JOURNAL OF BACTERIOLOGY, Oct. 1967, p. 867-869

Vol. 94, No. 4
Printed in U.S.A.

Initial Step in Catabolism of Glucose by the Meningopneumonitis Agent

JOYCE VENDER AND JAMES W. MOULDER
Department of Microbiology, The University of Chicago, Chicago, Illinois 60637

Received for publication 1 July 1967

The relative rates of catabolism of glucose and glucose-6-phosphate by intact-cell suspensions of the meningopneumonitis agent, a member of the psittacosis group (*Chlamydia*), and the properties of the hexokinase and glucose-6-phosphate dehydrogenase of these suspensions were investigated. It is proposed that the hexokinase is a host enzyme bound to the surface of the meningopneumonitis cell and that glucose-6-phosphate is the first substrate in the conversion of hexose to pentose to be attacked by enzymes synthesized by the meningopneumonitis agent.

Microorganisms of the psittacosis group (*Chlamydia*) degrade glucose with the liberation of carbon dioxide from the C1 and the C3-C4 positions but not from the C6 (6, 7). Whole-cell suspensions derived from infected allantoic fluid produce carbon dioxide from the C3-C4 positions via pyruvate as an intermediate and from the C1 position by means of a partial pentose phosphate pathway. Cell-free extracts of the meningopneumonitis agent, a typical representative of this group of obligate intracellular parasites, contain dehydrogenases for glucose-6-phosphate and 6-phosphogluconate that are qualitatively different from the corresponding enzymes of the host (4). Although hexokinase could not be found in these extracts (4), its presence in whole-cell preparations was strongly suggested by the absolute requirement for exogenous adenosine triphosphate (ATP) and Mg^{++} for release of carbon dioxide from the C1 position of glucose (6). Moreover, the essential role of glucose-6-phosphate as an intermediate in production of carbon dioxide from the C1 position was shown by the observations that in whole cells unlabeled glucose-6-phosphate was a strong competitive inhibitor of carbon dioxide released from C1-labeled glucose and that glucose-*1*-^{14}C-6-phosphate supported a higher rate of $^{14}CO_2$ production than did glucose-*1*-^{14}C (6).

The absence of hexokinase in cell-free extracts of the meningopneumonitis agent, and the ability of intact cells of this organism to liberate carbon dioxide from the C1 position of glucose-6-phosphate, suggested that this compound, not glucose, is the first substrate in the pathway from hexose to pentose to be metabolized by enzymes of the meningopneumonitis agent. It suggested also that the degradation of glucose by whole-cell preparations in the presence of ATP and Mg^{++} depends on the binding of host hexokinase to the surface of the meningopneumonitis cells. The purpose of this investigation was to examine this possibility. It has already been demonstrated that another intracytoplasmic particle, the mitochondrion, reversibly binds hexokinase to its external surface (5).

MATERIALS AND METHODS

Preparation of whole-cell suspensions and cell-free extracts. The Cal 10 strain of meningopneumonitis agent was purified from infected allantoic fluid as described elsewhere (4) except that 0.02 M tris(hydroxymethyl)aminomethane buffer, *p*H 7.2, was used for washing the preparations during the course of purification. The final pellet of meningopneumonitis cells was suspended in the K36 medium of Weiss (6). Cell-free extracts of meningopneumonitis cells and homogenates and extracts of uninfected chorioallantoic membranes were made in K36 medium according to Moulder, Grisso, and Brubaker (4).

Assay of enzymes. Because hexokinase activity could not be demonstrated in meningopneumonitis extracts by standard spectrophotometric methods of assay, a new method for measurement of hexokinase activity was devised. It has the advantages of sensitivity and applicability to whole cells as well as to extracts. It has the disadvantages of laboriousness and dependency on the uncertain purity of successive lots of commercially available enzymes. The method is based on the release of $^{14}CO_2$ from glucose-*1*-^{14}C when the phosphorylation of glucose to glucose-6-phosphate is rate-limiting; that is, in the presence of excess glucose-6-phosphate dehydrogenase (Sigma Chemical Co., St. Louis, Mo.) and 6-phosphogluconate dehydrogenase (Boehringer, Mannheim, Germany). The reaction mixture consisted of 3 ml of K36 medium containing 3 μmoles of Mg^{++}, 0.5 mg of bovine plasma albumin (Armour Pharmaceutical

Co., Kankakee, Ill.), 3 μmoles of nicotinamide adenine dinucleotide phosphate, 15 μmoles of ATP, 1.25 units of glucose-6-phosphate dehydrogenase, 0.4 unit of 6-phosphogluconate dehydrogenase, 10 μmoles (1 μcurie) of glucose-*1*-^{14}C (New England Nuclear Corp., Boston, Mass.), and the sample to be assayed for hexokinase activity. This reaction mixture was placed in the outer chamber of a Conway microdiffusion dish containing in its inner chamber 0.2 ml of 15% KOH adsorbed on filter paper. The closed Conway dish was incubated for 1 hr at 37 C; then the reaction was stopped and the bound carbon dioxide was liberated by the addition of 10 mmoles of H_2SO_4. After an additional 1-hr incubation at 25 C, the filter paper was transferred to a vial containing 15 ml of Bray's (1) scintillation mixture, and the inner chamber was rinsed with 1 ml of scintillation mixture. The $^{14}CO_2$ was then measured in a liquid scintillation counter. Under the conditions of assay, the release of $^{14}CO_2$ from glucose-*1*-^{14}C was linear with respect to time of incubation and to the volume of meningopneumonitis cells or extract added.

Glucose-6-phosphate dehydrogenase was assayed in the same manner with the omission of ATP and glucose-6-phosphate dehydrogenase and the substitution of glucose-*1*-^{14}C-6-phosphate (Nuclear-Chicago Corp., Des Plaines, Ill.) for glucose.

RESULTS

Enzyme activity of intact cells. Hexokinase was readily demonstrated in intact meningopneumonitis cells by the radioisotopic assay just described (Table 1 and Fig. 1). The glucose-6-phosphate dehydrogenase activity was always greater than that of hexokinase. This is in accord with the observations of Weiss (6) that the addition of yeast hexokinase to meningopneumonitis cells may increase the rate of glucose degradation and that glucose-6-phosphate is a better substrate for intact cells than glucose itself.

TABLE 1. *Effect of sonic disruption on hexokinase and glucose-6-phosphate dehydrogenase activity of meningopneumonitis cells*[a]

Prepn assayed	Hexokinase		Glucose-6-phosphate dehydrogenase	
	CO_2 released from glucose	Fraction of whole-cell activity	CO_2 released from glucose-6-phosphate	Fraction of whole-cell activity
	mμmoles/hr	%	*mμmoles/hr*	%
Intact cells	37	100	113	100
Disrupted cells	35	94	204	181
Cell-free extract	7	17	85	75

[a] Meningopneumonitis cells were disrupted at 0 C by a 4-min treatment in a sonic oscillator (MSE ultrasonic generator; Instrumentation Associates, New York, N.Y.). The disrupted cells were assayed immediately with no further treatment. The cell-free extract was obtained by centrifuging the disrupted cells for 30 min at 0 C and 20,000 × *g*. The intact cell suspension contained 4 mg of protein/ml as determined with the Folin phenol reagent (3); 0.3-ml samples of intact cells, disrupted cells, and cell-free extract were assayed for the two enzymes.

Enzyme activity of disrupted cells and cell-free extracts. Table 1 shows that hexokinase activity was unaffected by disruption of meningopneumonitis cells with sonic energy. When the disrupted cell suspensions were centrifuged, most of the activity remained with the cell debris; only a small fraction of the hexokinase activity was recovered in the cell-free extract.

In contrast, the glucose-6-phosphate dehydrogenase activity of the disrupted cells was almost double that of the intact cells, suggesting that

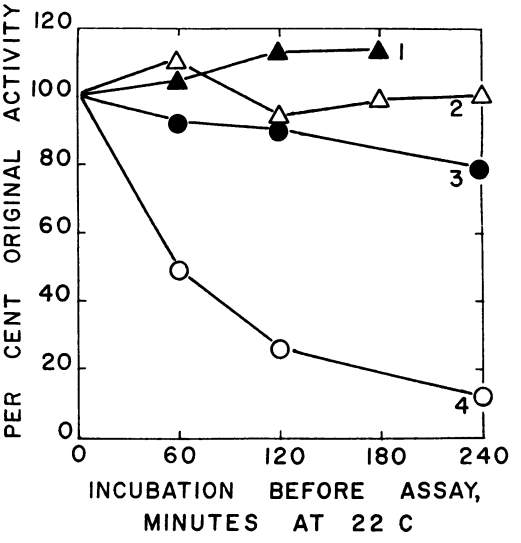

FIG. 1. *Effect of trypsin on the hexokinase and glucose-6-phosphate dehydrogenase activity of intact meningopneumonitis cells. To 0.5-ml suspensions of intact meningopneumonitis cells was added 0.1 ml of either trypsin (20 mg/ml, three times recrystallized, 50% $MgSO_4$; Worthington Biochemical Corp., Freehold, N.J.) or $MgSO_4$ (10 mg/ml). The suspensions were incubated at 22 C. After the desired incubation period, tryptic action was stopped by addition of 0.1 ml of a 30 mg/ml solution of soybean trypsin inhibitor (Sigma Chemical Co., St. Louis, Mo.) and the meningopneumonitis cells were assayed for the two enzymes. The initial hexokinase activity was 58 mμmoles of CO_2 per mg of protein per hr; the initial glucose-6-phosphate dehydrogenase activity was 130 mμmoles of CO_2 per mg of protein per hr. There was no measurable loss of infectivity at 22 C, either in the presence or absence of trypsin. (1) Glucose-6-phosphate dehydrogenase, no trypsin. (2) Glucose-6-phosphate dehydrogenase, plus trypsin. (3) Hexokinase, no trypsin. (4) Hexokinase plus trypsin.*

transport of glucose-6-phosphate into the intact cells had been rate-limiting. A much higher proportion of the glucose-6-phosphate dehydrogenase activity appeared in the cell-free extract. This, together with the higher initial activity of this enzyme in intact cells, resulted in the cell-free extract assaying more than 10 times as high for glucose-6-phosphate dehydrogenase as for hexokinase. This is obviously why hexokinase cannot be found in cell-free extracts by spectrophotometric assay whereas glucose-6-phosphate dehydrogenase can.

Effect of trypsin on the enzyme activity of intact cells. Figure 1 shows that treatment of intact meningopneumonitis cells with trypsin had no effect on glucose-6-phosphate dehydrogenase but that it rapidly destroyed the hexokinase of intact-cell suspensions. One enzyme was clearly accessible to the proteolytic action of trypsin and the other was not.

At 37 C, trypsin destroyed virtually all hexokinase activity in 30 min without affecting the activity of the dehydrogenase. However, at 37 C hexokinase was appreciably destroyed even in the absence of trypsin. Therefore, the experiment of Fig. 1 was conducted at 22 C, a temperature at which hexokinase is not significantly inactivated. The addition of bovine plasma albumin greatly stabilized hexokinase during assay at 37 C.

Addition of crystalline yeast hexokinase (Boehringer) to an assay system containing trypsin-treated meningopneumonitis cells as the sole source of dehydrogenases resulted in a rate of $^{14}CO_2$ production from glucose-*1*-^{14}C equal to or greater than that achieved by untreated meningopneumonitis cells in the same assay system without added hexokinase. This suggests that the main enzymatic deficiency in trypsin-treated cells was the lack of hexokinase.

Enzyme activity in uninfected chick embryos. Uninfected chorioallantoic membranes and allantoic fluid contained significant amounts of hexokinase that was destroyed by digestion with trypsin. When suspensions of intact meningopneumonitis cells were heated in K36 medium at 55 C for 1 hr, their hexokinase activity was completely destroyed. These heat-inactivated suspensions were mixed with normal allantoic fluid and held overnight at 0 C. When the meningopneumonitis cells were then repurified by the usual procedure, it was found that the final purified preparations had bound host hexokinase in amounts equivalent to 10 to 100% of the activity of the corresponding unheated cell suspension. This bound host hexokinase was also destroyed by trypsin.

Although Moulder, Grisso, and Brubaker (4) did not find glucose-6-phosphate dehydrogenase in uninfected chorioallantoic membranes and allantoic fluids by spectrophotometric assay, the radioisotopic assay easily demonstrated the presence of this dehydrogenase in these materials.

DISCUSSION

These results do not unequivocally demonstrate that glucose-6-phosphate and not glucose is the first substrate attacked by meningopneumonitis enzymes in the pathway from hexose to pentose or that the hexokinase activity of intact-cell suspensions is a host contaminant, but they are most reasonably interpreted in this manner. There appears to be no necessity for this organism to phosphorylate glucose with its own hexokinase and no reason for the inability to synthesize hexokinase to be selected against in an intracellular environment. It is possible that the hexokinase of intact-cell suspensions is a true meningopneumonitis enzyme located on the cell surface and thus accessible to the action of trypsin, but we consider it more probable that this organism lacks hexokinase, like mutants of *Escherichia coli* which utilize glucose-6-phosphate but not glucose (2).

ACKNOWLEDGMENTS

This research was supported by U.S. Public Health Service research grant AI-01594 from the National Institute of Allergy and Infectious Diseases. We wish to thank Grazina R. Musteikis for technical assistance.

LITERATURE CITED

1. BRAY, G. A. 1960. A simple efficient liquid scintillator for counting aqueous solutions in a liquid scintillation counter. Anal. Biochem. **1**:279–285.
2. FRAENKEL, D. G., F. FALCOZ-KELLY, AND B. L. HORECKER. 1964. The utilization of glucose-6-phosphate by glucokinaseless and wild type strains of *Escherichia coli*. Proc. Nat. Acad. Sci. **52**:1207–1213.
3. LOWRY, O. H., N. J. ROSEBROUGH, A. L. FARR, AND R. J. RANDALL. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. **193**:265–275.
4. MOULDER, J. W., D. L. GRISSO, AND R. R. BRUBAKER. 1965. Enzymes of glucose catabolism in a member of the psittacosis group. J. Bacteriol. **89**:810–812.
5. ROSE, I. A., AND J. V. B. WARMS. 1967. Mitochondrial hexokinase. Release, rebinding, and location. J. Biol. Chem. **242**:1635–1645.
6. WEISS, E. 1965. Adenosine triphosphate and other requirements for the utilization of glucose by agents of the psittacosis-trachoma group. J. Bacteriol. **90**:243–253.
7. WEISS, E., W. F. MEYERS, H. R. DRESSLER, and H. CHUN-HOON. 1964. Glucose metabolism of the psittacosis-trachoma group. Virology **22**:551–562.