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IMMEDIATE STOICHIOMETRIC APPEARANCE OF β-GALACTOSIDASE PRODUCTS IN THE MEDIUM OF *ESCHERICHIA COLI* CELLS INCUBATED WITH LACTOSE

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SUMMARY

Products of β-galactosidase action on lactose by intact *E. coli* cells appeared in the medium as soon as lactose was added and the amount of product was equal to the lactose used. No detectable levels of β-galactosidase were found in the medium and lactose was not significantly broken down unless *lac* permease was present. The appearance did not depend upon the presence of any of the commonly known galactose or glucose permease systems. The Km of product appearance from whole cells was equal to the K_t for lactose transport by *lac* permease. When the cells were broken the Km became the normal β-galactosidase Km.

INTRODUCTION

In an earlier study in our laboratory (1) it was shown that when lactose was fed to various strains of *E. coli* growing on various energy sources, or to starved cells, the products of the reaction of β-galactosidase (E.C.3.2.1.23) on lactose (galactose, glucose and allolactose) were found mainly in the medium Control experiments indicated that the products were being released from the cells. We have now extended those studies and have found that the release of the products is immediate upon lactose addition, is stoichiometric in relation to the depletion of lactose, is independent of the presence of permeases for glucose and galactose and has a Km equal to the K_t for transport of lactose by *lac* permease. This is a report of those observations.

MATERIALS AND METHODS

Bacterial Strains - *E. coli* strains K-12(*Lac*$^+$) and ML-308(*LacI*$^-$) were obtained from the American Type Culture Collection. Strain JM-1098 (*Lac*$^+$ *ptsF ptsM ptsG galP mgl*) (2) was a gift from Dr. Jones-Mortimer (Cambridge) while ML-35 (*LacI*-Z^+Y-A^+) was a gift from Dr. Kepes (Paris).
Growth of Bacteria - Cells were grown (37° with aeration) in M63 minimal medium (3) with 0.2% glycerol as a carbon source and supplemented as necessary (for K12: 10 μg/ml vitamin B_1; for JM-1098: 90 μg/ml histidine and 100 μg/ml

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thymine). When IPTG was used, it was added to a final concentration of 0.5 mM. Cells were inoculated from LB medium into minimal medium, grown to saturation and then subcultured into fresh minimal medium and regrown to saturation. They were then diluted into fresh minimal medium and grown to mid-log phase (A_{600}= 0.4 to 0.5) for use in the experiment. If IPTG was present in the growth medium, it was removed by two centrifugations (3 min, 8,000 g) in fresh medium without IPTG.

Turbidity (A_{600}) was related to dry weight by a curve of A_{600} vs dry wt.

Assays for Glucose and Galactose in the Medium - Coupled assays were performed at 30°. In both cases, *E. coli* cells were pre-incubated for 2 min at 30° before a 200 µl aliquot was added to the reaction mixture in a cuvette. The cuvette contents were mixed and the change in A_{340} with time was recorded. The reaction mixture for glucose contained hexokinase (0.3 units), glucose-6-phosphate dehydrogenase (2.5 µg), $NADP^+$ (0.5 mg), lactose, and buffer (0.1 M sodium phosphate, 0.006 M ATP, 0.007 M $MgSO_4 \cdot 7H_2O$, pH 7.6). The reaction mixture for galactose contained galactose dehydrogenase (0.05 mg), NAD^+ (0.5 mg) and buffer (0.1 M sodium phosphate, 0.001 M $MgSO_4 \cdot 7H_2O$, pH 7.6). The final reaction volumes, including the *E. coli* cells, were 1 ml. Lactose concentrations ranged from 0.1 to 10 mM. Since coupled assays were used, only galactose and glucose in the medium were measured by these procedures.

ONPG Hydrolysis Assay - The total β-galactosidase activity in *E. coli* cells which were made permeable was determined by a method described by Miller (4). Three drops each of 0.1% SDS and chloroform were added to 1 ml of culture. The tube was shaken vigorously for about 10 sec and the chloroform layer was allowed to partially separate. Aliquots of the aqueous layer were used to assay for β-galactosidase activity at 30°. Aliquots (100 µl) were added to 1.5 ml phosphate buffer (0.1 M sodium phosphate, 0.001 M $MgSO_4$, pH 7.6). The reaction was initiated by the addition of 0.5 ml of assay buffer containing 12 mM ONPG. The change in A_{420} was recorded.

Total Lactose Concentration Assay - The reaction mixture was 1 mM lactose in 1.70 ml of 0.01 M sodium phosphate buffer (pH 7.6) containing 0.001 M $MgSO_4 \cdot 7H_2O$. The reaction mixture and an aliquot (600 µl) of mid-log phase ML-308 cells were pre-incubated separately at 30° for 3 min, then mixed at zero time. Samples (100 µl) were removed at 30 sec intervals for 10 min, prepared for gas-liquid chromatography and then analyzed as described previously (1).

Assay for Allolactose in the Medium - Cells were added to 1 mM lactose assay medium. At 0,1,3,5 and 7 min, 1.5 ml samples were removed and filtered through 0.45µ Millipore filters. Aliquots (300 µl) of the filtrates were immediately frozen in liquid N_2. Samples were freeze-dried, silylated and the sugar concentrations determined by GLC as previously described (1). The rates at each of the times were calculated and extrapolated back to zero time to account for the hydrolysis of allolactose.

RESULTS

Figure 1 shows that when lactose was added to ML-308 cells in the coupled assay solutions for glucose and galactose, glucose and galactose began to appear in the solution as soon as lactose was added. The short lags noted were dependent upon the concentration of the coupled enzymes used in the assay. The reason for the lags is that coupled assays require a steady state concentration of products to be accumulated before reaction occurs at a maximum rate. This has been discussed by Rudolph et al.(5). Equivalent lags also occurred

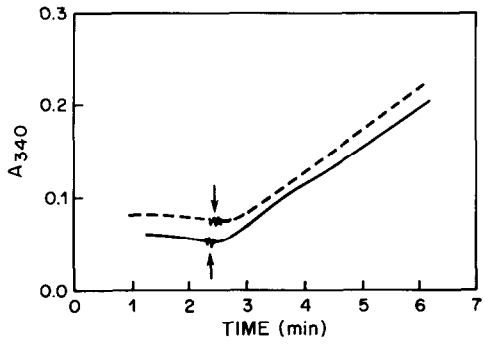

Figure 1 - Tracings of galactose and glucose coupled assays before and after 1 mM lactose was added to ML-308 *E. coli* cells. The arrows represent the point at which the lactose was added (---, galactose coupled assay; ___, glucose coupled assay).

when pure β-galactosidase of similar activity was assayed by the coupled enzymes. The overall results indicated that galactose and glucose began to appear in the medium as soon as lactose was added.

Table 1 indicates that the rates at which galactose, glucose and allolactose appeared in the medium (calculated as total hexose equivalent units; hexose equivalent units refers to the total amount of hexose whether tied up in glycosidic bonds or not) were essentially equivalent to the rate at which lactose was depleted. The allolactose rate was determined by extrapolating the results back to zero time and thus allolactose also seemed to appear in the medium as soon as lactose was added.

A Hofstee plot (6) for the production of glucose and galactose with whole *E. coli* (ML-308) cells is shown in Figure 2. A plot for the production of

TABLE 1

Rates of lactose decrease and of formation of products brought about by the addition of cells to 1 mM lactose. The results are expressed as μmoles of hexose equivalent used or produced per minute per mg dry weight (± standard deviation).

Compound	hexose equivalent
lactose	-0.196 ± 0.034
glucose	+0.072 ± 0.009
galactose	+0.073 ± 0.009
allolactose	+0.059 ± 0.006
total products	+0.204 ± 0.014

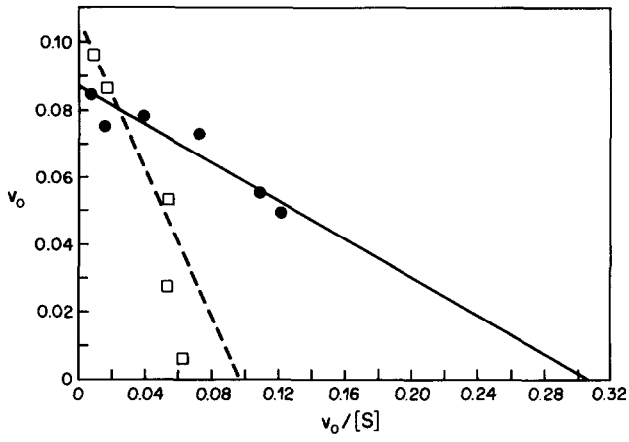

Figure 2 - ●—●, Hofstee (6) plot of the production of glucose or galactose from whole *E. coli* (ML-308) cells incubated with various concentrations of lactose (each point represents the average of at least 2 galactose and at least 2 glucose determinations by the coupled methods); □---□, Hofstee plot of the production of galactose by broken *E. coli* (ML-308) cells incubated with various concentrations of lactose (each point represents the average of at least 3 galactose determinations). In each case the line shown was fitted by the "simple" weighted method recommended by Cornish-Bowden (10).

galactose with broken *E. coli* cells (two passages through a French Pressure cell at 15,000 psi) is also shown. With the whole cells the Km was 0.28 ± 0.05 mM while for the broken cells it was 1.11 ± 0.12 mM. The Vmax values were quite similar (0.087 ± 0.003 μmoles/min/mg dry weight and 0.106 ± 0.003 μmoles/min/mg dry weight for whole and broken cells, respectively).

The rates of glucose and galactose production from lactose with various strains and conditions are listed in Table 2. Also indicated are the ONPG hydrolysis rates for these strains after the cells were made permeable with SDS and chloroform (4). The ratios of glucose and galactose release rates to the ONPG hydrolysis rates are also shown. We assumed that the permeable ONPG hydrolysis rates were proportional to the total β-galactosidase present in the cell and could therefore be used to compare results between different strains with different amounts of *lac* operon components. The glucose and galactose rates were zero when filtrates of ML-308 cells was assayed and very low when ML-35 (no *lac* permease) was studied. (The results with ML-35 were unusual in that the initial slow rates shown became even slower and essentially became zero after a few minutes). The ratios of rates of glucose and galactose

TABLE 2

Rates (μmoles/min/mg dry weight) of glucose and galactose appearance from whole cells incubated with 10 mM lactose and rates of ONPG hydrolysis by permeable cells. The ratio of these activities is also shown. Standard deviations are shown.

Strain or Condition	Monosaccharide rates (glucose plus galactose)	ONPG hydrolysis rates	Ratio of monosaccharide rates to ONPG rates
ML-308	0.167 ± 0.022	3.14 ± 0.39	0.053 ± 0.009
K-12	0.214 ± 0.020	3.76 ± 0.42	0.057 ± 0.007
JM-1098	0.141 ± 0.016	2.11 ± 0.11	0.067 ± 0.008
ML-35	<0.020	3.33 ± 0.25	<0.006
ML-308 MEDIUM	no detectable activity	no detectable activity	-

appearance to ONPG hydrolysis with ML-308, K-12 and JM-1098 were not signficantly different. (In the case of K-12 and JM-1098 the *lac* operon was induced by IPTG).

DISCUSSION

The results showed that galactose, glucose and allolactose (products of the action of β-galactosidase on lactose) appeared in the medium as soon as lactose was added to intact *E. coli* cells and that the rate of appearance was stoichiometric with the depletion of lactose. Earlier studies (1) indicated that the products were found mainly in the medium but there was no indication that the appearance in the medium was immediate and that it was stoichiometric with lactose depletion. The results indicate that galactose, glucose and allolactose are first released into the medium before being taken back into the cells. The fact that filtrates of *E. coli* showed no β-galactosidase activity when lactose was added indicated that the results were not caused by β-galactosidase which might have leaked out of the cells into the medium.

The occurrence of products in the medium required the presence of *lac* permease and the Km for the appearance of galactose and glucose in the medium was 0.28 ± 0.05 mM, very close to the K_t reported for transport of lactose (0.26 ± 0.03 mM) by *lac* permease (7). The Km became 1.11 ± 0.12 mM when the

cells were broken. This value is similar to the Km of pure β-galactosidase (1.24 ± 0.13 mM) acting on lactose (8). The overall rates with whole cells, however, were not significantly slower than with broken cells (i.e. the Vmax values were similar). The fact that the Km for the appearance of the products in the medium is similar to the K_t for lactose transport by *lac* permease cannot, therefore, be due to the *lac* permease uptake of lactose being rate limiting. The reason must be more complex.

The use of JM-1098 (2) showed that the well studied glucose and galactose permeases (gene products of *ptsG, galP and mgl*) are not involved in the efflux of glucose and galactose since the rates of efflux of glucose and galactose in relation to ONPG hydrolysis by JM-1098 were not significantly different from the rates of efflux by K-12 or ML-308, which have these permeases.

The results suggest that an interaction between *lac* permease and the cytoplasmic β-galactosidase occurs and that this causes the immediate efflux of products into the medium. Indeed, Villarejo and Ping (9) have presented some evidence that some β-galactosidase is normally found loosely bound to the inside of the inner membrane of *E. coli*.

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