ACHOLEPLASMA LAIDLAWII MUTANT DEFECTIVE IN GLUCOSE TRANSPORT SYSTEM

Mark A. TARSHIS, V. G. LADYGINA and T. P. A. ABAEVA
Department of Biochemistry, Medico-Biological Faculty, 2 Moscow Medical Institute, Moscow-21, USSR

Received 6 October 1976

1. Introduction

The most reliable information on the nature of bacterial transport systems is considered to be available from transport mutants [1]. We succeeded recently in isolating in *Acholeplasma laidlawii* mutant strain with a sharply decreased capability for glucose and 3-*O*-methylglucose (3-*O*-MG) uptake [2]. Both extracts and cells of the mutant remained capable of phosphorylating glucose without the uptake of other carbohydrates (fructose and ribose) being affected. We suggested that mutation gave rise directly to inactivation of a glucose and 3-*O*-methylglucose transport carrier. The present report is concerned with the elucidation of this point.

2. Materials and methods

The *Acholeplasma laidlawii* strain (originally obtained from Prof. Köller, Jena GDR) was grown in tryptose broth and harvested as described before [3]. The *A. laidlawii* mutant strain PTL-S was isolated after mutagenesis with nitrosoguanidine and platings on 2,3,5-triphenyltetrazolium-bromide indicator agar containing glucose as has been reported [2]. The ability of cells to accumulate 3-*O*-[^{3}H]MG was measured as described earlier [3]. The glucose, erythritol and glycerol permeability was estimated according to a procedure of Read and McElhaney [4] by measuring the swelling of cells in 200 mM permeant solution. The *A. laidlawii* cells have been shown to behave as ideal osmometers [5]. For measurement of 'entrance counterflow' the procedure described by Wong and Wilson [6] was used.

3. Results and discussion

It will be seen from fig.1 that the rate of osmotic swelling of PTL-S mutant in glucose is very low at both temperatures under investigation. It has recently become known that at a 200 mM external concentration no more than 8% of intracellular glucose is phosphorylated in 1 min in wild and mutant strains (unpublished data). We are thus now justified in comparing the transport activity of both types of cells for glucose in terms of the swelling rates. The absolute sensitivity of swelling to inhibitors of thiol groups (*N*-ethyl-maleimide) and amino groups (2,4-dinotrofluorobenzene) speaks partly for glucose being

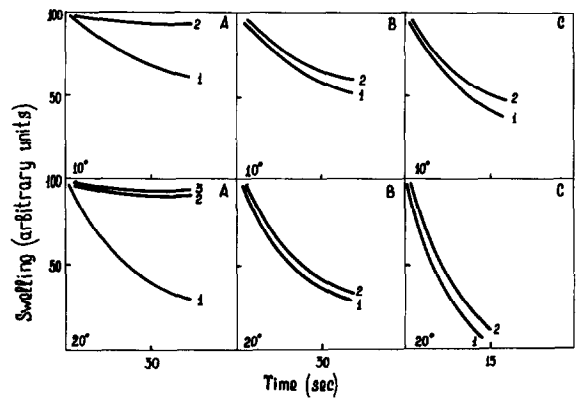

Fig.1. Osmotic swelling of *A. laidlawii* cells in glucose (A) erythritol (B) and glycerol (C). 50 μl of cell suspension in 200 mM sucrose were added with stirring to 4.5 ml of 200 mM permeant and a decrease in the light absorbance at 450 nm was measured. (1) Wild strain (2) mutant strain PTL-S (3) wild strain plus 1 mM *N*-ethylmaleimide or 1 mM 2,4-dinitrofluorobenzene.

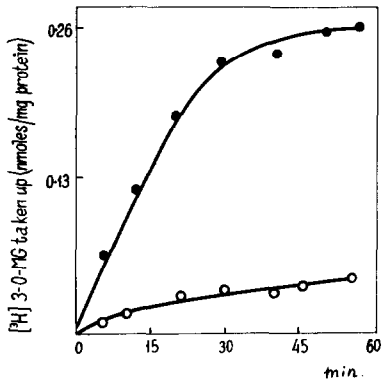

Fig.2. Uptake of 10 μM 3-*O*-[^{3}H]MG by *A.laidlawii* cells (•) wild strain; (○) mutant strain PTL-S.

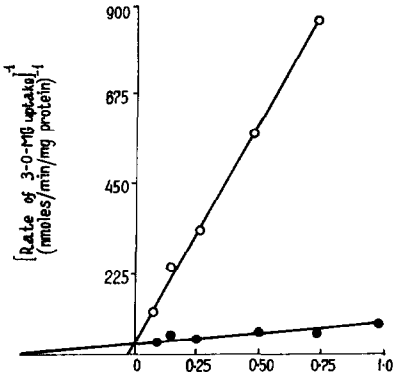

Fig.3. The initial rate of 3-*O*-MG uptake by *A.laidlawii* cells as a function of 3-*O*-MG concentration (a double-reciprocal plot). The cells were incubated at 37°C for 20 min in the presence of increasing 3-*O*-[^{3}H]MG concentrations. (•) wild strain; (○) mutant strain PTL-S.

transported via mediated transport rather than through simple diffusion. This is in accord with our previous data [7] and data of Read and McElhaney [4]. Swelling rates in glycerol and erythritol for the wild strain and PTL-S mutant are rather similar at both temperatures studied (fig.1). Glycerol and erythritol permeation into *A.laidlawii* cells proceeding by simple diffusion [4,5], it could be suggested that PTL-S mutant involves a defective carrier rather than other defective membrane components, whose interaction could have indirectly lowered transport activity. It has indeed been recently found that the activity of membrane-bound enzymes (ATPase and NADH-oxidase) as well as the intensity of temperature-dependent fluorescence of the hydrophobic 4-dimethylaminochalcone membrane probe remain unaffected in the mutant [8].

As expected, the mutant PTL-S is also markedly impaired in its ability to take up 3-*O*-MG (fig.2). The Lineweaver-Burk plot for the initial rate of 3-*O*-MG uptake by wild strain, presented in fig.3, indicates that this process occurs according to saturation kinetics with apparent K_m value of 22 μM. In contrast to wild-type cells, mutant cells require much higher 3-*O*-MG concentrations for uptake at significant rates (apparent K_m is of the order of 1 mM), indicating thereby a lowered substrate affinity to the carrier. These data unequivocally point to the defect being in a 3-*O*-MG and glucose carrier.

Table 1 shows no competitive inhibition of 3-*O*-MG transport by glucose and 6-deoxyglucose occurs in the mutant, in contrast to their transport in the wild

Table 1
Specificity of 3-*O*-MG transport by *A.laidlawii* cells

Competitor	Concentration (mM)	Transport activity (% control)	
		Wild strain	Strain PTL-S
D-Glucose	1.0	69	109
	0.1	38	111
6-Deoxy-D-glucose	1.0	54	103
	10.0	39	117

Unlabelled competitors were added to the uptake medium 1 min before 3-*O*-[^{3}H]MG (10 μM). Uptake of 3-*O*-[^{3}H]MG was measured after 20 min of incubation with cells.

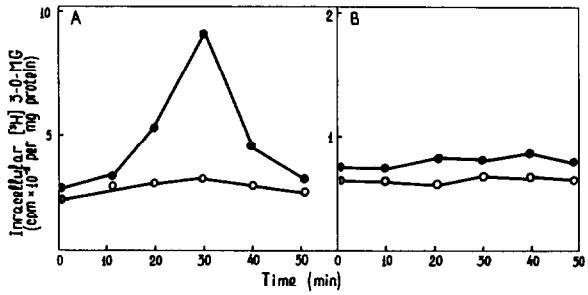

Fig.4. 3-*O*-MG 'counterflow' in *A.laidlawii* cells. Cells were exposed to 0.02 mM carbonylcyanide *m*-chlorophenylhydrazone (CCCP) for 60 min at 37°C in the presence of 10 mM unlabelled 3-*O*-MG. Following centrifugation pellet was resuspended in medium with 20 μM 3-*O*-[^{3}H]MG. Samples of 0.5 ml were removed and counted. A, wild strain; B, mutant strain PTL-S. (•) Cells, preloaded with unlabelled 3-*O*-MG. (○) Cells, not preloaded with unlabelled 3-*O*-MG.

strain which has repeatedly been found to be competitive [3,7]. Inhibition of 3-*O*-MG transport in wild strain by high glucose concentration (1 mM) is less than by small (0.1 mM) concentration, since intracellular glucose metabolism produces energy that facilitates 3-*O*-MG uptake, thus partially eliminating the competitive effect of glucose [3].

And, finally, the defect in the carrier is substantiated by our study of the 'entrance counterflow' that is known to provide a reliable test for the presence of a functionally active membrane carrier [6]. It is seen from fig.4 that the preloading of the cells with nonradioactive 3-*O*-MG in the presence of CCCP resulted in an increase in the transport rate of 3-*O*-[^{3}H]MG.

Effect of the 'entrance counterflow' is practically absent in the PTL-S mutant under study.

The experimental data lead thus to the conclusion that the PTL-S muation involves a serious defect in the glucose transport system.

Acknowledgements

We thank Professor L. F. Panchenko for helpful discussions.

References

[1] Kornberg, H. L. (1976) FEBS Lett. 63, 3–9.
[2] Ladygina, V. G., Bourd, G. I. and Tarshis, M. A. (1976) Dokl. Acad. Nauk SSSR, 229, 220–222 (in Russian).
[3] Tarshis, M. A., Panchenko, L. F., Bekkouzjin, A. G. and Ladygina, V. G. (1976) J. Bacteriol. 125, 1–7.
[4] Read, B. D. and McElhaney, R. N. (1975) J. Bacteriol. 123, 47–55.
[5] McElhaney, R. N., De Gier, J. and Van der Neut-Kok, E. C. M. (1973) Biochim. Biophys. Acta 298, 500–512.
[6] Wong, P. T. S. and Wilson, T. H. (1970) Biochim. Biophys. Acta 196, 336–350.
[7] Tarshis, M. A., Migoushina, V. L., Panchenko, L. F., Fedotov, N. S. and Bourd, G. I. (1973) Eur. J. Biochem. 40, 171–175.
[8] Ladygina, V. G., Migoushina, V. L., Abaeva, T. P., Logatchev, A. P. and Tarshis, M. A. (1976) Biokhimiya, in press (in Russian).