CARBOHYDRATE ASSIMILATION IN ACTIVELY GROWING YEAST, *SACCHAROMYCES CEREVISIAE*

II. SYNTHESIS OF POLYSACCHARIDES FROM [1-^{14}C]GLUCOSE

S. L. CHEN

Biochemistry Laboratory, Research Department, Red Star Yeast and Products Co., Milwaukee, Wisc. (U.S.A.)

(Received July 14th, 1958)

SUMMARY

Synthesis of the yeast mannan, glycogen and trehalose from [1-^{14}C]glucose was investigated in yeast grown at aeration rates permitting high, low and moderate oxygen absorption. Similar specific radioactivites were obtained in the hexoses isolated from the acid hydrolysates of these carbohydrates in all yeast samples. However, their activities were lower than that of the [1-^{14}C]glucose substrate. The degradation results revealed that the distribution pattern of radioactivity in the isolated hexoses was similar to that of the substrate, namely in the C^1 position. The specific activity of the C^1 fractions was about six times greater than that of the hexose carbon. The implications of these results were discussed.

INTRODUCTION

Several well-defined carbohydrates of yeast, such as trehalose[1], mannan[2,3], glycogen[4,5] and glucan[6,7], have been isolated and characterized. The carbohydrate content in yeast varies from 25 to 40% of the dry matter, depending upon the composition of the growth medium and the conditions under which the yeast is grown[8]. Similarly, its composition also varies substantially. Thus, in one strain of baker's yeast used in the present study, the trehalose content varies from 2% of the total cellular carbohydrates under essentially anaerobic conditions to 38% under aerobic conditions[9]. The purpose of this study is to investigate the synthesis of these carbohydrates in yeast grown with [1-^{14}C]glucose during fermentation aerated at three different levels of oxygen absorption.

EXPERIMENTAL

Isolation of mannan, trehalose and glycogen from ^{14}C-containing yeast

The yeast cells grown with [1-^{14}C]glucose as described in a previous paper[10] were harvested by centrifugation and fractionated by a method similar to that of NORTHCOTE[4]. The yeast cake obtained was extracted with several 100-ml portions of 3% NaOH solution for 96 h at 70 to 75° until no more carbohydrate was removed, as shown by a negative anthrone reaction. The combined NaOH extract was adjusted to pH 4.2 to 4.5 with HCl to precipitate proteins. More proteins were precipitated and

References p. 483/484.

removed upon neutralization and concentration *in vacuo*. The addition of two volumes of ethanol to the neutralized concentrate resulted in the formation of a precipitate on standing overnight at 2°. It was separated and re-dissolved in 100 ml of 3% NaOH solution. Upon the addition of 50 ml of Benedict solution, a mannan–copper complex was formed, and this was removed by centrifugation and decomposed in 25 ml of 2 *N* HCl. Mannan was precipitated with 2 volumes of ethanol. The mannan isolated with this method was found to be contaminated with glycogen even after repeated purification by alkaline washing and ethanol re-precipitation. A small amount of glucose was found to be present in the acid hydrolysate of the mannan so isolated. However, pure mannose was obtained from the mannan hydrolysate by chromatographing the latter on a cellulose powder column and eluting with *n*-butanol–water solvent (Fig. 1)[11].

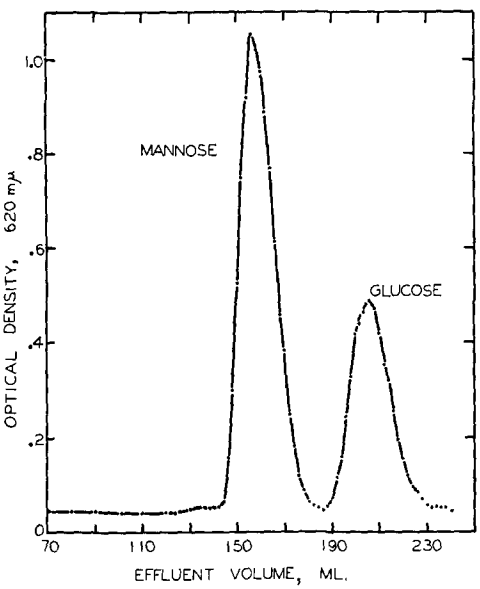

Fig. 1. Separation of glucose (glycogen) contaminant from mannose in the mannan hydrolysate by cellulose power column chromatography (Solvent: *n*-butanol–water). The sugars in the effluent were determined by an anthrone method[10], after the butanol was removed by evaporation *in vacuo*, and identified with paper chromatography method[12] and optical rotation.

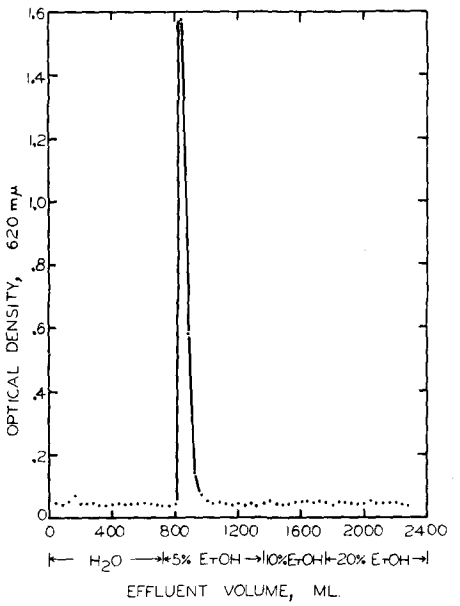

Fig. 2. Isolation of trehalose from the 3% NaOH extract by charcoal–celite column chromatography method. Trehalose was identified both by paper chromatography method[12] and optical rotation.

Trehalose was also isolated from the NaOH extract. After the removal of mannan by ethanol precipitation from the neutralized NaOH extract, the latter was freed of ethanol by evaporation *in vacuo* and from NaCl by ion exchange resin adsorption (Dowex 50 and Dowex 3). The de-salted extract was then concentrated to a small volume and chromatographed on a charcoal–celite column[13]. The trehalose, which was eluted with 5% ethanol (Fig. 2), was isolated, crystallized and hydrolyzed to glucose with 0.5 *N* H_2SO_4 in sealed tubes for 24 h at 100°.

After the extraction of yeast cells with 3% NaOH solution, the residue was

References p. 483/484.

treated with several 250-ml portions of 0.5 *N* acetic acid at 70 to 75° with constant stirring for a period of 5 to 7 days. The combined acetic acid extract was concentrated *in vacuo* and the glycogen present therein was precipitated with 2 volumes of ethanol. Subsequent to the purification of glycogen by 10% trichloroacetic acid extraction and repeated ethanol precipitation, it was hydrolyzed with 0.5 *N* H_2SO_4 as previously described. Glucose was then isolated from the acid hydrolysate for degradation experiments.

Degradation of [^{14}C]-hexoses

The purified [^{14}C]hexoses, *i.e.* mannose and glucose, isolated from the acid hydrolysates of yeast mannan, trehalose and glycogen were degraded by *Leuconostoc mensenteroides* No. 39[14,15]. For mannose, a pre-adapted culture was used. About 40 mg of cell solids was used per Warburg flask, which contained from 50 to 100 μmoles of hexose in 3 ml of 0.05 *M* phosphate buffer at pH 6.0. The CO_2 liberated from the C^1 of the hexose molecule was absorbed with NaOH in the center well. Fermentation was usually completed in 4 to 5 h, and the cells were then removed from the fermented medium by centrifugation. The ethanol produced was distilled off from the neutralized fermented medium and converted to acetic acid by a $K_2Cr_2O_7$–H_2SO_4 oxidation method[16]. The residue was then acidified and the lactic acid remained therein was extracted by ethyl ether. The lactic acid and the acetic acid so isolated were degraded by the method of KATZ *et al.*[17].

The results on the determination of the specific radioactivities of the hexoses isolated from the acid hydrolysates of yeast mannan, trehalose and glycogen are shown in Table I. Almost identical specific radioactivities were obtained in those hexoses which were isolated from the same yeast sample. However, in all cases, their specific activities were lower than that of the substrate glucose molecules.

The specific radioactivities of the C^1 of the degraded mannan–mannose and glycogen–glucose are also shown in the same table. It is noteworthy, that the specific

TABLE I

MEASUREMENT OF SPECIFIC ACTIVITY OF MANNOSE, GLUCOSE AND THEIR DEGRADATION PRODUCTS

These hexoses were isolated from the acid hydrolysates of yeast mannan, glycogen and trehalose. The C^1 fractions of these compounds were obtained by degrading the hexoses with the Leuconostoc fermentation method.

Hexose	*Yeast sample**					
	Sample 1		*Sample 2*		*Sample 3*	
	counts/min/mmole C in sugar	*counts/min/mmole C^1*	*counts/min/mmole C in sugar*	*counts/min/mmole C^1*	*counts/min/mmole C in sugar*	*counts/min/mmole C^1*
Mannose	3,140	19,200	3,100	18,300	3,340	18,300
Glucose (Glycogen)	3,200	20,000	2,940	17,200	3,750	22,300
Glucose (Trehalose)	3,130	—	3,170	—	3,100	—

* These yeast samples were grown at three different aeration levels with [1-^{14}C]-Glucose substrate[10]: Sample 1, 69 mmoles O_2/l/h; Sample 2, 15 mmoles O_2/l/h; Sample 3, 7 mmoles O_2/l/h. The specific activity of the [1-^{14}C]-glucose substrate was 24,300 counts/min/mmole glucose or 4050 counts/min/mmole C in the glucose substrate. The dilution due to the seed yeast was corrected in all measurements of the specific activities.

References p. 483/484.

radioactivity of the C^1 in all samples was about six times greater than that of the hexose carbon. The amount of radioactivity detected in any other carbon atoms was negligible. This shows that substantially all of the radioactivity was present in C^1 of the isolated hexose molecules.

DISCUSSION

The experimental results of Table I showed that the same radioactivity distribution pattern as the [1-^{14}C]glucose substrate was obtained in the hexose molecules isolated from the acid hydrolysates of yeast mannan and glycogen. Such findings are in agreement with the results reported by GILVARG[18] and SOWDEN AND FRANKEL[19]. These data indicate that direct polymerization of hexose units is involved during the synthesis of these polysaccharides in yeast. This is apparently true in all yeast samples whether grown at high or low aeration levels. The formation of polysaccharides by condensation of triose units via the reversal of the Embden-Meyerhof-Parnas pathway must be quantitatively insignificant, otherwise a re-distribution of the radioactivity in the hexose molecules should be detected.

The fact that similar specific radioactivities were obtained in the mannose isolated from yeast mannan and the glucose isolated from trehalose and glycogen, together with their similar distribution patterns, indicate that mannose is formed directly from [1-^{14}C]glucose. Since the specific activity of mannose is not lower than that of the isolated glucose, the presence of a non-radioactive mannan fraction, as shown by SOWDEN *et al.* in Torula yeast[20], is doubtful, unless its quantity is extremely small or it can not be extracted by the methods employed.

Contrary to the results reported by GILVARG[18], the specific activities of the isolated mannose and glucose were found to be lower than that of [1-^{14}C]glucose substrate. This radioactivity dilution in the isolated hexoses was explained by the synthesis of low activity glucose via the hexose monophosphate shunt[19]. It is significant that in these three yeast samples, the radioactivities of the isolated hexoses were diluted to a similar extent, and the metabolic CO_2 produced via the hexose monophosphate shunt was constant, *i.e.* 4 to 6%, at different aeration levels[10], under which the yeast was grown.

ACKNOWLEDGEMENTS

The author is indebted to Dr. I. C. GUNSALUS, University of Illinois, for his gift of *Leuconostoc mesenteroides* culture No. 39. The technical assistance of Messrs. KENNETH LAUER and PHILIP BURCK is gratefully acknowledged.

REFERENCES

1 E. M. KOCH AND F. C. KOCH, *Science*, 61 (1925) 570.
2 W. N. HAWORTH, E. L. HIRST AND F. A. ISHERWOOD, *J. Chem. Soc.*, (1937) 784.
3 W. N. HAWORTH, R. L. HEATH AND S. PEAT, *J. Chem. Soc.*, (1941) 833.
3 D. H. NORTHCOTE, *Biochem. J.*, 53 (1953) 348.
5 S. PEAT, W. H. WHELAN AND T. E. EDWARDS, *J. Chem. Soc.*, (1955) 355.
6 W. E. HASSID, A. M. JOSLYN AND R. M. MCGREADY, *J. Am. Chem. Soc.*, 63 (1941) 295.
7 D. J. BELL AND D. H. NORTHCOTE, *J. Chem. Soc.*, (1950) 1944.
8 E. J. COOPER AND S. L. CHEN, unpublished data.
9 S. L. CHEN AND K. J. H. LAUER, unpublished data.

[10] S. L. CHEN, *Biochim. Biophys. Acta*, 32 (1959) 470.
[11] P. SCHAMBYE, H. G. WOOD AND M. KLEIBER, *J. Biol. Chem.*, 226 (1957) 1011.
[12] S. M. PARTRIDGE, *Biochem. J.*, 42 (1948) 238.
[13] R. L. WHISTLER AND D. F. DURSO, *J. Am. Chem. Soc.*, 72 (1950) 677.
[14] I. C. GUNSALUS AND M. GIBBS, *J. Biol. Chem.*, 194 (1952) 871.
[15] I. A. BERNSTEIN, K. LENTZ, M. MALM, P. SCHAMBYE AND H. G. WOOD, *J. Biol. Chem.*, 215 (1955) 137.
[16] W. D. MAXSON AND M. J. JOHNSON, *Ind. Eng. Chem.*, 45 (1953) 2554.
[17] J. KATZ, S. ABRAHAM AND J. L. CHAIKOFF, *Anal. Chem.*, 27 (1955) 177.
[18] C. GILVARG, *J. Biol. Chem.*, 199 (1952) 57.
[19] J. C. SOWDEN AND S. FRANKEL, *J. Biol. Chem.*, 221 (1956) 587.
[20] J. C. SOWDEN, S. FRANKEL, B. H. MOORE AND J. E. MCCLARLY, *J. Biol. Chem.*, 206 (1954) 547.

IN VITRO EFFECT OF TOCOPHEROL METABOLITES ON RESPIRATORY DECLINE IN DIETARY NECROTIC LIVER DEGENERATION*

KLAUS SCHWARZ AND WALTER MERTZ

Laboratory of Nutrition and Endocrinology, National Institute of Arthritis and Metabolic Diseases National Institutes of Health, Bethesda, Md. (U.S.A.)

AND

ERIC J. SIMON

Department of Medicine, Cornell University Medical College and New York Hospital, New York, N. Y. (U.S.A.)

(Received July 10th, 1958)

SUMMARY

The latent phase of necrotic liver degeneration in the rat, produced by dietary deficiency of vitamin E and Factor 3, is characterized by respiratory decline, *i.e.*, a failure of respiration of liver slices in the Warburg. Tocopherol readily reverses this defect when injected intravenously but not when added to the Warburg medium.

The *in vitro* effect of various tocopherol derivatives on respiratory decline was investigated. DL-α-tocopherol, *d*-α-tocopheryl polyethylene glycol-1000 succinate, DL-α-tocopherylhydroquinone, DL-α-tocopherylquinone, and DL-"α-tocopheroxide" (acetal of α-tocopherylquinone) did not influence the breakdown of respiration. Two tocopherol metabolites, isolated from the urines of rabbits and humans, 2-(3-hydroxy-3-methyl-5-carboxy)-pentyl-3,5,6-trimethylbenzoquinone and its γ-lactone, were found to prevent respiratory decline when added to the Warburg medium. A dose response curve was obtained. The 50% effective dose was at approx. 6.25 μg. The diacetate of the hydroquinone of the lactone was active at higher dose levels. The significance of the findings is discussed.

In addition to the effect on respiratory decline, the γ-lactone at levels of 100 μg per flask produced a stimulation of the initial O_2 consumption. The stimulatory effect was less pronounced, but present, with liver slices from normal controls.

* An account of this work has been presented Nov. 2, 1956, at the Symposium on Approaches to the Quantitative Description of Liver Function at San Francisco, Calif.

References p. 491.