JOURNAL OF BACTERIOLOGY, Oct., 1965
Vol. 90, No. 4

Printed in U.S.A.

Effect of 2-Deoxyglucose on *Schizosaccharomyces pombe*

ROLAND MEGNET

Institut für Allgemeine Mikrobiologie der Universität, Bern, Switzerland

Received for publication 20 April 1965

ABSTRACT

MEGNET, ROLAND (Institut für Allgemeine Mikrobiologie der Universität, Bern, Switzerland). Effect of 2-deoxyglucose on *Schizosaccharomyces pombe*. J. Bacteriol. **90**:1032–1035. 1965.—Cultivation of *Schizosaccaromyces pombe* in a medium containing 2-deoxyglucose (100 μg/ml) results in the death of the cells after an initial period of apparently normal growth. At higher deoxyglucose concentration (400 μg/ml), the cells die immediately after inoculation. Only growing cells are killed, and microscopic inspection of the cultures reveals cell-wall fragments of lysed cells. A mutant resistant to 2-deoxyglucose, which cannot use glucose as a carbon source, was found to be partially deficient in hexokinase. The data constitute evidence for the inhibition of some reaction(s) in the synthesis of cell-wall polysaccharides by metabolites of 2-deoxyglucose in this organism.

During an investigation of procedures designed for the selection of mutants in *Schizosaccharomcyes pombe* which are deficient in the enzyme hexokinase, 2-deoxyglucose (2-DG) was tried as a screening agent. This compound, a substrate for yeast hexokinase (Crane, 1962), is known to inhibit the growth and fermentation of yeast (Woodward, Cramer, and Hudson, 1953). It was assumed that the inhibitory effect of 2-DG on this organism is due to a block of the glucose phosphate isomerase by 2-deoxyglucose-6-phosphate, formed by the hexokinase reaction (Wick et al., 1957). Hexokinaseless mutants, growing on a carbon source which is not a substrate of hexokinase, were expected to be resistant to 2-DG because these mutants would not synthesize 2-deoxyglucose-6-phosphate. This expectation was later confirmed, but the assumption on the mechanism of the 2-DG inhibition seemed not to be well founded. Cells of *S. pombe* grown in the presence of 2-DG formed fragile cell walls, resulting in the death of the cells upon lysis. Thus, the primary effect of 2-DG on this organism is the inhibition of some reaction(s) in the synthesis of capsular polysaccharides. Similar observations were recently reported in *Saccharomyces* (Heredia, de la Fuente, and Sols, 1964). In this paper, the use of 2-DG for studies of cell-wall biosynthesis, the screening of auxotrophic mutants, and the selection of hexokinaseless mutants in *S. pombe* are described.

MATERIALS AND METHODS

Strains. The strains of *S. pombe* used in this study were: wild-type 975; a purple adenine-requiring mutant, 975/50 (kindly supplied by U. Leupold); and a hexokinaseless mutant, *hk* 5.25, of spontaneous origin which was derived from 975.

Culture media. The defined medium of Megnet (1959) was supplemented with 0.01 M L-malate. YGM medium contained (per liter): yeast extract (Difco), 5 g; glucose, 10 g. Gluconate medium contained the salts and vitamin base on the defined medium, 0.2 M gluconic acid, and 0.2 M KH_2PO_4. The 2-DG (Aldrich Chemical Co., Inc., Milwaukee, Wis.) was dissolved in water and sterilized by filtration.

Culture methods. Cells were grown at 30 C with aeration in test tubes containing 10 ml of liquid medium. Plates were incubated for 4 days at 30 C. A pretreatment of 2 hr in liquid synthetic medium was used to deplete the adenine-requiring cells of endogenous adenine. Optical densities of tube cultures were measured with a Lumetron colorimeter with filter 650.

Survival curves were constructed from colony counts on YGM plates. The adenine mutant colonies were differentiated from wild-type colonies by their purple color.

Screening of spontaneous hexokinaseless mutants. Wild-type cells were plated on solid gluconate medium supplemented with 2-DG, 100 μg/ml. Plates incubated for 10 days at 30 C were replicated on glucose-medium plates. Cells from colonies which did not grow on the glucose medium were purified by streaking on gluconate plates, picked, and stored on gluconate agar slants.

Hexokinase assay. Cells of the wild-type and the hexokinaseless mutant were grown in liquid gluconate medium and broken up by repeated freezing at −20 C and thawing at 4 C. Enzyme activities in crude, cell-free extracts were determined spectrophotometrically with glucose-6-phosphate dehydrogenase as indicator enzyme,

according to a procedure outlined by Slein (1962). Substrates and glucose-6-phosphate dehydrogenase were from Boehringer, Mannheim, Germany. Protein concentrations were determined by use of the biuret method (Layne, 1955).

RESULTS

Wild-type cells of *S. pombe* cultivated in defined medium supplemented with 2-DG, 100 μg/ml, grew in the first 18 hr at a rate which was not much different from the rate of the control culture. After 18 hr, the optical density values remained constant and the viable count started to drop exponentially (Fig. 1). After 30 hr of cultivation, the titer of viable cells dropped below the value at the start of the experiment; the culture began to foam, and microscopic inspection of the suspension showed mostly empty halves of cell walls. Figure 2 shows a representative sample of such cell-wall fragments.

Only growing cells of *S. pombe* were killed by 2-DG (Fig. 3). A mixture of wild-type and ade-

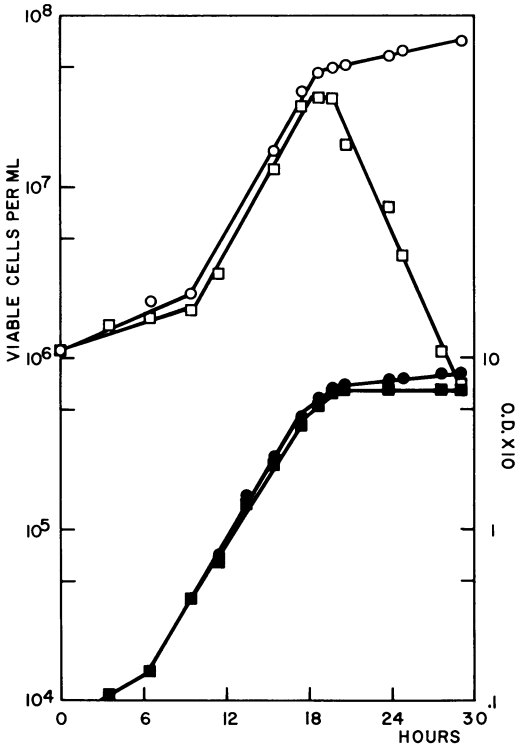

FIG. 1. *Effect of 2-DG on the growth of the wild type of Schizosaccharomyces pombe in defined medium. Viable cells per milliliter: control, ○; 2-DG (100 μg/ml), □. Optical density: control, ●; 2-DG (100 μg/ml), ■.*

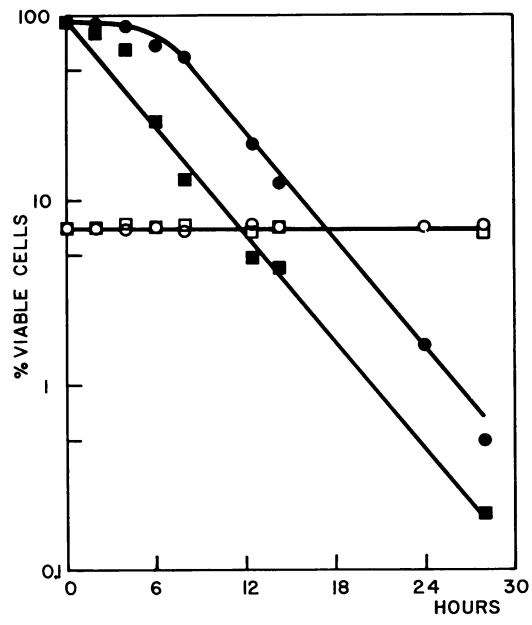

FIG. 3. *Selective killing of wild-type cells of Schizosaccharomyces pombe in a mixture of wild-type (●, ■) and adenine-requiring (○, □) cells, in synthetic medium plus 2-DG, 400 μg/ml, squares; 600 μg/ml, circles.*

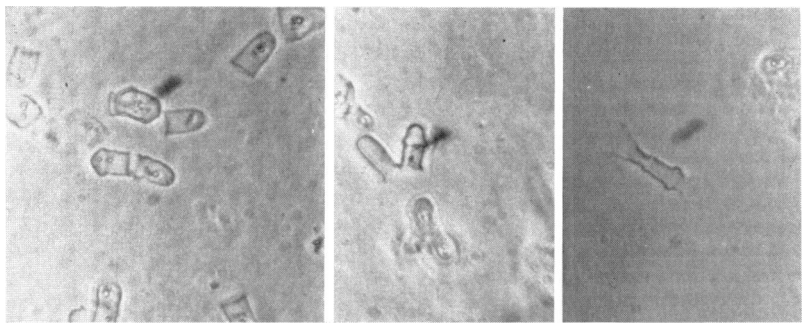

FIG. 2. *Cell-wall fragments of a 30-hr culture of wild-type cells of Schizosaccharomyces pombe in synthetic medium plus 2-DG (100 μg/ml). Water mounts, phase contrast, × 800*

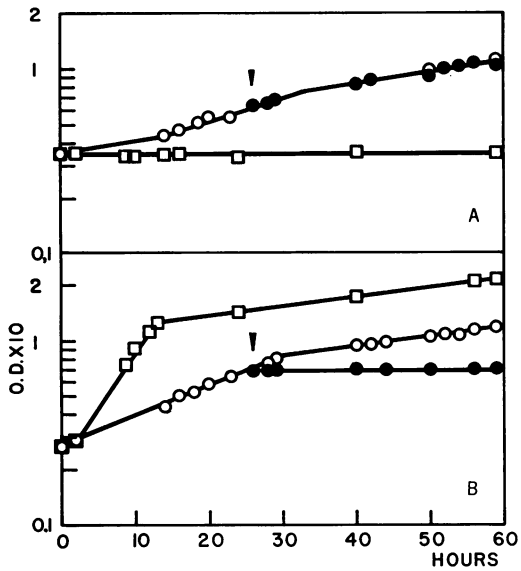

FIG. 4. *Growth of (A) a hexokinase-deficient mutant, hk 5.25, and (B) of the wild type of Schizosaccharomyces pombe. Both types of cells were grown in gluconate-phosphate medium, ○; glucose-phosphate medium, □; and a parallel culture of gluconate-phosphate medium, ●, to which 200 μg/ml of 2-DG were added (arrow).*

nine-requiring cells was treated in synthetic medium with 2-DG at 400 and 600 μg/ml. There was an immediate drop of viability of the growing wild-type fraction at 400 μg/ml of 2-DG. At 600 μg/ml of 2-DG, there was first a lag, followed by the same rate of inactivation as in the lower 2-DG concentration. In contrast to the wild-type cells, the nongrowing adenine-requiring cells were not affected by 2-DG.

The growth behavior of the hexokinaseless mutant and the wild type is shown in Fig. 4. The cells were incubated in two different media; one was the defined glucose medium supplemented with potassium phosphate, and the other was the gluconate medium to which, after 26 hr of cultivation, 2-DG at a final concentration of 200 μg/ml was added. (The relatively high concentration of potassium phosphate, used as a buffer in the gluconate and glucose media, reduces the growth rate and final yield of the cultures. By using citrate instead of phosphate the growth rate in these media can be improved.) In contrast to the wild-type, the hexokinaseless mutant did not grow in the glucose medium and was not inhibited by 2-DG during growth on gluconate. The specific activities of the hexokinase in wild-type and mutant extracts are given in Table 1. When mutant and wild-type extracts were mixed, no inhibitor could be demonstrated in the mutant extract.

TABLE 1. *Hexokinase activity* of crude extracts of wild type and mutant hk 5.25*

Strain	Hexokinase activity (units/ml)†	Protein‡	Specific activity (units/mg of protein)
		mg/ml	
Wild type 975	690	11.90	57.70
Mutant *hk* 5.25	26	2.31	11.25

* Reaction mixture contained: triethanolamine buffer, *p*H 7.6 (0.1 M), 0.2 ml; glucose (2.18 M), 0.1 ml; $MgCl_2$ (65.6 μM), 0.1 ml; adenosine triphosphate (6.46 μM), 0.1 ml; nicotinamide adenine dinucleotide phosphate (8.83 μM), 0.1 ml; glucose-6-phosphate dehydrogenase (20 μg/ml), 0.1 ml; extract diluted in 1% serum albumin, 0.1 ml; water, 0.2 ml.

† One unit of hexokinase activity = change in optical density at 340 mμ of 0.1 per minute at 30 C.

‡ Bovine serum albumin was used as standard.

DISCUSSION

A mutant of *S. pombe* partially deficient in hexokinase is resistant to a substrate of this enzyme, 2-DG. This indicates that the first requirement for the inhibitory action of 2-DG on this organism is its enzymatic conversion into 2-deoxyglucose-6-phosphate. The partially deficient mutant cannot use glucose as a carbon source and, in addition, does not utilize mannose and fructose, both of which are substrates of the hexokinase (Megnet, *unpublished data*). The reason why the residual hexokinase activity does not allow the mutant strain to use substrates of this enzyme is unclear and needs further investigation.

The reactions after the phosphorylation of 2-DG have yet to be investigated in *S. pombe*. Thus, only speculations on the probable enzymatic block produced by metabolites of 2-deoxyglucose-6-phosphate can be offered. The 2-deoxyglucose-6-phosphate formed in wild-type cells could be expected to inhibit at least three reactions. These are the reactions catalyzed by glucose phosphate isomerase, glucose-6-phosphate dehydrogenase, and phosphoglucomutase. The first two enzymes can be excluded from the discussion because their inhibition would probably only lead to a reduction of the growth rate and not to the death of the cells. On the other hand, a block of the phosphoglucomutase reaction would result in a reduced supply of glucose-1-phosphate, a precursor of cell-wall polysaccharides (Ginsburg, 1964). Consequently, weak cell walls would be formed which could not sustain the pressure exerted by the growing protoplasm. The inhibition of the synthesis of structural polysaccharides beyond the phosphoglu-

comutase seems even more probable, since 2-deoxyglucose-6-phosphate is a substrate of the rabbit-muscle phosphoglucomutase (Együd and Whelan, 1963). The trapping of uridine nucleotides [used in the synthesis of polysaccharides (Ginsburg, 1964)] by 2-deoxyglucose-1-phosphate formed by the phosphoglucomutase reaction could account for the observed effect (Heredia et al., 1964).

The selective killing of growing cells by 2-DG is a further indication that the synthesis of structural polysaccharides is the primary target of metabolites of 2-DG. A procedure, similar to the penicillin method for bacteria, for the screening of auxotrophic mutants of *S. pombe* based on this observation was recently devised (Megnet, 1965).

A study of the shapes of cell-wall fragments formed by cells treated with 2-DG confirms the data of Mitchison (1957) on the growth zones of the cell wall of *S. pombe*. The wall fragments of cells grown in 100 μg/ml of 2-DG consisted mainly of thimble-shaped structures. A few cylinders open at both ends were also obsrved. Mitchison (1957) found that walls of cells in the process of division grow in the central region, and the walls of separated daughter cells at the ends. In this study, the thimble-shaped forms were found mainly in exponentially growing cultures and the cylinders during the lag and stationary phase of growth. These structures are expected from growing cells in which cell-wall biosynthesis is blocked. Such cells will burst at places where the cell wall is most fragile, i.e., at the zones where new cell-wall material is deposited. A dividing cell will break up in the middle, and a growing, nondividing cell will burst open at the ends.

ACKNOWLEDGMENTS

This investigation was supported by grant no. 2987 from the Swiss National Science Foundation.

The technical assistance of Ruth Blaser and Heinz Läuffer is gratefully acknowledged.

LITERATURE CITED

CRANE, R. K. 1962. Hexokinase and pentokinases, p. 47–66. *In* P. D. Boyer, H. Lardy, and K. Myrbäck [ed.], The enzymes, vol. 6. Academic Press, Inc., New York.

EGYÜD, L. G., AND W. J. WHELAN. 1963. The substrate specificities of phosphoglucomutase and phosphoglucose isomerase. Biochem. J. **86**:11P–12P.

GINSBURG, V. 1964. Sugar nucleotides and the synthesis of carbohydrates. Advan. Enzymol. **26**:35–38.

HEREDIA, C. F., G. DE LA FUENTE, AND A. SOLS. 1964. Metabolic studies with 2-deoxyhexoses. Biochim. Biophys. Acta **86**:216–223.

LAYNE, E. 1955. Spectrophotometric and turbidimetric methods for measuring proteins, p. 447–454. *In* S. P. Colowick and N. O. Kaplan [ed.], Methods in enzymology, vol. 3. Academic Press, Inc., New York.

MEGNET, R. 1959. Untersuchungen über die Biosynthese von Uracil bei *Schizosaccharomyces pombe*. Arch. Julius Klaus-Stift. Vererbungsforsch. Sozialanthropol. Rassenhyg. **33**:299–334.

MEGNET, R. 1965. Screening of auxotrophic mutants of *Schizosaccharomyces pombe* with 2-deoxyglucose. Mutation Res. **2**:328–331.

MITCHISON, J. M. 1957. The growth of single cells. *Schizosaccharomyces pombe*. Exptl. Cell Res. **13**:244–262.

SLEIN, M. W. 1962. D-Glucose, Bestimmung mit Hexokinase und Glucose-6-phosphat-Dehydrogenase, p. 117–123. *In* H.-U. Bergmeyer [ed.], Methoden der enzymatischen Analyse. Verlag Chemie, Weinheim.

WICK, A. N., D. R. DRURY, H. I. NAKADA, AND J. B. WOLFE. 1957. Localization of the primary metabolic block produced by 2-deoxyglucose. J. Biol. Chem. **224**:963–969.

WOODWARD, G. E., F. B. CRAMER, AND M. T. HUDSON. 1953. Carbohydrate analogs as antagonists of glucose in carbohydrate metabolism of yeast. J. Franklin Inst. **256**:577–589.