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LETTERS TO THE EDITOR

Induction of the L-Arabinose Operon

In *Escherichia coli* the presence of arabinose and the *C* gene protein leads to induction of enzymes required for growth on arabinose (Sheppard & Englesberg, 1967). The induction level of these enzymes as a function of the arabinose concentration in the cell provides information from which one can calculate (a) the affinity of *C* protein for arabinose and (b) the most likely oligomeric structure of *C* protein.

A convenient strain for such induction measurements would have equal intracellular and extracellular arabinose concentrations. Such a strain is one in which (a) active transport is abolished so that arabinose is not concentrated intracellularly, (b) the maximum rate that arabinose can pass into the cell is large compared to both the rate of arabinose utilization and the rate of dilution by growth. A strain lacking the active transport has been constructed (Schleif, 1969). By introducing a metabolic block in the utilization of arabinose and growing the cells on glycerol as a carbon source, the requirement for an appreciable rate of arabinose entry was accordingly reduced. It was convenient to block the ribulokinase, leaving the arabinose isomerase functional as the indicator of induction level, since the isomerase assay is very sensitive. The conversion of some intracellular arabinose to ribulose by isomerase is not a significant drain on the intracellular arabinose since this conversion is readily reversible; at equilibrium there is a ninefold excess of arabinose compared to ribulose. (It should be mentioned that I assume ribulose does not diffuse out of the cells at a rate greatly exceeding the rate arabinose leaks in.) A complication introduced by many ribulokinase defects is that the remaining arabinose enzymes become hyperinducible (Englesberg, 1961). The particular kinase mutation used in this study, which was put into the transport-deficient mutant by P1 transduction, was chosen from the least-hyperinducible of the kinase-deficient mutants I have found. It is inducible to 50% higher arabinose enzyme levels than wild-type strains. At present there is no explanation of the hyperinducibility phenomenon, and I assume the kinase deficiency is not greatly altering the induction curve.

Are these cells sufficiently permeable that the intracellular and extracellular arabinose concentrations are equal? The following measurements show that arabinose can enter these cells much more rapidly than it is consumed or diluted by growth, and it follows that the internal and external arabinose concentrations are equal. The rate of arabinose entry into the active transport negative cells was measured in a strain with an active ribulokinase instead of the inactive one used in the induction measurements. This strain can therefore use all arabinose finding its way into the cell. The rate of arabinose consumption was also measured in the ribulokinase-negative strain. For these measurements cells growing on glycerol with a doubling time of 60 minutes were pre-induced by the presence of 10^{-3} M-arabinose for two hours; they were then spun and washed twice. After growth for ten minutes, radioactive arabinose was added to 10^{-5} M, and its uptake assayed each five minutes for 30 minutes. It was found that arabinose at this concentration is incorporated at the rate of 7×10^{-18} mole/cell/30 minutes and 1×10^{-18} mole/cell/30 minutes in the strain

with and without active kinase, respectively†. Thus even at the low concentration of 10^{-5} M, arabinose can enter the cell at least seven times faster than it is utilized. Intracellular arabinose at 10^{-5} M is diluted by cell growth; cell volume 10^{-12} cm^3, doubling time 60 minutes at a rate of 10^{-20} mole/cell/30 minutes. The rate of arabinose entry, 7×10^{-18} mole/cell/30 minutes, is considerably greater than its consumption or dilution rate, and therefore intracellular and extracellular arabinose concentrations are nearly equal.

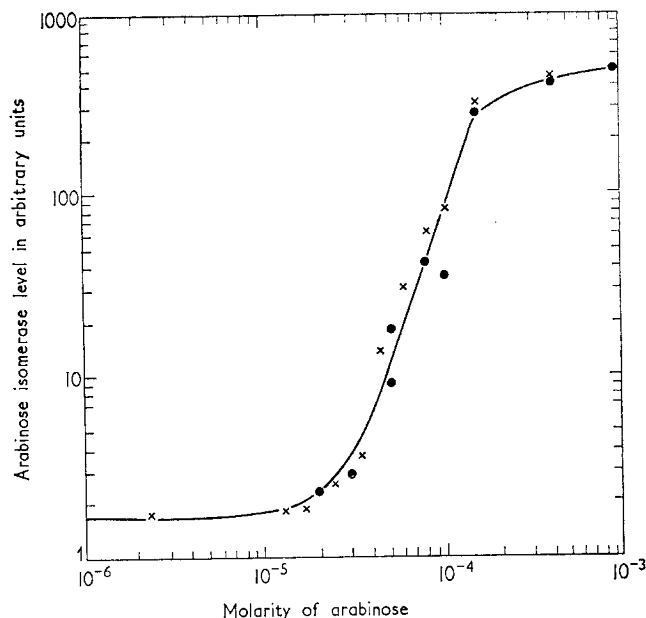

FIG. 1. The induction of arabinose enzymes as a function of intracellular arabinose concentration.

The strain lacking both ribulokinase and active arabinose transport (RFS 496) was grown to stationary phase in M9 minimal salts, 0·2% glycerol, B1, 1% Casamino acids. 0·1 ml. of cells was then added to 250-ml. flasks containing 50 ml. of the above medium and arabinose at various concentrations. In 6 hr of growth the density was 3×10^8/ml., a growth of approximately 100-fold. The cells were spun down at 4°C, resuspended in 0·4-ml. assay mix and immediately mixed vigorously with 1 drop toluene. Duplicate samples of various volumes were diluted to 0·20 ml. in assay mix. To one of the samples 0·8 ml. of 0·5 N-HCl was added whereas the other sample was incubated at 37°C for 50 min before addition of the HCl. The isomerase was taken to be proportional to the ribulose synthesized during the incubation. For further details see the preceding paper. Plotted are results from two independent series of measurements, (●) and (×).

This strain, lacking active transport and ribulokinase, shows a sigmoidal induction curve. Figure 1 shows the results of two series of measurements of the level of arabinose isomerase as a function of arabinose concentration in the medium. The important features of this induction curve are: (a) once induction begins, it rises approximately cubically with arabinose concentration, (b) arabinose concentrations of importance to induction are 10^{-5} M to 10^{-4} M.

Two conclusions can be drawn from these data: first, the cubic induction dependence

† This rate of diffusion, 7×10^{-18}, is sufficiently high that at 10^{-2} M-arabinose these cells can grow; a requisite 10^{-15} mole/cell diffusing in per doubling time of 3 hr.

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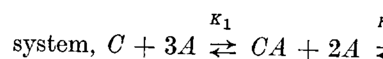

system, $C + 3A \overset{K_1}{\rightleftarrows} CA + 2A$

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10^{-4} M in arabinose leads to
$K_1K_2 > 10^{-9}$ M^2, $K_1K_2K_3 \approx 10$
is $K_1 = K_2 = K_3 = 10^{-4}$ M.

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Biological Laboratories
Harvard University
Cambridge, Mass. 02138, U.S.A.

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on arabinose concentration shows that more than one arabinose molecule must interact with more than one site before appreciable induction occurs, making it likely that the C protein is an oligomer, possibly a tetramer; second, the dissociation constant between arabinose and the C protein is approximately 10^{-4} M. This estimate depends critically upon the control being positive. The essential argument is that in such a case the half-maximum induction level corresponds to the inducing substance being half charged with inducer. When various specific models are fitted to the data, it is found that the dissociation constant does not vary greatly with the model. For example, in one class of models induction is taken to be proportional to the fraction of tetrameric C protein molecules binding three molecules of arabinose. In the following system, $C + 3A \overset{K_1}{\rightleftarrows} CA + 2A \overset{K_2}{\rightleftarrows} CA_2 + A \overset{K_3}{\rightleftarrows} CA_3$, the fraction of C molecules binding three arabinose molecules is $\dfrac{A^3/K_1K_2K_3}{1 + a_1A/K_1 + a_2A^2/K_1K_2 + a_3A^3/K_1K_2K_3}$, where a_1, a_2 and a_3 are statistical factors on the order of unity. The requirement that this relationship leads to a cubic induction relationship in the range $A = 10^{-5}$ to 10^{-4} M in arabinose leads to the following requirements: $K_1 > 3\times10^{-5}$ M, $K_1K_2 > 10^{-9}$ M^2, $K_1K_2K_3 \approx 10^{-12}$ M^3. One set of K_i satisfying these constraints is $K_1 = K_2 = K_3 = 10^{-4}$ M.

Any combination of dissociation constants consistent with the above constraints would make detection of the arabinose C protein by its binding to arabinose very difficult. Hence it is not likely that the equilibrium dialysis assay used to isolate the *lac* repressor can be used to isolate the arabinose C protein (Gilbert & Müller-Hill, 1966).

The cubic induction curve seen here is analogous to the non-linear induction curves seen in the negatively controlled lactose system. Early measurements showed approximately a quadratic induction with inducer (Boezi & Cowie, 1961; Sadler & Novick, 1965) and a later measurement suggests the induction is closer to cubic (Cohn-Bourgeois, 1966). In neither the positive nor the negative control systems does the non-linearity require co-operative binding of inducer, and in both cases induction can be understood as mass action effects on an oligomer.

Biological Laboratories
Harvard University
Cambridge, Mass. 02138, U.S.A.

ROBERT SCHLEIF

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