THE LOCALIZATION OF ALKALINE PHOSPHATASE IN E. COLI K_{12}

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The alkaline phosphatase of E. coli is produced in response to low levels of inorganic phosphate (P_i) in the growth medium (1, 2) and permits the cells to utilize organic phosphate esters to satisfy their need for phosphorus. Since phosphate esters are considered not to enter the cell as such, it follows that the enzyme must be located at the surface. We have now obtained evidence that the enzyme lies outside of the cell membrane, since it is liberated quantitatively into the surrounding medium when the cells are converted to protoplasts.

Our first indication that the substrate was in intimate association with the cell at the time of hydrolysis came from studies of the uptake of radioactivity with glucose 6-P^{32} as substrate[2]. In the absence of P_i in the medium this radioactivity is taken up rapidly and quantitatively by resting cells (Figure 1). In the presence of a large excess of P_i the entry of radioactive phosphate derived from glucose 6-P^{32} into the cell is reduced but by no means eliminated. About 30 per cent of the isotope added appears in the

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[2] The P^{32} labeled glucose 6-phosphate used in these experiments was kindly provided by Dr. A. Panek.

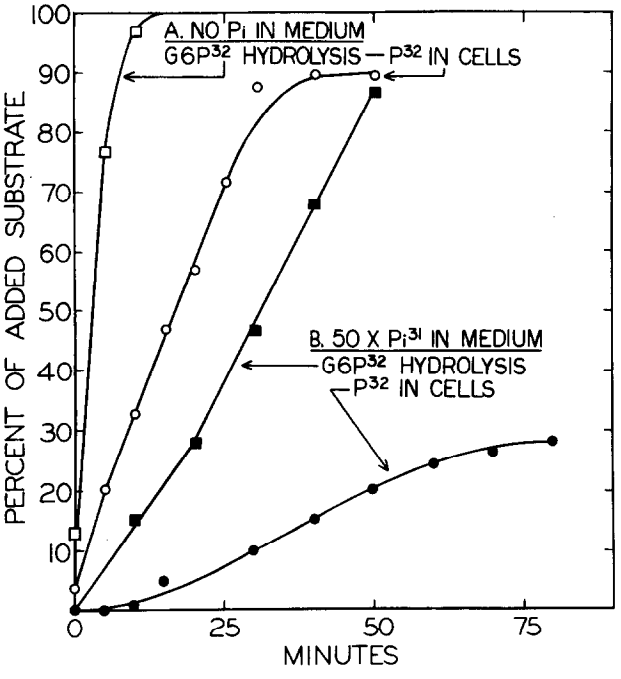

Figure 1. Hydrolysis of glucose 6-P^{32} (G6P^{32}) and uptake of P^{32} by E. coli (K_{12}) cells. The reaction mixtures contained, per ml of growth medium, 1.5×10^9 cells and 100 μgrams of chloramphenicol. The mixture was incubated at 37° with shaking for 10 minutes. At zero time 10 μmoles of glucose and 0.1 μmole of G6P^{32} were added. In experiment B, 5 μmoles per ml of inorganic phosphate ($P_i{}^{31}$) was also added at zero time. The experiment was carried out at 37° with shaking. Glucose 6-phosphate was measured with glucose 6-phosphate dehydrogenase and TPN. For P^{32} uptake, aliquots of cells were filtered on a membrane filter, washed thoroughly with water, dried and counted.

In experiment B the final concentration of P_i in the medium at 80 min was 5 μmoles per ml, unchanged from the initial level.

cell, without a corresponding decrease in total inorganic phosphate in the medium. Two interpretations are possible: either glucose 6-phosphate enters the cells as such, or else the P^{32} derived from glucose 6-P^{32} is not in complete equilibrium with $P_i{}^{31}$ in the medium. The results described below provide a basis for the second alternative.

Protoplasts prepared from phosphatase-positive cells are almost devoid of activity, all of which appears in the medium. No enzyme is de-

tected in lysates of these protoplasts (Table I). By contrast, the enzymes glutamic dehydrogenase, glucose 6-phosphate dehydrogenase and β-galactosidase remain in the protoplasts, with little or no activity appearing in the medium (Table I).

Experiments are now in progress to determine whether alkaline phosphatase lies between the cell wall and membrane, or is associated with the wall itself. Both have been suggested for invertase in yeast (4-7). Location of the enzyme between the wall and membrane would account for the lack of complete equilibration of liberated P_i^{32} and P_i^{31} in the medium. Our results also explain the ability of the cell to preserve its intracellular pool of phosphate ester intermediates when the phosphatase is present, since the interior of the cell appears to be completely devoid of the enzyme. This is a type of compartmentalization which has often been suggested to account for coexistence of enzymes and substrate in bacterial cells and it will therefore be of particular interest to examine the cells for other enzymes similarly situated.

Experimental

Cells were grown on salt medium of the following composition: triethanol amine buffer, 0.05 M, pH 7.5; phosphate buffer, 10^{-4} M, pH 7.0; $(NH_4)_2SO_4$, 0.2%; $MgSO_4 \cdot 7H_2O$, 0.02%; $FeSO_4 \cdot 7H_2O$, 0.00005%; KCl, 0.0075%; methionine, 0.004%. Glucose was sterilized separately and added to a concentration of 0.5% as carbon source. Cells grown overnight at 37° with shaking were harvested by centrifugation, washed at 0° with phosphate- and glucose-free medium and resuspended in phosphate-and glucose-free medium containing chloramphenicol as indicated (Figure 1).

For the protoplast experiment (Table I) cells were washed with

Table I

Loss of Alkaline Phosphatase Activity on Formation of Lysozyme Protoplasts

Experiment No.	Enzyme assayed[1]	Toluenized cells	Protoplasts	Liberated into Medium
1[6]	Alkaline phosphatase[2]	-	5	68
	G 6 P dehydrogenase[3]	-	34	10
	Glutamic dehydrogenase[4]	-	30	6
2[7]	Alkaline phosphatase	55	1	70
	β-Galactosidase[5]	60	84	4

1 Expressed as total enzyme units per 10 ml of cell suspension.

2 Each cuvette, (1 ml), contained 0.1 mg of p-nitrophenyl phosphate in 0.5 M TRIS (hydroxymethyl) amino methane (TRIS) buffer, pH 8.0, and enzyme solution or cell suspension. The reaction was run at room temperature and measured at 420 $m\mu$ in the Beckman DU spectrophotometer. A unit of enzyme is equivalent to a change in optical density of 1.0 per min.

3 Each cuvette, (1 ml), contained 0.05 M TRIS buffer, pH 7.65, 0.01 M $MgCl_2$, 1.0 μmole of glucose 6-phosphate, 0.4 μmole of TPN and enzyme solution. TPN reduction was followed at 25° at 340 $m\mu$. A unit of enzyme represents 1.0 μmole of TPN reduced per hour.

4 Conditions as for glucose 6-phosphate dehydrogenase except that 1.0 μmole of glutamic acid was substituted for glucose 6-phosphate.

5 Each cuvette, (1 ml), contained 0.04 M phosphate buffer, pH 7.5, 0.5 μmole of o-nitrophenyl galactoside and enzyme solution or cell suspension. The reaction was measured as for alkaline phosphatase. A unit of enzyme is equivalent to a change in optical density of 1.0 per hour.

6 The cell concentration was 1.2×10^{10} cells per ml.

7 The cell concentration was 5.8×10^{9} cells per ml.

distilled water, resuspended in 40 ml of 20% sucrose and 20 ml of 0.1 M TRIS buffer, pH 8.0. In experiment 2 the growth substrate was 0.5% glycerol instead of glucose and methyl thiogalactoside (8×10^{-5} M) was added as inducer of β-galactosidase. An aliquot (10 ml) of sucrose-TRIS buffer cell suspension

was removed before the formation of protoplasts and shaken at 25° for 2 minutes with toluene (0.05 ml per ml of cell suspension) before assay. The remaining sucrose-TRIS buffer cell suspension (50 ml) was chilled to 0° and 0.5 ml of 0.1 M ethylene diamine tetraacetate (EDTA), pH 8, was added followed by 0.05 ml of lysozyme (Worthington Biochem. Corp.) suspension containing 5 mg per ml (3). Protoplast formation was followed by diluting aliquots with distilled water and measuring the decrease in optical density at 600 mμ. When protoplast formation was complete (no further decrease in optical density of diluted aliquots) an aliquot of suspension (10 ml) was centrifuged at high speed. The supernatant solution was decanted and the protoplasts washed once with 10 ml of 20% sucrose. After recentrifugation, the protoplasts were lysed by the addition of 10 ml of distilled water. Each fraction was assayed as indicated.

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