GALACTOSIDE ACCUMULATION ASSOCIATED WITH ION MOVEMENTS IN STREPTOCOCCUS LACTIS

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SUMMARY: Active transport of thiomethyl-β-galactoside by Streptococcus lactis 7962 occurred during valinomycin-induced potassium ion movement, in the absence of metabolizable substrate.

Harold and his co-workers have suggested that energy metabolism in the anaerobe Streptococcus faecalis is coupled to the active transport of a variety of molecules by way of a hydrogen ion movement (1,2). These authors propose that during fermentation of glucose proton extrusion generates a membrane potential of 150-200 mV (inside negative) and a pH gradient (inside alkaline) (2,3,4). The addition of uncoupling agents collapses both the potential gradient and hydrogen ion gradient and at the same time abolishes active transport of several nutrients (1,2,4). The authors infer from these and other experiments that the active transport of solutes is coupled to an inward migration of hydrogen ions, the gradient for this ion being maintained by an electrogenic proton extrusion pump. A membrane-bound ATPase is considered to be the enzyme complex effecting proton ejection and hence the membrane potential during metabolism in streptococci (5). An analogous Mg^{2+}-,Ca^{2+}-ATPase may perform proton extrusion in Escherichia coli (6).

These views of Harold and his co-workers are consistent with the chemiosmotic hypothesis of Mitchell (7), who postulated proton movement associated with membrane transport of a variety of substrates including lactose in Escherichia coli (8). Experimental evidence for a proton-lactose symporter has been provided by West (9) and by West and

Mitchell (10) who showed that non-metabolizing E. coli cells take up protons during transport of galactosides (probably in a one to one ratio). Eddy and Nowacki (11) have shown that accumulation of several amino acids can be effected by proton uptake and potassium extrusion in ATP-depleted yeast cells.

The experiments to be reported were carried out with the anaerobe Streptococcus lactis 7962 which lacks oxidative metabolism and is incapable of active transport of β-galactosides without the addition of metabolizable substrates, although it possesses functional transport carriers (12). In the present study the energy for active transport of thiomethyl-β-galactoside (TMG) in the absence of added substrate was provided by net ionic movements.

S. lactis 7962 cells were tested for ability to accumulate ^{14}C-TMG after addition of valinomycin, the K^+-specific ionophore (13). After one minute of incubation, valinomycin-treated cells accumulated TMG to an internal concentration 14 times higher than that in the external medium (Fig. 1). The TMG concentration then declined over a 20 minute period and approached that in the external medium. In the absence of valinomycin no accumulation of TMG was observed. Similar effects were observed when the cells were suspended in 0.1M sodium phosphate buffer, pH 7.0, or 0.1M glycylglycine, pH 7.0, both with valinomycin at $1x10^{-5}$M. A smaller peak (3 fold accumulation) was observed when 100 mM NaSCN was added instead of valinomycin, in 0.1 M Tris buffer, pH 7.6.

The effect of valinomycin may be attributed to the efflux of internal K^+ from the cells into the external medium. In several preliminary experiments the intracellular K^+ concentration decreased from approximately 400 mM to about 200 mM during the 18 min. experimental period. That the ratio of K^+ concentration in/out is important for this effect is illustrated in Fig. 1. Increasing the concentrations of

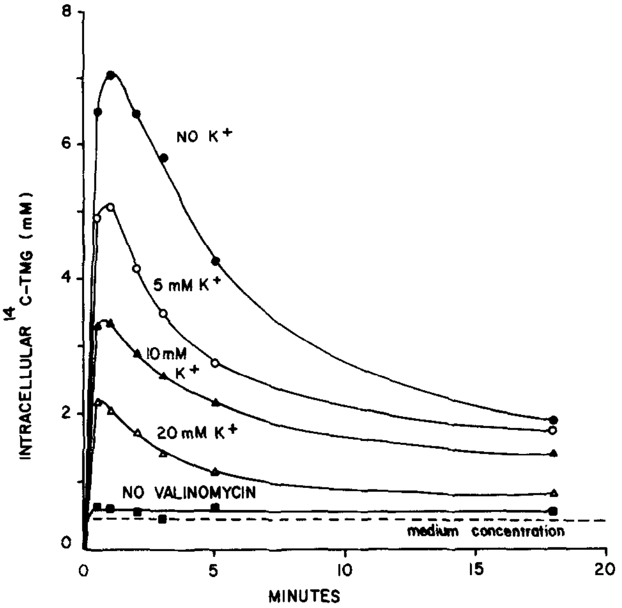


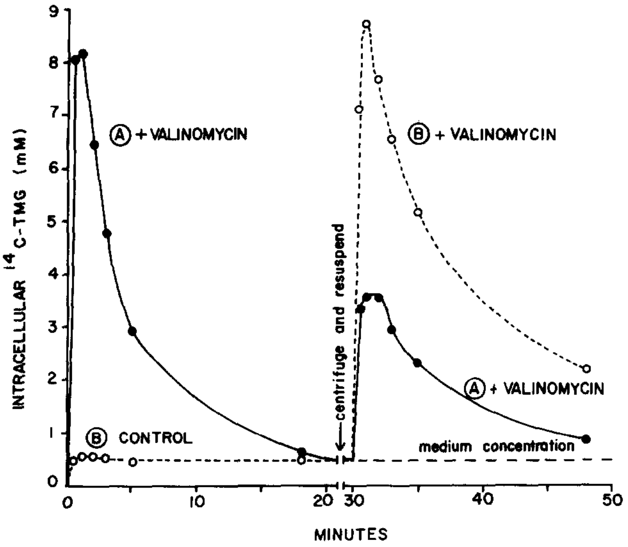
Figure 1: Accumulation of ^{14}C-TMG on addition of valinomycin in media containing various amounts of K^+. *S. lactis* (ATCC No. 7962) cells were grown in the complex medium of Citti *et al.* (14) supplemented with 1% galactose and 30mM arginine. Cells from logarithmic growth were washed and resuspended in 0.1M tris (hydroxymethyl) aminomethane (Tris)•HCl buffer, pH 7.6. Each vessel contained: 0.2 ml of washed exponential-phase *S. lactis* 7962 cells at 18 mg dry weight cells/ml, 0.1 to 0.4 ml 50mM KCl, and sufficient 0.1M Tris•HCl buffer, pH 7.6, to give a final volume of 1.0 ml; 1 µl of 1×10^{-2}M valinomycin dissolved in 95% ethanol was added, and, within 10 sec., 0.1 ml of 5mM ^{14}C-TMG at 1µCi/ml to start the reaction. Portions of 0.1 ml were removed at indicated intervals; the cells were collected on membrane filters, washed and counted, as described before (12). All manipulations were carried out at 25°C.

K^+ in the medium reduces the extent of TMG accumulation. At external K^+ concentrations greater than 100mM no TMG accumulation occurred in the presence of valinomycin. Sodium ions in the medium were not inhibitory, as accumulation of TMG in 100mM sodium phosphate buffer reached a peak of 20 fold concentration over that of the medium. Thus, net K^+ efflux from the cell, associated with cation entry or anion efflux, is the source of energy for active transport of TMG.

If this view were correct, the loss of TMG from the cell following accumulation is related to reduction or cessation of K^+ efflux. Such considerations predict that a second burst of TMG accumulation would result from reestablishing conditions for rapid K^+ efflux. Figure 2 shows the results of an experiment in which following one episode of transient accumulation the cells were centrifuged and resuspended in K^+-free incubation medium containing TMG. A second burst of TMG accumulation resulted. Its reduced magnitude compared with the first event may be due to the loss of about half of the intracellular K^+ during the first period.

When the proton conductors carbonylcyanidefluoromethoxyphenylhydrazone (CCFP) (1×10^{-6}M) or tetrachlorosalycilanilide (TCS) (1×10^{-5}M) were used instead of valinomycin, no TMG accumulation was observed. These inhibitors also prevented the valinomycin-induced galactoside accumulation.

One possible explanation for these results is that K^+ efflux from valinomycin-treated cells gives rise to a large membrane potential which permits H^+ to enter the cell via the membrane ATPase which, in turn, generates ATP. According to this view it is ATP and not ionic movement *per se* which is the immediate source of energy for galactoside transport. This hypothesis was tested by blocking the ATPase with N,N'-dicyclohexylcarbodiimide (DCCD), a compound known to inhibit the membrane bound



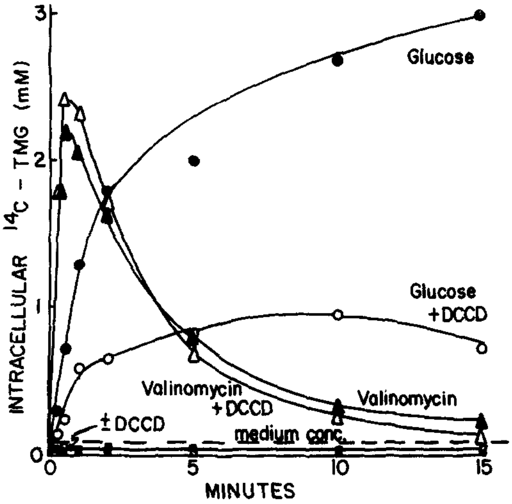


Figure 3: *Effect of DCCD on glucose- and valinomycin-effected TMG accumulation*. *S. lactis* 7962 cells were grown to exponential phase, harvested, washed in 0.1M sodium phosphate buffer, pH 7.0, and resuspended in this buffer as described for Fig 1. Two 2 ml cell suspensions at 12,300 Klett units were incubated 25°C for 20 min, one tube containing 4µl 1x10^{-1}M DCCD in 95% ethanol. The cells were centrifuged at 4°C for 10 min. at 24,000 x *g*, the supernatant fluids discarded, and the pellets resuspended in 1.0 ml of buffer. For TMG accumulation, each vessel contained: 0.1 ml of 1mM ^{14}C-TMG at 0.5µCi/µmole, 0.2 ml 100mM glucose where indicated, 2µl 0.1M DCCD for DCCD-pretreated cells, 2µl 0.01 M valinomycin where indicated, and sufficient buffer for a final total volume of 1.0 ml. The reaction was started on addition of 0.2 ml cell suspension and carried out at 25°C. Aliquots of 0.1 ml were removed for filtration on Millipore filters as described for Fig 1.

ATPase in the closely related organism *S. faecalis* (5). When DCCD (2x10^{-4}M) was added to cells of *S. lactis* inhibition was observed of the active transport of TMG in the presence of glucose, but no effect was found on the valinomycin-induced sugar accumulation (Fig.3). This suggests that the membrane bound ATPase is not involved in the

Figure 2: *Accumulation of ^{14}C-TMG on addition of valinomycin*. Each tube contained 0.5 ml of washed exponential-phase *S. lactis* 7962 cells at 24.9 mg dry wt/ml and 1.3 ml 0.1M Tris·HCl buffer, pH 7.6; 2 µl of 1x10^{-2}M valinomycin was added to tube A only, and within 10 sec. 0.2 ml of 5mM ^{14}C-TMG (1µCi/µmole) was added to both tubes to start the reaction. Aliquots of 0.1 ml were removed from each tube for assay of intracellular ^{14}C-TMG. At 20 min. 1.0 ml of each mixture was centrifuged at 12,000 x *g* for 5 min at 4 C, the supernatant fluids decanted, the sides of the tube swabbed, and the pellets resuspended at 30 min in a mixture of 0.2 ml 5mM ^{14}C-TMG (1µCi/µmole), 1.8 ml 0.1M Tris·HCl buffer, pH 7.6, and 2 µl of 1x10^{-2}M valinomycin. Again 0.1 ml aliquots were removed at indicated intervals and analyzed for intracellular ^{14}C-TMG concentration.

valinomycin-induced active transport of TMG.

These experiments demonstrate a relationship between potassium ion translocation and the accumulation of an uncharged molecule. These data are compatible with the view that under these conditions efflux of K^+ is associated with the entry of H^+ with the TMG on the sugar transport carrier. Experiments are now in progress to test the possible movement of protons associated with TMG transport.

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