ENERGY COUPLING FOR GALACTOSIDE ACCUMULATION IN Escherichia coli*

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Galactosides are transferred rapidly across the cell boundary of strains of E. coli which contain a functioning y gene in the lactose operon (1-3). The rapid transfer into or out of the cell is mediated by a specific carrier, the product of the y gene, and in the absence of the carrier the cell is relatively impermeable to galactosides. Recently, Fox and Kennedy (4) have identified a protein which appears to be the specific galactoside carrier or a portion thereof. We consider here the question of how energy is coupled to the carrier mechanism to produce active transport into the cell.

Consider a suspension of E. coli cells with functional galactoside carriers but unable to accumulate because energy metabolism is inhibited. Galactoside added to the suspending medium rapidly penetrates the cells via the carrier-mediated transfer, and at equilibrium the internal and external concentrations are equal. The dissociation constants for the carrier-galactoside complex at the outer boundary of the cell membrane (K_o) and at the inner boundary (K_i) are also equal. Now, allow the cells to generate phosphate-bond energy. An energy coupling mechanism could produce active transport into the cell either by selectively decreasing K_o (i.e. enhancing affinity of the carrier for the galactoside at the outer boundary of the cell membrane) or by increasing K_i. Decreasing K_o would increase the fraction of membrane carriers bound to galactoside, and

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active transport into the cell would result from the movement of these bound carriers to the inner surface of the membrane, i.e. from a push mechanism. Increasing K_i would decrease the fraction of carriers bound to galactoside at the inner surface of the cell membrane, and active transport would thus result primarily from a pull mechanism. The experiments below demonstrate that energy metabolism is associated with an increased proportion of carriers bound to galactoside, indicating that the former, push mechanism is operative.

A method was developed to estimate the proportion of carriers associated with galactoside, based on the observation (4) that interaction of carrier and thiodigalactoside protects the carrier from inhibition by N-ethylmaleimide (NEM), a sulfhydryl binding agent. The degree of protection from NEM provides an estimate of the carrier-galactoside interaction and is determined as follows. E. coli are grown aerobically at 37° C in a defined medium (5) with 0.4% succinate as carbon source and with (strain ML 30, inducible for the lactose operon) or without (constitutive strains ML 308 and 3300) 5×10^{-4} M isopropylthiogalactoside as inducer. Cultures in the log phase of growth are centrifuged and the cells suspended in "wash" solution, 0.1 M potassium phosphate of pH 7.0 containing 0.005 M $MgCl_2$ and 100 µg/ml of chloramphenicol, to a cell density of approximately 1.4×10^9. Two aliquots are then incubated in the absence and two in the presence of 2×10^{-4} M thiodigalactoside (TDG) for 20 minutes at 23° C. (To test the effects of metabolic inhibitors an additional 4 samples are treated identically, except that the reaction mixtures contain 0.02 M KCN or 0.01 M 2, 4-dinitrophenol in addition). One aliquot of each pair is then treated with 2×10^{-4} M NEM for 20 minutes at 23° C and the reaction terminated by addition of a 5-fold excess of β-mercaptoethanol to all tubes. The cells are washed 3 times with 20 ml wash solution, harvested by centrifugation, and tested for galactoside transport by either of two procedures. The rate of hydrolysis of o-nitrophenyl galactoside (ONPG) by intact E. coli is limited by the influx rate into the cells and provides an estimate of entry (3). Thus cells are incubated in wash solution in the presence of 0.008 M ONPG for 15 minutes at 23° C,

the reaction terminated by addition of 2 volumes of 1.0 M Na_2CO_3, and the optical density at 420 mμ determined (6). Each sample is read against a blank prepared identically except that ONPG is added after Na_2CO_3. In the second procedure, aliquots of cells in wash solution are incubated with 0.001 M thiomethylgalactoside (TMG) and 0.05 μC C^{14}-TMG (New England Nuclear Corp., specific activity 2.9 mC/mMole) for 5 minutes at 23° C, the cells separated and washed by rapid filtration on millipore filters (pore size, 0.45μ), and the filters suspended in Bray's solution (7) and counted in a liquid scintillation spectrometer. If a equals the per cent inhibition of galactoside transport by NEM in the absence of protecting galactoside, TDG, and b equals the per cent inhibition in the presence of TDG, "protection" is defined as (a - b)/a X 100.

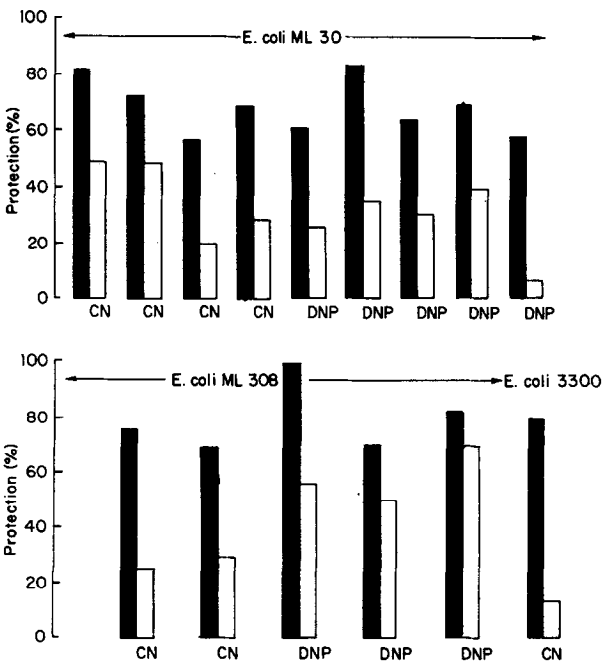

Fig. 1. Effect of KCN or 2, 4-dinitrophenol on thiodigalactoside protection of galactoside carriers from inhibition by N-ethylmaleimide. Each set of bars represents one experiment in which protection (defined in text) was determined with (light bars) or without (dark bars) the inhibitor.

Fig. 1 illustrates the results of 15 experiments in which protection was determined in the absence and presence of KCN or dinitrophenol (DNP), and ONPG hydrolysis was the final assay of galactoside transport. The inhibitors consistently decreased protection in all three strains of E. coli, with mean values

in the control and inhibited cells, respectively, of 73.2% and 35.0% (st. error = 5.34%, $P < 0.001$). Moreover, similar results were observed when strains ML 30 and ML 308 were tested using both ONPG hydrolysis and C^{14}-TMG uptake as assays (Table I). Treatment with the metabolic inhibitors in the absence of TDG did not change significantly the values for ONPG hydrolysis in these experiments. Further, the per cent inhibition of ONPG hydrolysis by NEM was unchanged by DNP and increased by 16.2% ($P < 0.01$) by KCN. Thus the metabolic inhibitors did not increase cell permeability nonspecifically, nor did they influence galactoside carrier function significantly in the absence of TDG.

Table I

Effect of metabolic inhibitors on protection as tested by ONPG hydrolysis and C^{14}-TMG uptake

Strain	Assay	Protection (%)	
		control	inhibitor
ML 30	ONPG	45.6	28.4*
	C^{14}-TMG	46.9	21.6
ML 308	ONPG	100.0	55.8*
	C^{14}-TMG	93.5	31.9
ML 308	ONPG	68.6	29.2+
	C^{14}-TMG	70.8	28.9

* 2, 4-dinitrophenol was the inhibitor.
+ KCN was the inhibitor.

Although the foregoing results demonstrate that energy metabolism is required for optimal protection by TDG, they do not define the precise role of the energy. Is metabolism required to enhance carrier affinity for TDG or to convert TDG to a metabolite which binds the carrier efficiently? The latter possibility seems excluded by two sets of observations. The protection procedure described above was modified so that all cells treated with TDG were exposed initially without KCN for 15 minutes to allow formation of the hypothet-

ical metabolite. Subsequently, the cells were incubated with NEM alone or with KCN plus NEM. The mean value for protection in 5 experiments with strain ML 30 was again clearly greater in the absence of KCN, 60.1% versus 41.0% with cyanide treatment (st. error = 5.40%, $P < 0.01$). If thiogalactosides are converted to metabolites of high affinity for the transport carriers, the initial influx rate for added C^{14}-TMG should decrease progressively as cells are incubated with C^{12}-TMG. Therefore strains ML 30 and ML 308 were incubated with 0.001 M C^{12}-TMG in wash solution as described above, and C^{14}-TMG was added to separate portions at 0, 3, and 20 minutes. Thereafter, samples were filtered at 0.3, 0.6, and 0.9 minutes and the initial influx rate estimated. In 2 experiments with ML 30 the mean influx rates at 0, 3, and 20 minutes, respectively, were 1.7, 2.9, and 3.3 cpm X 10^3/minute, and corresponding values for ML 308 were 1.7, 2.5, and 2.5. (The initial increase in influx probably results from counter-transport).

Finally, additional evidence that energy metabolism enhances affinity of the carrier for galactosides was obtained by estimating the apparent Km for ONPG hydrolysis by intact cells. The cells were incubated initially in wash solution with or without 0.02 M KCN for 20 minutes at 23° C. Thereafter, the initial rates of hydrolysis observed with various concentrations of ONPG were estimated and the results plotted by the method of Lineweaver and Burk (8). Linear plots were observed for both KCN treated and untreated cells with initial ONPG concentrations of 0.005-0.015 M, and the values for apparent Km are listed in Table II. The mean ratios Km in cyanide/Km control for strains ML 30, ML 308, and 3300, respectively, were 4.92, 2.46, and 6.37. Thus inhibition of metabolism appears to decrease the affinity of the transport carrier for ONPG. (The relatively high concentrations of ONPG used in these experiments minimize the possibility that KCN might decrease hydrolysis by preventing intracellular accumulation and thereby efficient hydrolysis by β-galactosidase).

The present results indicate that energy coupling for galactoside accumulation involves increased affinity of the carrier for galactoside, an increased

Table II

Effect of KCN on apparent Km for ONPG hydrolysis by intact E. coli

Strain	No. experiments	Apparent Km, mean values (mM)			Ratio Vmax in KCN/ Vmax control
		control (a)	KCN (b)	Ratio (b)/(a)	
ML 30	3	0.89	4.38	4.92	1.21
ML 308	3	1.23	3.02	2.46	0.99
3300	2	1.25	7.96	6.37	1.24

concentration of carrier galactoside complexes in the membrane, and a resulting push mechanism. This conclusion agrees with the suggestion of Kepes (2), but disagrees with the pull mechanism proposed more recently by Koch (3). The latter model was based on the observation that metabolic inhibitors seem to increase efflux of C^{14}-TMG more than they decrease entry of ONPG (10, 3). However, a selective effect on efflux is equally compatible with a push mechanism, if the energy coupling reactions influence the carriers in any region of the cell membrane other than the innermost boundary. A push mechanism seems clearly involved in the accumulation of glucosides in yeast, as described by Okada and Halvorson (9), and may be a common device for energy coupling in active transport.

References

1. Cohen, G. N. and Monod, J., Bacteriol. Rev. 21, 169 (1957).
2. Kepes, A. and Cohen, G. N., in The Bacteria, I. C. Gunsalus and R. Stanier, Eds. (Academic Press, New York, 1962), vol. IV, p. 179.
3. Koch, A. L., Biochim. Biophys. Acta 79, 177 (1964).
4. Fox, C. F. and Kennedy, E. P., Proc. Natl. Acad. Sci. U.S. 54, 891 (1965).
5. Rickenberg, H. V., Cohen, G. N., Buttin, G. and Monod, J., Ann. Inst. Pasteur 91, 829 (1956).
6. Kuby, S. A. and Lardy, H. A., J. Am. Chem. Soc. 75, 890 (1953).
7. Bray, G. A., Anal. Biochem. 1, 279 (1960).
8. Lineweaver, H. and Burk, D., J. Am. Chem. Soc. 56, 658 (1934).
9. Okada, H. and Halvorson, H. O., Biochim. Biophys. Acta 82, 547 (1964).
10. Osborn, M. J., McLellan, W. L. and Horecker, B. L., J. Biol. Chem. 236, 2585 (1961).