A Method for the Determination of the Content of Glycogen-like Polysaccharides in Small Amounts of Bacteria

H. PALMSTIERNA and R. ELIASSON

Chemistry Department I, Karolinska Institutet, Stockholm, Sweden

In order to obtain a survey of the amount of glycogen-like polysaccharides * present in bacterial cells at different times of growth and under different cultivation conditions, it was found desirable to use a less time-consuming and less elaborate method than that previously employed [1,2]. The method described in the following involves no precipitation of the polysaccharides and it is therefore possible to use small amounts of cells. The test organism was *Escherichia coli* B.

Method: Between 5 and 20 mg of lyophilized cells were hydrolysed in 0.2 ml 3 N KOH for two hours in small Pyrex glass test tubes (Length 100 mm, inner diameter 6 mm). The upper end of the test tube was drawn to a capillary in order to avoid losses of fluid. After the alkaline hydrolysis, the pH was adjusted to about 1 with 4 N sulfuric acid and hydrolysed for thirty minutes. Both hydrolyses were performed on a boiling water-bath. The glucose was isolated from the hydrolysate by means of ion-exchangers. The hydrolysate was first passed through Dowex 50 in the H^+-form, and thereafter through Dowex 2 in the acetate-form; both were thoroughly washed. It is necessary to use the ion-exchangers in the order mentioned, since the reverse order seriously affects the yield of glucose. The ion-exchangers were placed in a column between thin layers of Pyrex glass wool. The inner diameter of the column was 6 mm and the length of each of the ion-exchange layers was 90 mm. The mesh size of the ion-exchangers was between 20 and 40. The level of the fluid in the column was kept constant at the top of the ion-exchangers. The hydrolysate was slowly washed through the column. The total volume of the effluent amounted to 15 ml. When required, the volume was reduced by blowing thoroughly filtered air over the fluid, which was heated to between 50 and 60° C. It is necessary to keep the temperature at this high level, since the yield of glucose might otherwise be reduced by bacterial contaminations. The glucose was determined by a modification of Dische's carbazol method [3].

Results. The effluent from the column contained no ninhydrin positive substances, and was free from ultraviolet absorption between 240 and 320 mμ. Ascending paper chromatograms (butanol saturated with water) were performed. Slight traces of ribose were found in most instances. For the same reasons as mentioned previously, the presence of galactose could not be excluded [2].

Forty determination were performed. The results are shown in Table 1.

Table 1.

Sample	Glycogen-glucose in per cent of the dry weight. Precipitation method.	Glycogen-glucose in per cent of the dry weight. Method described in this paper.
A	1.2	1.7 ± .5
B	2.9	3.2 ± .7
C	3.5	3.5 ± .6
D	7.4	7.9 ± .9
E	8.0	8.9 ± 1.2
F	9.3	9.4 ± .7
G	10.1	10.8 ± 1.2
H	15.0	16.2 ± 1.0

As previously shown by the isotope dilution method [2], the values obtained by the precipitation method are about 5 per cent too low.

Discussion. If other bacteria than *E. coli* B are under examination, it is necessary to ascertain that the glucose obtained is substantially free from other carbohydrates. The method is not as accurate as that previously used by the authors, but since the variations in the glycogen content of *E. coli* B are very large, the method described gives useful information. It is easily performed on small amounts of bacteria and is not as time-consuming as methods involving precipitation of the alkalistable polysaccharides.

* For the sake of brevity called glycogen in the following.

1. Palmstierna, H. *Acta Chem. Scand.* **9** (1955) 195.
2. Palmstierna, H. *Acta Chem. Scand.* **10** (1956). *In press.*
3. Gurin, S. and Hood, D. B. *J. Biol. Chem.* **131** (1939) 211.

Received March 27, 1956.

Isolation of ^{32}P-Labeled Phosphoserine from a Yeast Hexokinase Preparation, Incubated with Labeled ATP or Glucose-6-Phosphate

GUNNAR ÅGREN and LORENTZ ENGSTRÖM

Institute of Medical Chemistry, University of Uppsala, Sweden

In earlier communications from this laboratory we reported the incorporation of radioactive inorganic phosphate into phosphoserine isolated from the Schneider protein residues of several rat organs [1]. The maximum incorporation for all organs investigated occurred at approximately three hours after injection of radioactive phosphate [2]. This seemed to infer that some intracellular phosphoproteins may function as transphosphorylating enzymes.

The incorporation of radioactive inorganic phosphate into a new phosphorylated amino acid [3], prepared from the protein of *Lactobacillus casei*, was also compared with the phosphorus uptake of nucleotide and nucleic acid fractions in an attempt to trace the source and the destination of the amino acid phosphorus [4]. However, all these measurements can only give an average value for presumably different kinds of phosphoproteins of different loci within the cell.

As an alternative it seemed of interest to study pure phosphoproteins, as hexokinase or phosphoglucomutase, which are known to be involved in the transfer of phosphorus. The possibility that this transfer might include the formation of an enzyme-phosphate intermediate has been suggested by many authors [5]. Recently this hypothesis received support from the demonstration by Najjar and Pullman [6], that phosphoglucomutase exists in two states, a phosphoenzyme and a dephosphoenzyme. The former transfers phosphate to glucose-1-phosphate or glucose-6-phosphate. Glucose-1,6-diphosphate is formed in the process.

In the present study we have shown the formation of an enzyme -phosphate intermediate in the hexokinase reaction by isolating phosphoserine from the acid hydrolysate of the enzyme, incubated with ^{32}P-labeled ATP [7] in the presence of glucose. In another experiment we also obtained phosphoserine from the enzyme incubated with glucose-6-phosphate isolated from the reaction mixture of the enzyme and AT^{32}P.

The enzyme was purified according to the first six steps of the methods of Berger *et al.*[8] and contained one per cent of glucose *. About 20 mg of this protein was incubated for a few seconds with 1—2 mg ATP, containing about 1—2 mC of phosphorus activity and in the presence of 0.01 M $MgCl_2$. The enzyme was precipitated with 10 % trichloroacetic acid. The protein was hydrolysed and fractionated on a Dowex 50-column according to Ågren *et al.*[1]. The peak eluted after the inorganic phosphate in the expected position of phosphoserine weighed less than 0.1 mg. A part of this material was run through a Dowex 1 formate column according to Busch *et al.*[9] with the addition of non-labeled phosphoserine. The ninhydrin colour from the non-labeled phosphoserine and the activity from the isolated fraction were eluted in the same column volume. Other parts of the activity together with non-labeled phosphoserine were analyzed with one-dimensional paper chromatography using *iso*butyric acid-ammonia and benzene-propionic acid systems. After development of the ninhydrin colour, the paper strips were scanned in the apparatus previously described [2]. The ninhydrin colour from the non-labeled phosphoserine and the activity from the isolated fraction completely agreed.

The trichloroacetic acid centrifugate from the reaction mixture of enzyme and AT^{32}P was run through a Dowex 1 formate column according to Siekevitz and Potter[10], and the column volume containing the radioactive glucose-6-phosphate was incubated with more enzyme solution, con-

* The enzyme was kindly furnished by professor O. Lindberg, Wenner-Gren Institute, Stockholm.