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Enzymatic degradation of sugars

E.STURANI and S. COCUCCI

Laboratorio di Fisiologia Vegetale dell'Istituto di Scienze Botaniche della Università di Milano.

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SUMMARY

A quick and convenient procedure for the degradation of sugars has been worked out. The quantitative conversion of glucose to lactic acid by the use of commercially available enzymes, followed by the chemical degradation of the latter compound, allows to discriminate between the carbons 1 and 6, 2 and 5, 3 and 4 of the molecule. Moreover the enzymatic conversion of glucose to glycerol-1-phosphate and to 3-phosphoglyceric acid allows to separate the glucose molecule into the two moieties, that is to discriminate between carbons 1, 2, 3 and 4, 5, 6.

The problems and the results of this degradation procedure are discussed.

INTRODUCTION

Several chemical and biological methods of degradation of sugars [1, 2, 3] giving the positions of the labeled carbon atoms in the molecule have been reported.

We have worked out a rapid enzymatic degradation procedure which allows to determine the labeling of the carbons 1 and 6, 2 and 5, 3 and 4 of the glucose molecule.

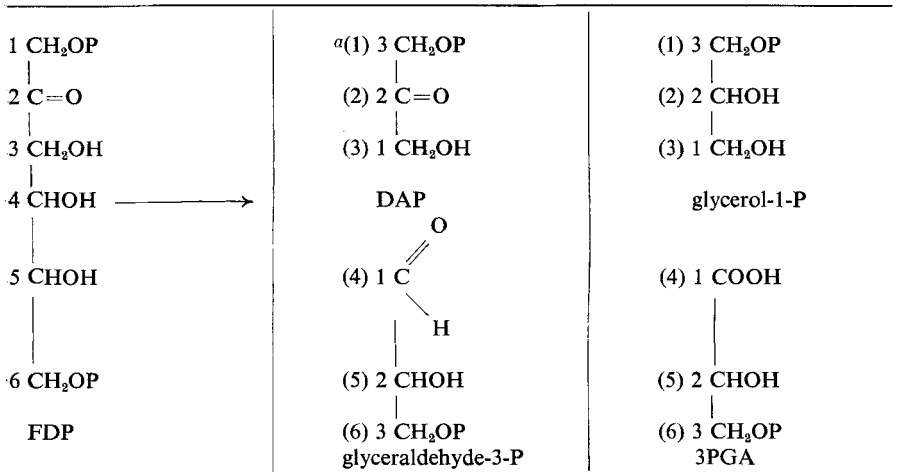
This degradation is based on the conversion of glucose into 3-phosphoglycerate (3PGA) by means of the reactions catalyzed by the following enzymes: hexokinase, hexosephosphate isomerase, fructose-6-P kinase, aldolase, triosephosphate isomerase, glyceraldehyde-3-phosphate dehydrogenase. The 3PGA is then isolated and converted into lactic acid by the action of phosphoglyceril mutase, enolase, pyruvate kinase and lactic dehydrogenase.

The lactic acid is then further degraded by chemical methods [4, 5]. The carbons 1 and 6, 2 and 5, 3 and 4 of glucose are found in the positions 3, 2 and 1 respectively of lactic acid.

It is also possible to separate the two moieties of the glucose molecule, that is carbons 1, 2, 3 and carbons 4, 5, 6.

The method we used is based on the conversion of glucose to fructose diphosphate (FDP), followed by the degradation of this compound to glycerol-1-P and 3PGA

Conversion of fructose diphosphate into glycerol-1-P and 3-P- glycerate



[a] The numbers between parentheses indicate the positions occupied by the carbons in the FDP (and therefore in the glucose) molecule.

The radioactivity of the two compounds separated by paper chromatography is then determined. The labelling of glycerol-1-P corresponds to the radioactivity of carbons 1, 2, 3 of glucose, the one of 3PGA corresponds to the radioactivity of carbons 4, 5, 6 of glucose.

To obtain reliable results it is important to avoid the presence of triosephosphate isomerase (even in traces) when splitting FDP by the aldolase reaction, and to convert immediately the triosephosphates to glycerol-1-P and 3PGA by the appropriate enzymes.

Experimental

Glycolytic enzymes and acidic phosphatase were purchased by the Biochemia s.r.l., NAD, NADH, ATP by the Sigma Chemical Co. The glycolytic compounds have been enzymatically determined by standard spectrophotometric methods [6].

The labeled compounds were localized on the chromatograms with a Vanguard radiochromatoscanner and the radioactivity of the purified compounds was determined in a Packard liquid scintillation counter.

(*a*) *Degradation of glucose to lactic acid.*

The glucose to be tested (0.2-0.5 μmoles) was incubated in a 2 ml final volume in the presence of 50 mM Tris-Cl pH 8.2, 2 mM ATP, 1 mM NAD, 1.5 mM sodium arsenate, 2 mM $MgCl_2$ with 20 μg of hexokinase 20 μg of hexose phosphate isomerase, 100 μg of fructose-6-P kinase, 100 μg of aldolase, 20 μg of triose phosphate isomerase, 100 μg of glyceraldehyde-3-P dehydrogenase. The reduction of NAD was followed spectrophotometrically and 10 % perchloric acid (PCA) was added when no more increase in optical density was observed for 20 minutes. The precipitate was removed and the supernatant was neutralized with KOH. Then 150 mM KCl, 1 mM ADP, 1 mM NADH together with 20 μg of enolase, 20 μg of phosphoglyceril mutase, 20 μg pyruvate kinase and 50 μg of lactic dehydrogenase were added to the neutralized solution. The addition of 10^{-5} M 2,3 DiPGA was essential in order to accelerate the mutase reaction. The overall reaction was followed spectrophotometrically as decrease in optical density at 340 mμ, and when the optical density did not change any more, 10 % PCA was added again as in the previous step. It was essential to interrupt the series of the reactions after the glyceraldehyde-3-P dehydrogenase step because of a slight contamination of the lactic dehydrogenase and of the pyruvate kinase by glycerol-1-P dehydrogenase. Moreover the procedure used allows to follow spectrophotometrically the reduction of NAD (corresponding to the synthesis of 3PGA) in the first reaction mixture and the oxidation of NADH (corresponding to the synthesis of lactic acid) in the second one.

The conversion of glucose to lactic acid was quantitative. The lactic acid so obtained was then further degraded by the chemical methods described in literature [4, 5].

(*b*) *Degradation of glucose to glycerol and glyceric acid.*

The glucose to be tested (0.1-0.3 μmoles) was incubated in 1 ml solution containing 20 mM Tris-Cl pH 8.2, 2 mM ATP, 2 mM $MgCl_2$, 20 μg of hexokinase, 20 μg of hexosephosphate isomerase and 100 μg of fructose-6-P kinase. After 90 minutes the reaction was stopped by the addition of 10 % PCA. After precipitation and neutralization with KOH, the solution (2 ml) containing FDP was adjusted again to pH 8.2 and the following additions were made : 0.3 mM NADH, 0.3 mM NAD, 1.5 mM sodium arsenate, 100 μg of aldolase, 20 μg of glycerol-1-P dehydrogenase and 100 μg of glyceraldehyde-3-P dehydrogenase.

After 90 minutes the reaction was stopped as usual and the compounds, after treatment with acidic phosphatase, were chromatogrammed on No. 1 Whatman paper with a phenol-water solvent system. The substrate was transformed quantitatively in the final products.

Using this procedure triosephosphate isomerase was absolutely absent from the second reaction mixture : as this enzyme slightly contaminates com-

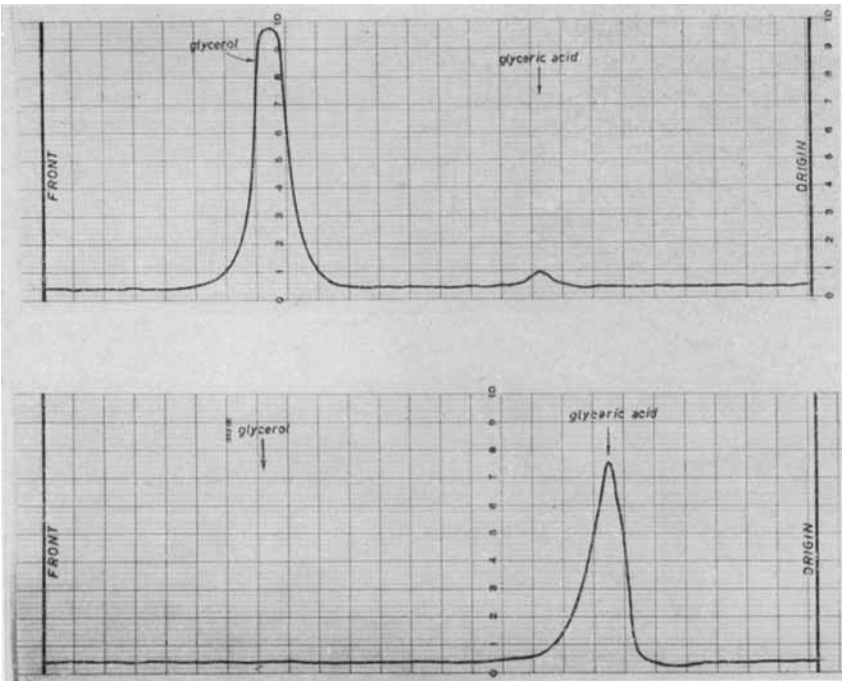


FIG. 1. — Upper : chromatogram of the products of the degradation of glucose-3^{14}C. Lower : chromatogram of the products of degradation of glucose-4^{14}C.

mercial hexokinase and fructose-6-P kinase it is necessary to interrupt the reactions sequence after the formation of FDP; aldolase, glycerol-1-P dehydrogenase and glyceraldehyde-3-P dehydrogenase do not contain this contaminant activity. Moreover dihydroxyacetonephosphate (DAP) and glyceraldehyde-3-P were immediately substracted from the reaction mixture by the addition of the two driving enzymes : no mixing of triosephosphates under this condition was observed.

Glucose-3-^{14}C and glucose-4-^{14}C have been degraded by this method. The resulting 3PGA and glycerol-1-P were treated with acidic phosphatase and chromatogrammed on N° 1 Whatman paper with a phenol-water (4 : 1) solvent system at 20 °C. The resulting radiochromatograms are shown in figure 1. Following the degradation of glucose 3^{14}C, 97 % of the radioactivity is localized in a spot having an RF of 0.7, characteristic of glycerol, while 3 % of it has RF 0.34, characteristic of glyceric acid.

The chromatogram of the products of the degradation of glucose-4-^{14}C shows the whole radioactivity localized in the glyceric acid spot, while no labeling is found in the position of glycerol. The results of the degradations reported indicate that the procedure used allows to distinguish efficiently between the two moieties of the molecule of sugars.

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