hydroxyl group is identically bound at every step except the first one. Formulae were derived for the molecular distribution of the polymer product. The following scheme illustrates the reaction:

$$ROH + \underset{\diagdown\,O\,\diagup}{CH_2CH_2} \rightarrow ROCH_2CH_2OH$$

$$ROCH_2CH_2OH + \underset{\diagdown\,O\,\diagup}{CH_2CH_2} \rightarrow ROCH_2CH_2OCH_2CH_2OH \; \textit{etc.}$$

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According to Hehre [1] the corresponding scheme for the synthesis of dextran from sucrose should be (Gl = glucose or anhydroglucose, Fr = fructose or anhydrofructose):

$$(\text{Gl-})_n + \text{Gl-Fr} \rightarrow (\text{Gl-})_{n+1} + \text{Fr}$$
dextran sucrose dextran fructose

Unfortunately the initial steps are not known, so it is not possible to apply the distribution formulae derived for the ethylene oxide reactions. A great many other enzymic polysaccharide syntheses have been investigated and also corresponding syntheses of oligosaccharides [4], but practically none of the results published lend themselves to a theoretical interpretation on these lines*. However, results were recently published concerning the synthesis of methyl *α-iso*-maltoside and its homologues from methyl α-D-glucoside and sucrose by means of dextransucrase [5]. The compounds formed were quantitatively separated by means of chromatographic methods as far as the oligosaccharide containing five glucose residues. The data given are well suited to test Hehre's hypothesis and, more specifically, the distribution formulae derived for ethylene oxide reactions. The role of the enzyme will not be considered — it is assumed to act as an ideal catalyst.

THEORETICAL

The derivation of the distribution formulae will not be repeated here. For convenience the final result is reproduced. The following notation accords with that previously used [2].

N_i molecule with i added monomer units (in this case glucose units)
m number of moles of monomer donor (in this case sucrose)
n_{00} » » » » acceptor or starting material (in this case methyl glucoside)
n_i » » » » N_i in the reaction product
c distribution constant = ratio of the equal velocity constants of the higher steps to that of the first step
$v = m/n_{00}$ average degree of polymerisation

The distribution formulae are:

$$v = c \ln \frac{n_{00}}{n_0} - (c - 1)\left(1 - \frac{n_0}{n_{00}}\right) \quad (1)$$

$$\frac{n_i}{n_{00}} = \frac{c^{i-1}}{(c-1)^i}\left\{\frac{n_0}{n_{00}} - \left(\frac{n_0}{n_{00}}\right)^c \sum_{j=0}^{i-1} \frac{1}{j!}\left[(c-1)\ln\frac{n_{00}}{n_0}\right]^j\right\} \quad (2)$$

Calculations

The present calculations are based on the data given in Table II of the paper mentioned [5]. For the sake of convenience this material is reproduced in Table 1 using the notation given above. Further, v_0 is the molar ratio of sucrose

* *Added in proof:* W. J. Whelan and J. M. Bailey (*Biochem. J.* 58 (1954) 560) investigated the molecular distribution of amylose synthesised with potato phosphorylase. They found agreement with the distribution calculated for a stepwise polyaddition.

to methyl glucoside at the beginning of the reaction, N_F, N_G, and N_L represent fructose, glucose and leucrose (5-*O*-α-D-glucopyranosyl-α-D-fructopyranose). Experiments 1, 2, 3, and 5 were carried out on a small scale and the analyses made by quantitative paper chromatography while experiment 4 was made on a preparative scale and the reaction product separated on a carbon-celite column. In experiment 4, 98 % of the total carbohydrate was soluble in 50 % methanol. About 2 % of these solubles contained more than five glucose residues and were not quantitatively determined. The insolubles were more highly polymerised material, presumably dextran.

Table 1. Composition of 50 % methanol-soluble fraction of the glycoside mixture, as glucose in % of total.

Expt. No.	v_0	N_0	N_1	N_2	N_3	N_4	$N_F + N_G$	N_L
1	3.0	11.8	4.6	8.8	10.8	7.2	51.5	5.2
2	1.5	15.5	10.6	11.4	11.4	7.8	40.8	2.4
3	0.75	27.5	12.9	13.5	10.5	5.1	29.9	0.6
4	0.75	26.0	12.7	13.9	10.9	5.1	30.2	1.2
5	0.3	51.0	17.3	10.1	3.5	0.5	17.5	—

Since the data in Table 1 refer to only a part of the reaction product, there is no direct connection between them and the amounts of sucrose and methyl α-glucoside at the beginning of the reaction. These amounts must therefore be indirectly calculated from the material balances. The following set of equations is self-explanatory (n_F, n_G, n_S, and n_L are the number of moles of fructose, glucose, sucrose and leucrose):

$$n_{00} = \sum n_i \quad \text{material balance for the methyl groups} \tag{3}$$

$$m = \sum i n_i \quad \text{» » » » glucose units added to methyl glucoside} \tag{4}$$

$$n_S = n_F + n_L \quad \text{» » » » fructose units} \tag{5}$$

$$n_S = n_{00} v_0 \quad \text{» » » » sucrose} \tag{6}$$

$$v = m/n_{00} \quad \text{definition of } v \tag{7}$$

Eqns. (5) and (6) give:

$$n_F = n_{00} v_0 - n_L \tag{8}$$

In the material balances the amounts of the higher homologues (N_5 *etc.*) could generally not be disregarded. Since they were not experimentally determined, they have been estimated by extrapolation from the amounts of the lower homologues. This was not possible for experiment 1 since the amounts in this case seem to be rather large. Table 2 contains recalculated figures from Table 1 together with extrapolated data (within parentheses) and figures calculated by means of formulae (3), (4), (7), and (8). For simplicity the figures are based on the amounts of N_0 given in Table 1.

The data in Table 2 were used to calculate the molar ratios n_i/n_{00}. Using the values for v and n_0/n_{00}, the distribution constant c' was calculated by means of formula (1). An independent value, c'', was calculated by means

Table 2. Composition of the glycoside mixture, molar proportions.

Expt. No.	v_0	n_0	n_1	n_2	n_3	n_4	n_5	n_6	n_7	n_F+n_G	n_L	n_{00}	m	v	n_F
1	3.0	11.8	2.3	2.9	2.7	1.4				51.5	2.6	21.2	22.0	1.0	60.9
2	1.5	15.5	5.3	3.8	2.9	1.6	(0.8)	(0.3)	(0.1)	40.8	1.2	30.3	34.4	1.138	44.2
3	0.75	27.5	6.5	4.5	2.6	1.0	(0.3)	(0.04)	—	29.9	0.3	42.4	29.1	0.685	31.5
4	0.75	26.0	6.4	4.6	2.7	1.0	(0.3)	(0.04)	—	30.2	0.6	41.0	29.5	0.719	30.2
5	0.3	51.0	8.7	3.4	0.9	0.1	—	—	—	17.5	—	64.0	18.4	0.288	19.2

of formula (2) using the values for n_0/n_{00} and n_1/n_{00}. These figures are given in Table 3. The mean value, $c = 3.80$, was used together with the values for v to calculate theoretical distributions by means of formulae (1) and (2). The results are given in Fig. 1. The experimental figures are indicated as points.

Table 3. Distribution constants for the synthesis of methyl *α-iso*maltoside and its homologues from methyl α-D-glucoside and sucrose using dextransucrase.

Expt. No.	2	3	4	5
c′	3.59	4.07	3.91	3.55
c″	3.29	4.22	4.10	3.69

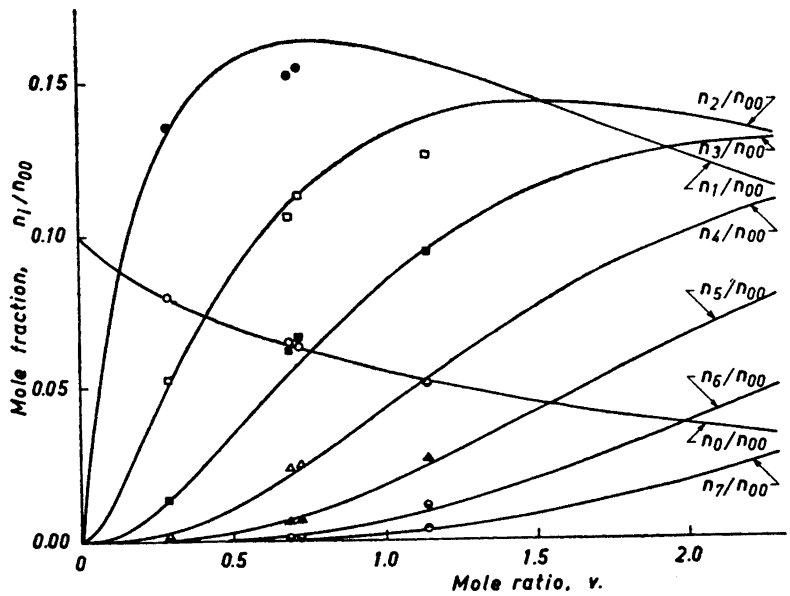

Fig. 1. Distribution of saccharides of the methyl *α-iso*maltoside series, synthesised using dextransucrase; n_i/n_{00} = mole fraction of saccharide with i added glucosyl groups; v = mole ratio of reactants, sucrose to methyl α-glucoside. For convenience, the scale for n_0/n_{00} is ten times smaller than that for the other mole fractions.

DISCUSSION

If all sucrose was used up for the formation of methyl saccharides, the proportion of sucrose to methyl glucoside at the beginning of the experiments, v_0, should equal the corresponding proportion, v, calculated from the analysis of the reaction product. As is seen in Table 2 this is approximately the case in experiments 3, 4, and 5. With the larger amounts of sucrose in experiments 2 and 1, only part of the glucose from the sucrose is bound in the methyl saccharides. Part of the remainder is accounted for as leucrose, but most of it should be bound in the dextran.

The fructose figures in Table 2 reveal a discrepancy in the experimental material. The amounts of fructose calculated from the material balances generally are higher than the experimentally determined sum of fructose and glucose. The discrepancy increases with increasing proportion of sucrose. Since practically all sucrose reacted, there is a loss of fructose which is not accounted for.

Inspection of Table 3 shows that the calculated distribution constants, c' and c'', are rather similar in each experiment. The differences between the constants of the various experiments are also rather small. It thus seems warranted to use a mean value of these figures to calculate the distribution of all the methyl saccharides. As is seen, this calculation gives a rather good picture of the experimental distribution. Of course the experimental material is rather small. It is regrettable that the figures from experiment 1 do not permit an interpretation. However, it seems permissible to state that the reactivity of the primary hydroxyl group of methyl α-D-glucoside is smaller than that of the corresponding groups of at least the next higher homologues, these latter appearing to be of equal reactivity. This conclusion is only a formal one since the details of the enzyme action are not considered.

Unfortunately these conclusions cannot be directly applied to the formation of dextran from sucrose using dextransucrase, in which the initial steps are not known. However, it would be very strange if this reaction was not essentially analogous, *i.e.* a stepwise addition of glucose units to a growing polymer. It seems most simple and natural to assume that sucrose itself is the primary acceptor. The main difficulty is that no intermediates in the form of oligosaccharides have been identified since high molecular dextran is almost the only product even at low degrees of conversion of sucrose [6]. One is then forced to conclude that the enzyme has a very low affinity to sucrose as an acceptor and favours very much the transfer of glucosyl groups to higher saccharides. The significance of branching should not be overlooked in this connection. All natural dextrans are branched but the exact nature of the branching is not settled. However, if a significant part of the branch end groups can function as glucosyl acceptors, the dextran molecule will grow like an avalanche.

Several recent results are in accord with the view that sucrose is the primary acceptor. Hehre [7] has shown one type of natural dextran with a low molecular weight to contain a sucrose unit as end group. Bailey, Barker, Bourne, Stacey, and Theander [8] have synthesised the glucosylsucrose corresponding to the first step of this hypothetical scheme and shown it to be an

excellent acceptor for glucosyl groups transferred from sucrose by dextransucrase. In most experiments described in the literature, crude preparations of dextransucrase were used. In particular, the enzyme was not freed from small amounts of dextran which may act as an acceptor. Recently Bailey, Barker, Bourne, and Stacey [9] have shown that an enzyme preparation with only traces of dextran has a low activity towards sucrose, which can however be increased many times if a suitable acceptor is added. Recent work with the analogous sucrose — levansucrase — levan system is also in accord with the view that sucrose is the primary acceptor in the building up of the polysaccharide.[10-12]

It should be pointed out that formulae (1) and (2) apply only if the amount of acceptor is constant throughout the polymerisation. If the acceptor and monomer donor are identical, as is assumed in the dextran system, very much more complicated formulae are obtained. The occurrence of branching complicates the situation still further. Without more experimental material it seems, however, not worth while to speculate on these problems.

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