ACTA CHEMICA SCANDINAVICA 12 (1958) 1559—1563

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

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Previous investigations have shown that the molecular weight of glycogen from *E. coli B* changes during growth. The glycogen has now been fractionated in different molecular weight fractions. It was found that the glycogen consisted mainly of two fractions, one of high molecular weight, $40-90 \times 10^6$, and one of low molecular weight, $<2 \times 10^6$. The high molecular weight fraction is more active in metabolism than is the low molecular one. The changes in the overall molecular weight observed during growth are mainly attributable to changes in this fraction.

In earlier experiments the synthesis and breakdown of the glycogen in cells of *Escherichia coli B* has been investigated [1-3]. Isotopic techniques have been employed in some of these experiments [2]. By the use of these techniques it has been shown that the glycogen was not homogeneous with respect to its metabolic activity. In later experiments variations in the molecular weight of the glycogen were shown to be correlated to the physiological state of the bacterial cells [3].

This paper reports the additional information obtained by the application of isotopic techniques to the study of the metabolic activity of the different molecular weight fractions obtained by ultracentrifugal fractionation of the glycogen.

METHODS

Bacteriological methods. A medium with glucose as the carbon source and ammonium chloride as the sole nitrogen source was used [4] with appropriate modifications for one-factor-starvation [1]. In the nitrogen-deficient media the ammonium chloride concentration was 200 mg/l giving a population density of approximately 380 mg of dry cells per litre of culture upon exhaustion of the nitrogen source.

The cells were cultivated as described earlier [1] in a nitrogen-deficient medium and allowed to enter the "starvation phase" at which time isotopic carbon was added (uniformly labelled ^{14}C-glucose). After this addition, the cells were allowed to metabolize for 45 min. The culture was then rapidly cooled and the cells quantitatively recovered by

Table 1. Isotopic incorporation and molecular weight of glycogen isolated from cells of *E. coli B* during different conditions of growth. For an explanation of the symbols, see the text.

	Glycogen, per cent of dry weight of bacteria	Counts per min per μg glycogen-glucose	Weight average molecular weight of soluble glycogen
Experiment E			
I	9.9	—	24×10^6
II	13.7	129	50×10^6
III	11.4	36	44×10^6
Experiment F			
I	9.2	—	40×10^6
II	10.2	133	51×10^6
III	7.2	27	42×10^6

centrifugation. The cells were resuspended in a carbon-free medium, which contained the nitrogen source in excess. After 90 min additional incubation, the cells were spun down. Three samples were taken for analysis; one immediately before the addition of the isotopic carbon (I), the second 45 min after this addition (II), and the third 90 min after the resuspension in the carbon-free, nitrogen-containing medium (III). The dry weight of the cells per litre of culture and their nitrogen and glycogen in per cent of the dry weight was determined.

Chemical methods. The glycogen was isolated as previously described [3]. Light-scattering was used for the molecular weight determinations [3].

Before the molecular weight was determined, the glycogen was fractionated in five fractions by means of centrifugation. The first fraction was obtained as a precipitate after centrifugation in a common laboratory centrifuge at about 2 000 r.p.m. This fraction was insoluble in water and accounted for 10—20 % of the total glycogen. It contained silicate impurities and therefore no estimation of the molecular weight could be made. Three fractions were obtained as precipitates after ultracentrifugation in a Spinco preparative ultracentrifuge at 20 000, 40 000 and 105 000 × *g*. The fifth fraction was the supernatant remaining after these centrifugations.

For the measurements of radioactivity, the samples were spread on aluminium planchets and counted at infinite thinness in a windowless gas flow counter, flushed with a helium-*iso*butane mixture. Since the layer of material did not exceed 0.05 mg per cm^2 no correction was made for self-absorption.

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

RESULTS

The results reported in earlier communications have been confirmed [2, 3]. During a phase in which *E. coli B* cells accumulated glycogen, the molecular weight of the glycogen increased. During a subsequent utilization of the accumulated glycogen, the molecular weight decreased (Table 1).

The results of the fractionation experiments are given in Table 2. On account of the different centrifugation times the experiments E and F are not strictly comparable. In both cases, however, the soluble glycogen was divided into four fractions of quite distinct molecular weight. About 50 % of the soluble glycogen has a very high molecular weight, 40—90 × 10^6. Between 25—40 % was found in the low molecular weight, "supernatant fraction" ($< 2 \times 10^6$). The fractions between these extremes contained a

Table 2. Distribution of glycogen in different molecular weight fractions and the specific activity of these fractions. In experiment E the time of each centrifugation was 60 min, while in experiment F the time of centrifugation was 20 min.

Sample	Fraction	Per cent of total soluble glycogen	Counts per min per μg glycogen-glucose	Molecular weight × 10^{-6}
Experiment E				
I	Insoluble	—	—	—
	Precipitated at			
	20 000 *g*	50.5	—	40
	40 000 *g*	17.2	—	20
	105 000 *g*	5.3	—	8
	Supernatant	27.0	—	< 1.5
II	Insoluble	—	140	—
	Precipitated at			
	20 000 *g*	57.8	106	83
	40 000 *g*	2.3	102	18
	105 000 *g*	11.1	97	10
	Supernatant	28.9	93	< 1.5
III	Insoluble	—	40	—
	Precipitated at			
	20 000 *g*	52.0	21	82
	40 000 *g*	0.7	19	<35
	105 000 *g*	7.3	19	10
	Supernatant	39.9	27	< 1.5
Experiment F				
I	Insoluble	—	—	—
	Precipitated at			
	20 000 *g*	53.0	—	67
	40 000 *g*	5.3	—	45
	105 000 *g*	11.6	—	10
	Supernatant	30.1	—	< 1.4
II	Insoluble	—	146	—
	Precipitated at			
	20 000 *g*	49.2	120	92
	40 000 *g*	12.9	(187)	35
	105 000 *g*	15.8	105	10
	Supernatant	22.1	55	< 1.5
III	Insoluble	—	(not measured)	—
	Precipitated at			
	20 000 *g*	48.4	24	80
	40 000 *g*	8.2	24	21
	105 000 *g*	9.1	25	10
	Supernatant	34.3	37	< 1.9

rather small portion of the total glycogen. The high molecular weight fraction was the one having the highest specific activity at the end of the glycogen accumulation phase (the nitrogen starvation phase). At this time the specific activity in the low molecular weight fraction was considerably lower. At the end of the carbon starvation period the specific activity in the high molecular weight fraction was low. The low molecular weight fraction retained its specific activity to a higher extent than the high molecular one.

DISCUSSION

The results given in Table 1 are in good agreement with our earlier observations [2, 3]. The specific activity of the glucose incorporated in the glycogen during accumulation was the same as that of the medium, indicating that no large pool of intermediary metabolites was present in the bacterial cells. During a subsequent growth period, when the cells had been transferred to a nitrogen-containing and carbon-free medium, the glycogen concentration decreased. Mainly the labelled glucose residues were split off. These facts have been interpreted as depending upon a formation of a peripheral tier of labelled glucose around the glycogen molecules during the starvation phase. This tier was the first one to be split off, when the glycogen was utilized [2]. Furthermore the results might be explained by differing metabolic activities in different glycogen fractions. The present experiments have been performed to determine whether different molecular weight fractions have different metabolic activity.

The fraction with a molecular weight of $40—90 \times 10^6$ contained in all samples about 50 % of the total soluble glycogen. The low molecular weight fraction contained 20—40 % (molecular weight $< 2 \times 10^6$). Thus, the two fractions in between contained only small quantities of glycogen. The polydispersity of the glycogen has thus no Gaussian distribution. Instead there seems to be two different kinds of glycogen, one low molecular weight species and one high molecular weight species.

In an earlier report [3] the weight average molecular weight of the total soluble glycogen was followed during growth. It was found to increase during glycogen synthesis and to decrease during glycogen utilization. For comparison the over all weight average molecular weights were calculated from data in Table 2. They are given in Table 1. These data confirm the earlier results, and it is now obvious that the changes occur mainly in the high molecular weight fraction (Table 2).

The specific activity of the glycogen after nitrogen starvation was higher in the insoluble fraction than in any of the others. In the soluble glycogen the metabolic activity was greater in the high molecular weight fractions than in the low molecular weight ones. During the subsequent breakdown of the glycogen more of the labelled glucose was split off from the highest labelled fractions than from the less labelled fractions. This shows that of the soluble glycogen the high molecular weight fraction had the highest metabolic activity. This result may seem surprising in view of the relatively smaller surface of the large molecules.

Metabolic inhomogeneity of the same kind has been found in rabbit muscle glycogen, but in rat liver the low molecular fraction showed a higher metabolic activity [6].

The authors are grateful for the skilled technical assistance given by Mr. R. Eliasson and Mrs. Berit Edling.

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Received June 4, 1958.