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On the Glycogen in *Escherichia coli B*; Variations in Molecular Weight during Growth. III

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The glycogen formed by *Escherichia coli B*, cultivated in continuous culture at different growth rates, has been isolated. This glycogen has been subjected to ultracentrifugal fractionation and the molecular weight of the different fractions has been determined. Of the total glycogen isolated the rapidly dividing cells contained a high percentage of a *low* molecular weight fraction. Slowly dividing cells contained a high percentage of a *high* molecular weight fraction.

In experiments reported in a preceding article ultracentrifugal fractionation was used to obtain fractions of glycogen with different molecular weights [1]. In the present report this method has been applied to glycogen isolated from cells of *E. coli B* grown in continuous culture at different growth rates.

METHODS

The continuous culture methods used have been described earlier [2]. Nitrogen-deficient synthetic medium with sodium lactate as the carbon source was used [3]. The concentration of ammonium chloride (the sole nitrogen source) was 4×10^{-3} M. The data of the experiment reported here are given in Table 1.

Glycogen was isolated from the cells as described earlier [4]. Light-scattering was used for the molecular weight determinations [4]. Fractionation was effected by centrifugation in a Spinco preparative ultracentrifuge. Two fractions were obtained as precipitates after subsequent runs of 20 min at 20 000 *g* and at 105 000 *g*. The supernatant remaining after the last run constituted a third fraction.

A modification of Dische's carbazol method was used for the quantitative determinations of glucose [5].

RESULTS

The molecular weights of the fractions obtained by ultracentrifugal fractionation of the glycogen samples are given in Table 2. Of the three fractions prepared the low molecular weight fraction and the intermediate fraction showed small variations between the different samples. The high molecular weight fraction showed an increase in the molecular weight in rapidly dividing cells.

Table 1.

Dilution rate (h^{-1})	Number of cells per ml $\times 10^{-9}$	Dry weight of cells per litre of culture (mg)	Glycogen-glucose, % of dry weight	Total nitrogen, % of dry weight	Bacterial nitrogen per litre of culture (mg)	Synthetic rate of glycogen
0.09	2.5	401.7	18.6	11.7	47.0	14.3
0.09	2.5	428.5	19.5	11.2	48.0	15.6
0.14	2.5	415.7	15.0	11.5	48.8	18.2
0.14	2.5	423.9	15.4	11.7	49.6	18.4
0.27	2.3	399.0	12.1	12.6	50.3	25.9
0.36	2.2	384.7	6.8	13.4	51.5	18.3
0.36	2.0	382.1	6.7	13.3	50.8	18.1
0.47	1.9	378.9	4.7	13.6	51.5	16.2
0.68	1.8	356.9	2.4	14.1	50.3	11.6

Nitrogen-limited continuous culture of *E. coli B*. Input concentration of ammonium nitrogen: 56 mg/l.

In a continuous culture steady-state growth can be maintained for long periods of time and the growth rate of the bacteria controlled by the rate of addition of new medium. This "rate of addition" is called the *dilution rate*, defined as f/v, where f is the flow rate of the medium and v is the culture volume. The values given in the last column represent the amount of glycogen synthesized in 1 h per 100 mg bacterial nitrogen.

The glycogen content of these cells was low however, and a large amount of cell material had to be hydrolyzed to obtain the required amount of glycogen. Impurities might have been responsible for the increased molecular weight in these samples.

In Fig. 1 the distribution of glycogen between the different molecular weight fractions is shown. The values are expressed in per cent of the total glycogen of the cells. These values are given in Table 3.

In rapidly dividing cells, at a dilution rate of 0.68 h^{-1}, the amount of glycogen found in the low molecular weight fraction was 50 % of the total glycogen, the high molecular weight fraction contained 45 % and the remainder was found in the intermediate fraction. The glycogen content of these cells was 2.4 % of the dry weight (Table 1).

As reported earlier, the glycogen content of cells cultivated at different growth rates is not uniform [2]. Rapidly dividing cells have a low glycogen content while the reverse is true of slowly dividing cells.

Table 2. Molecular weight of different fractions of glycogen obtained by ultracentrifugal fractionation.

Dilution rate (h^{-1})	Molecular weight $\times 10^{-6}$ 20 000 *g*	105 000 *g*	supernatant
0.09	51	15	<2.1
0.14	67	16	<1.6
0.27	91	16	<1.4
0.36	109	21	<0.9
0.47	149	18	<1.1
0.68	262	17	<1.2

Table 3. Distribution of glycogen between different molecular weight fractions.

Dilution rate (h^{-1})	Per cent of total glycogen		
	20 000 *g*	105 000 *g*	supernatant
0.09	61.9	16.3	21.8
0.14	57.4	18.4	24.2
0.27	59.5	14.6	25.9
0.36	58.2	8.7	33.1
0.47	57.7	6.6	35.7
0.68	44.9	5.2	49.9

At a reduced growth rate of the cells, the percentage of glycogen found in the low molecular weight fraction decreased, while the percentage of it in both of the other two fractions increased (Fig. 1). At a dilution rate of 0.09 h^{-1} the low molecular weight fraction accounted for about 22 % of the total glycogen, the intermediate fraction for about 16 % and the high molecular weight fraction for about 62 %. The glycogen content of these cells was about 19 % of the dry weight (Table 1).

DISCUSSION

In the earlier reported experiments [1,4,6] concerned with the synthesis and breakdown of glycogen in *E. coli B*, a special cultivation technique was used involving complete nitrogen starvation. During periods of such starvation, glycogen accumulated in the cells. In the continuous culture, however, nitrogen limitation may be used as a rate-regulating factor and the growth rate of the cells can be varied within a wide range.

As shown earlier [2], the rate of glycogen synthesis increases when the growth rate of the cells is reduced in the nitrogen-limited continuous culture. In the

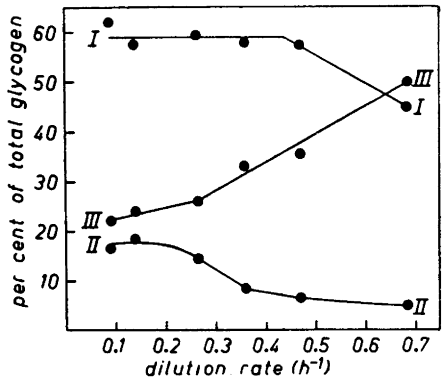

Fig. 1. Distribution of glycogen between different molecular weight fractions. Glycogen samples from *E. coli B* cells grown at different growth rates in continuous culture.

I: high molecular weight fraction.
II: intermediate fraction.
III: low molecular weight fraction.

last column of Table 1 this is confirmed; it can be seen that the rate of glycogen synthesis increases to a maximum at a dilution rate of 0.27 h^{-1}. At lower rates, however, a decrease is noted. This decrease may be the result of such a high content of glycogen in the cells that further formation is inhibited.

In the preceding communication [1] it was shown that there seems to exist two main fractions of glycogen in *E. coli B*, one with a high molecular weight (40—90 $\times$ 10^6) and one with a low molecular weight ($< 2 \times 10^6$). Corresponding fractions are found in the glycogen obtained from continuously cultivated cells, the intermediate fraction never exceeding 20 % of the total glycogen.

From the experiments reported here it can be concluded that the main part of the glycogen that accumulates in slow-growing cells, when their content of glycogen is high, is found in the high molecular weight fraction. When the cells grow at a higher rate, the glycogen content of the cells decreases and at the same time there is an alteration of the distribution ratio of the different molecular weight fractions; half of the total glycogen in these cells may be found in the low molecular weight fraction.

The results are of interest for studies of glycogen metabolism in *Escherichia coli B* since it was previously shown [1], that the different molecular weight fractions have different metabolic activities.

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