THE TRANSPORT OF METHYL-α-D-GLUCOPYRANOSIDE BY THERMALLY STRESSED SALMONELLA TYPHIMURIUM

Merle D. Pierson[1] and Z. John Ordal

Departments of Food Science and Microbiology
University of Illinois, Urbana, Illinois 61801

Received March 12, 1971

SUMMARY

Heat-injury altered the glucose transport system of Salmonella typhimurium. In the absence of an energy source the rate of methyl-α-D-glucopyranoside (α-MG) accumulation was less for heat-injured cells than for normal cells. The decreased level of α-MG accumulation was accounted for by a lower rate of endogenous metabolism in the injured cells. In the presence of an exogenous energy source (succinate), the rate of α-MG transport and the level of α-MG accumulation was much greater for injured cells in comparison to normal cells. The kinetics of α-MG accumulation differed between Trypticase Soy Broth and citrate grown cells.

Bacterial cell injury can be characterized by an alteration of the selective permeability mechanisms and/or the alteration of the biosynthetic capabilities of the stressed cells. The selective permeability alterations include: an increased salt sensitivity of a normally tolerant microorganism (3,8) and leakage of intracellular materials (1). Clark and Ordal (3) reported that injury could be demonstrated in Salmonella typhimurium if the heated cells were plated on Levine's Eosin Methylene Blue Agar containing 2% salt and this count compared to a Trypticase Soy Agar plate count.

Since heat-injury causes a disruption of the cell membrane, there may be an alteration of membrane bound permeases. Salmonella typhimurium has a glucose permease which is constitutive and which can be assayed by the use of the nonmetabolizable substrate methyl-α-D-glucopyranoside (α-MG) (5). The permease for α-MG and glucose are thought to be the same system (4). In this paper the rate of α-MG transport by normal and heat-injured Salmonella typhimurium is reported.

MATERIALS AND METHODS

Cell Preparation and Injury Procedure

Cultures of S. typhimurium were grown for either 12 hr in Trypticase

[1]Present address: Department of Food Science and Technology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

Soy Broth (TSB) or 21 hr in a citrate salts medium (CSM) (12) on a rotary shaker at 37 C. The cells from 200 ml of culture were harvested by centrifugation at 8,000 x g for 10 min at 0 to 2 C. The pellet was washed one time with 0.1 M phosphate buffer (pH 6.0). The washed pellet was resuspended in 10 ml of 0.1 M phosphate buffer (pH 6.0) and inoculated into 190 ml of 0.1 M phosphate buffer, pretempered at 48 C. The suspension was heated at 48 C for 30 min under constant agitation (3). The heat-injured cells were harvested by centrifugation at 8,000 x g for 10 min at 0 to 2 C. For the preparation of normal (unheated) cells the same procedure was followed except the heating step was omitted.

Cell Concentration Standardization

Normal cells were resuspended in distilled water to obtain a standard curve of O.D. at 600 nm versus dry weight of cells. For each of the following experiments normal and injured cells were resuspended in 0.1 M phosphate buffer (pH 7.2) to a common O.D. In this way equal numbers of cells could be compared (11). As a guideline, the amount of normal and injured cells was expressed as mg normal equivalent cells rather than an O.D. reading.

Warburg Manometry

Conventional Warburg manometry was used to determine the rate of oxygen uptake at 37 C by normal and injured cells. Each flask contained 1.0 ml of 0.1 M phosphate buffer (pH 7.2), 0.5 ml of substrate (10 μmoles), 1.0 ml of cell suspension (2.5 mg normal equivalent cells), 0.4 ml of water, and 0.1 ml of 20% KOH in the center well.

Transport of Methyl-α-D-Glucopyranoside

The transport of α-MG by normal and injured S. typhimurium was determined according to the method of Hoffee, et al.(7). α-MG(glucose-U-^{14}C) (Calbiochem, Los Angeles, Calif.) was diluted with α-MG to give a specific activity of 0.48 μc/μmole and α-MG concentration of 4 x 10^{-5}M in the final reaction mixture. When the transport of α-MG was determined in the presence of an energy source, succinate was added to the α-MG solution to give a concentration of 0.1% in the final reaction mixture.

For the measurement of transport, the normal and injured cells were adjusted to 0.25 mg normal equivalent cells/ml with phosphate buffer (pH 7.2). Five ml of cells were placed in a 50 ml flask and incubated for 3 min at 30 C in a shaker water bath. To the preincubated cells 1 ml of substrate was added. At time intervals, a 1 ml sample was removed from the reaction mixture and vacuum filtered onto a presoaked 0.45 μm pore size Millipore membrane filter (Millipore Corp., Bedford, Mass.). The retained cells were washed with 1 ml of cold buffer. The filters were

dried under a heat lamp and added to 15 ml of scintillation fluid composed of 5 g of 2,5-diphenyloxazole and 0.5 g of 1,4-bis-2-(4-methyl-5-phenyloxazolyl) benzene per liter of toluene.

Samples were counted with a Packard Tri-Carb Model 3320 liquid scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.) until at least 1000 counts were obtained. Correction for quenching was made by the Bush channel ratio method (2).

RESULTS AND DISCUSSION

The demonstration of heat-injury for both TSB and CSM grown S. typhimurium has been previously reported (3,12). When the injured cells were placed in a recovery medium such as TSB, recovery and subsequent growth was demonstrated.

In the presence of glucose, heat-injured cells consumed oxygen at a rate somewhat higher than that of normal cells (Table I). Therefore, heat-

TABLE I

Oxygen uptake at 37 C by normal and injured TSB and CSM grown Salmonella typhimurium.

	Oxygen Consumed (μmoles/hr[1])			
	TSB Grown Cells		CSM Grown Cells	
Substrate	Normal	Injured	Normal	Injured
Succinate	39.0	41.8	37.5	42.5
Glucose	33.9	36.6	32.6	35.7
Endogenous	1.07	0.35	1.29	0.53

[1]Rates of oxygen uptake after subtraction for endogenous.

injured cells were still able to transport and metabolize glucose. The rate and level of α-MG accumulation by normal TSB grown cells (Fig. 1A) was similar to that reported by Hoffee and Englesberg (6). In the absence of an external energy source, the rate of α-MG accumulation by TSB grown cells was greater for normal than for injured cells (Fig. 1A).

It would at first appear as though heat-injury might have caused a slight inactivation of the permease system. However, Hoffee, et al. (7) reported that endogenous metabolism provided the necessary energy for the transport of α-MG in the absence of an energy source. In the case of starved cells they found that an exogenous energy source was needed for α-MG accumulation. The normal cells probably had enough endogenous energy for a rapid uptake of α-MG. The injured cells had lost a considerable amount of

intracellular material (13) and there was probably endogenous energy used for the repair of heat induced lesions during and following heating. The result would be a decreased supply of endogenous energy in the injured cells and, therefore, a decreased rate of α-MG transport. To support this it was observed that the rate of endogenous metabolism was lower in injured cells in comparison to normal cells (Table 1.)

The accumulation of α-MG by CSM grown cells is given in Fig. 1B. The results were similar to those for TSB grown cells for the initial rate of α-MG uptake. However, after 3 min the level of α-MG started to decrease in the normal cells while the uptake continued in the injured cells. These data indicate that the glucose permease system varies with the growth conditions and that there was an alteration of the permease system due to heat-injury.

Hoffee, et al.(7) concluded that the glucose permease system of S. typhimurium consists of an energy requiring entrance reaction, an energy requiring exit reaction, and an exit reaction which acts by a diffusion mechanism. One of the exit reactions could have been responsible for the lower level of α-MG accumulation in the normal CSM grown cells.

To further evaluate this system, 0.1% succinate was used as an exogenous energy source. If endogenous energy was a limiting factor in α-MG transport

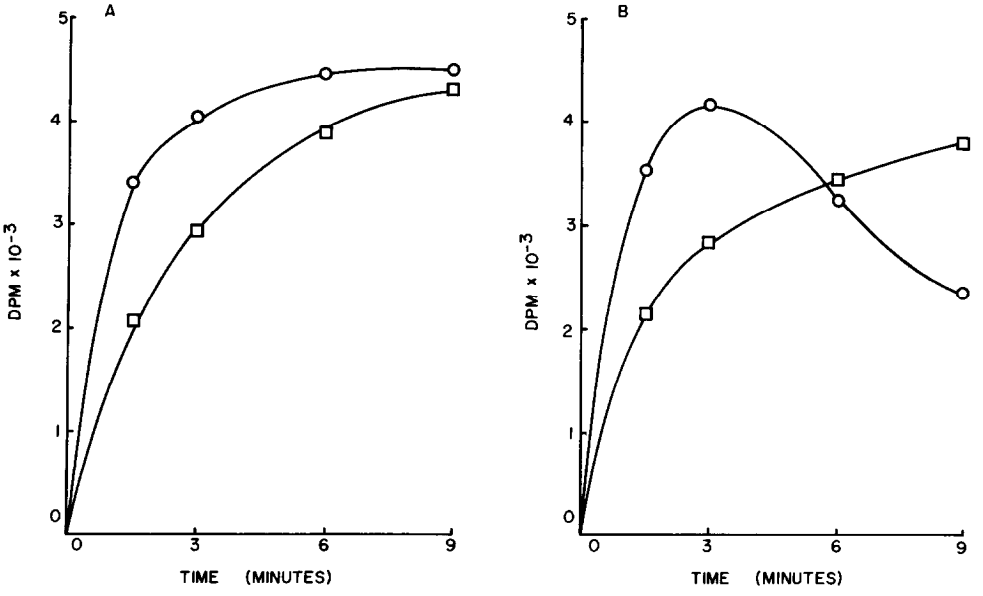

Fig. 1. The accumulation of methyl-α-D-glucopyranoside (glucose-U-C^{14}) at 30 C by normal (○) and heat-injured (□) Salmonella typhimurium. One ml samples were taken from a reaction mixture of 0.1 M phosphate buffer (pH 7.2) containing 0.21 mg normal equivalent cells/ml, and 4 x 10^{-5} M in α-MG (0.48 μc/μmole). A. TSB grown cells. B. CSM grown cells.

by injured cells, then the addition of an energy supply should relieve this deficiency. The injured cells oxidized succinate at a rate somewhat higher than that of normal cells (Table I) and, therefore, succinate could serve as an energy source.

For normal TSB grown cells there was a decrease in the level of α-MG accumulation when an external energy source was added (Fig. 2A) in comparison to the absence of an external energy source (Fig. 1A). This result agrees with the data of Hoffee and Englesberg (6). Injured TSB grown cells did not display a drop in the level of α-MG accumulation in the presence of an exogenous energy supply. The injured cells continued to accumulate α-MG in the presence of succinate much like the cells did in the absence of succinate. Also, the initial rate of uptake was somewhat greater for injured cells.

The results for α-MG accumulation in the presence of an exogenous energy source by CSM grown cells were somewhat similar to those for TSB grown cells (Fig. 2B). However, the change in the rate of uptake of α-MG by CSM grown cells was not as dramatic as seen with TSB grown cells.

Kaback (9) reported that isolated membranes of E. coli had a maximum rate of α-MG uptake at 46 C; however, the maximum steady state level of α-MG

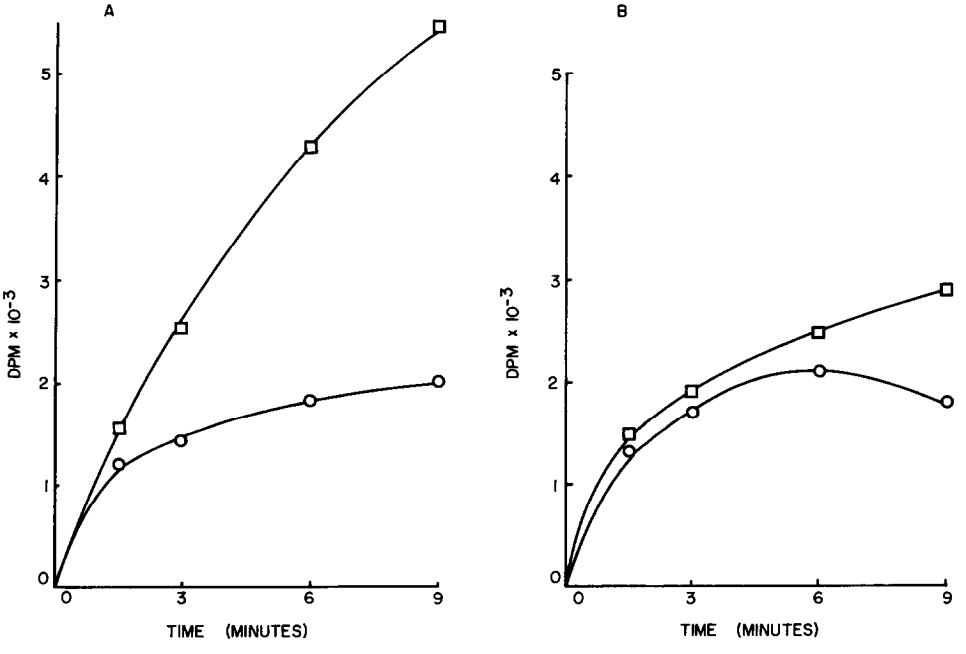

Fig. 2. The accumulation of methyl-α-D-glucopyranoside (glucose-U-C^{14}) at 30 C by normal (○) and heat-injured (□) Salmonella typhimurium in the presence of an energy source. Reaction mixture was the same as in Fig. 1 but with 0.1% succinate. A. TSB grown cells. B. CSM grown cells.

was attained at 27 C. He speculated that this increased rate of uptake was possible due to a physical alteration of the lipid membrane. An irreversible physical alteration of the lipid membrane could have resulted in the increased rate of α-MG transport in the presence of an energy source by heat-injured S. typhimurium. Hoffee and Englesberg (6) suggested that the low level of α-MG accumulation in the presence of an energy source was due to an enhanced exit reaction. If heat-injury caused an alteration of this reaction then there may be an increased accumulation of α-MG. The observed phenomenon could also relate to the inhibition of the α-MG transport system by a phosphorylated intermediate such as glucose-6-phosphate (10) or ATP. There could be a channeling of ATP to the repair of the heat-induced lesions of the cell but at the same time sufficient energy is being provided for an optimal rate of transport.

ACKNOWLEDGMENT

This investigation was supported by research grant 2-RO1-FD-00093 from the U.S. Food and Drug Administration.

REFERENCES

1. Allwood, M. C., and Russell, A. D. J. Bacteriol. 95, 345 (1968).
2. Bush, E. T. Anal. Chem. 35, 1024 (1963).
3. Clark, C. W., and Ordal, Z. J. Appl. Microbiol. 18, 322 (1969).
4. Cohen, G. N., and Monod, J. Bacteriol. Rev. 21, 169 (1957).
5. Englesberg, E., Watson, J. A., and Hoffee, P. S. Cold Spring Harbor Symp. Quant. Biol. 26, 261 (1961).
6. Hoffee, P., and Englesberg, E. Proc. Natl. Acad. Sci. U.S. 48, 1759 (1962).
7. Hoffee, P., Englesberg, E., and Lamy, F. Biochim. Biophys. Acta 79, 337 (1964).
8. Iandolo, J. J., and Ordal, Z. J. J. Bacteriol. 91, 137 (1966).
9. Kaback, H. R. J. Biol. Chem. 243, 3711 (1968).
10. Kaback, H. R. Proc. Natl. Acad. Sci. U.S. 63, 724 (1969).
11. Pierson, M. D. Ph.D. Thesis, Univ. of Illinois (1970).
12. Tomlins, R. I., and Ordal, Z. J. J. Bacteriol. 105, 512 (1971).
13. Tomlins, R. I. Personal Communication.