# ON THE LOCALISATION OF ENZYMES OF DEOXYNUCLEOSIDE CATABOLISM IN *ESCHERICHIA COLI*

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## 1. Introduction

*Escherichia coli* possesses a group of degradative enzymes which are specifically located at the surface of the cell and it is believed that these enzymes are located in the "periplasmic" space, a region between the inner cell membrane and the cell wall (see [1]). Four criteria have been used in the localisation of these enzymes. Firstly, they are released into the medium by the conversion of whole cells to spheroplasts [2, 3]. Secondly, they are released into the medium when the cells are subjected to an "osmotic shock" [3, 4]. Thirdly, they are active in whole cell preparations when the membrane is impermeable to the substrate [5, 6]. Finally, cytological techniques, using electron microscopy, indicate their surface localisation [7, 8]. Only two enzymes, namely alkaline phosphatase and 5'-nucleotidase, have been shown to fulfil all four criteria. Several other enzymes (e.g. ribonuclease, DNA endonuclease and cyclic phosphodiesterase) have been shown to be released by osmotic shock and/or by the conversion of cells to spheroplasts and are also believed to be surface-located.

In contrast, enzymes which show activity only when the cells are broken open or made permeable, are considered to be "internal" enzymes (e.g. β-galactosidase, glutamic dehydrogenase; see [1]).

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It has recently been reported that certain enzymes involved in deoxynucleoside catabolism (thymidine phosphorylase, purine nucleoside phosphorylase, deoxyriboaldolase and phosphodeoxyribomutase) are released by osmotic shock [9–11]. This suggested that these enzymes might also be periplasmic. In this report we show that three of these enzymes, though released by osmotic shock, are *not* released when cells are converted to spheroplasts. Neither do they display activity in whole cells when impermeable substrates are used.

## 2. Materials and methods

### 2.1. *Bacterial strains and culture conditions*

The two strains of *E. coli* K12 used were C600 (*thi–thr–leu–lac*Y) and J1 (*pho*R), a strain constitutive for alkaline phosphatase synthesis [12]. The cells were grown at 37° in appropriately supplemented M9 medium [13] with glucose (0.2%) as carbon source. Enzymes of deoxynucleoside catabolism were induced with thymidine (2 mM) and β-galactosidase with isopropylthiogalactoside (1 mM) for 1–2 generations.

### 2.2. *Osmotic shock treatment*

The method was essentially that described by Neu and Heppel [3]. Exponentially growing cells at about $5 \times 10^8$ cells/ml were washed by filtration with 3 volumes of 0.01 M tris-HCl (pH 8) containing 0.03 M NaCl, and resuspended in 1/20 volume of 0.03 M

Table 1
Release of enzymes by osmotic shock from cells of strain J1.

| Fraction assayed | Thymidine phosphorylase | Purine nucleoside phosphorylase | Deoxyribo-aldolase | β-Galacto-sidase | Glutamic dehydrogenase | Ornithine trans-carbamylase | Alkaline phosphatase |
|---|---|---|---|---|---|---|---|
| Supernatant of sucrose and EDTA-treated cells | 0 | 11.3 | 0 | 43.2 | 0 | –[a] | 359 |
| Supernatant of shocked cells | 367.5 (78.6) | 96.6 (49.1) | 436 (83.9) | 252 (5.5) | 1.6 (4.2) | 300 (56.1) | 6304 (91.8) |
| Resuspended shocked cells (sonicated) | 100 | 90 | 83.8 | 4300 | 36.4 | 235 | 203.5 |
| Control (sonicated) | 465 | 158.5 | 418 | 7750 | 31.4 | 500 | 8033 |

Figures in parenthesis are percent activity released.
[a] Not assayable in the presence of sucrose.

tris-HCl buffer (pH 8). A control sample was removed and diluted two-fold with the same buffer and the remaining culture was diluted two-fold in the same buffer containing 40% sucrose and disodium ethylenediaminetetraacetate (EDTA) added to a final concentration of 0.33 mM. After 10 min at room temperature the treated cells were centrifuged, the supernatant retained and the well drained pellet gently resuspended with ice-cold water. After 10 min in an ice bath the cells were centrifuged, the shock fluid retained and the precipitate resuspended in 0.03 M tris-HCl buffer (pH 8). The resuspended pellet and the control cells were disrupted by sonication.

2.3. *Preparation of spheroplasts*

Exponentially growing cells were washed as above and resuspended in 1/20 volume of 0.1 M tris-HCl (pH 8), and spheroplasts prepared by treatment with EDTA and lysozyme as described by Schlesinger [14].

2.4. *Treatment with EDTA*

The method of Leive was applied [15] using washed exponential cells resuspended in 0.1 M tris-HCl buffer (pH 8). Treatment with EDTA (0.2 mM) was terminated with $MgCl_2$ (2 mM).

2.5. *Enzyme assays*

*β-Galactosidase* was assayed as described by Pardee, Jacob and Monod [16].

*Alkaline phosphatase* was assayed as described by Schlesinger [17]. The reaction mixture (1.5 ml) contained *p*-nitrophenyl phosphate (1.2 mg) in 1 M tris-HCl buffer (pH 8) and 0.05 ml of enzyme extract.

*Thymidine phosphorylase* was measured by determining the rate of formation of thymine from thymidine, as previously described [18] except that 70 mM phosphate buffer (pH 5.9) was used. When the enzyme was assayed in the reverse direction the disappearance of deoxyribose was measured. The reaction mixture contained 2 mM deoxyribose-1-phosphate, 10 mM thymine and 50 mM phosphate buffer (pH 5.9). Samples were taken at intervals and deoxyribose measured according to Burton [19].

*Purine nucleoside phosphorylase* was measured as described previously [20].

*Deoxyriboaldolase* was assayed by measuring the disappearance of dRib-5-P [18].

*Ornithine transcarbamylase* was measured as described by Jones [21].

*Glutamic dehydrogenase* was measured according to Malamy and Horecker [2].

*Enzyme activities* are given as nmoles product formed (or substrate consumed) per minute per ml of concentrated cell extract.

Table 2
Release of enzymes by conversion of cells (strain J1) to spheroplasts.

| Fraction assayed | Thymidine phosphorylase | Purine nucleoside phosphorylase | Deoxyribo-aldolase | β-Galacto-sidase | Glutamic dehydro-genase | Ornithine[a] trans-carbamylase | Alkaline phosphatase |
|---|---|---|---|---|---|---|---|
| Supernatant of spheroplasts | 0 (0) | 9 (0) | 16.7 (9.3) | 12.2 (1.8) | 0 (0) | 7.1 (4.7) | 2568.7 (97.8) |
| Resuspended spheroplasts | 194.8 | 39.3 | 166.7 | 808.2 | –[b] | 135.5 | 63.5 |
| Resuspended spheroplasts (sonicated) | 248.8 | 80 | 162.5 | 676.8 | 38.3 | 142.7 | 57.6 |
| Control (resuspended cells, sonicated) | 232 | 73 | 324.2 | 1225.8 | 29 | 156.9 | 3317.9 |

Figures in parenthesis are percent activity released.
[a] Assayed after dialysis for 24 hr against 0.01 M tris-HCl (pH 8) to remove sucrose.
[b] Whole cells are not assayable by the method used.

Table 3
Enzyme activity in EDTA-treated cells of strain C600.

| Treatment | Thymidine phosphorylase | Deoxyribo-aldolase | β-Galactosidase | Ornithine transcarbamylase |
|---|---|---|---|---|
| Untreated cells | 0 | 0 | 124 (3.9) | 17 (2.6) |
| EDTA | 256 (73.1) | 150 (50) | 1600 (51) | 240 (36.1) |
| toluene | 350 (100) | 300 (100) | 2210 (70.4) | 688 (103.5) |
| EDTA + toluene | 350 (100) | 300 (100) | 3140 (100) | 665 (100) |

Values in parenthesis are percent activity of the samples treated with EDTA and toluene. β-Galactosidase and ornithine transcarbamylase are included as control enzymes (see [22]). Thymidine phosphorylase was assayed in reverse.

## 3. Results and discussion

The amount of various enzymes released into the medium after osmotic shock treatment is shown in table 1. Alkaline phosphatase was included as a known periplasmic enzyme and β-galactosidase and glutamic dehydrogenase as known internal enzymes [1]. It may be seen that whereas the release of alkaline phosphatase was almost complete (91.8%) that of the deoxynucleoside degrading enzymes was less extensive, that of purine nucleoside phosphorylase being only 49.1%. We have tested the effect of EDTA concentration during the shock treatment on the extent of release. It was found that when no EDTA was used 56% of the alkaline phosphatase was released (see also [4]) whereas there was no release of thymidine phosphorylase. At $10^{-5}$–$10^{-4}$ M EDTA 75–90% of alkaline phosphatase and 30–40% of thymidine phosphorylase was released, maximal release of the latter enzyme being obtained only at $3.3 \times 10^{-4}$ M EDTA and above. Thus there is a clear quantitative differ-

ence between the extent of release of alkaline phosphatase and thymidine phosphorylase (and presumably the other enzymes of deoxynucleoside catabolism) which is dependent on the concentration of EDTA used during the treatment.

Ornithine transcarbamylase is not a degradative enzyme and does not display activity in whole cells [22]; it would therefore be expected to behave like an internal enzyme. Surprisingly, however, 50% of this activity is released by osmotic shock (table 1).

If the deoxynucleoside degrading enzymes or ornithine transcarbamylase are periplasmic enzymes they would be expected to be released by the conversion of cells to spheroplasts [2, 3]. Table 2 shows, however, that while 97.8% of alkaline phosphatase is released, there is no detectable release of the deoxynucleosede degrading enzymes or of ornithine transcarbamylase.

We routinely find that, in contrast to alkaline phosphatase, preparations of intact cells display reduced activities of thymidine phosphorylase, purine nucleoside phosphorylase and deoxyriboaldolase (see also [10]). The latter enzyme in particular displays little or no activity (0–9%). This is most likely because the cell membrane acts as a permeability barrier and the substrate of deoxyriboaldolase is a phosphorylated compound (dRib-5-P) which cannot penetrate into the cell. In order to determine whether this reduced activity is only due to a permeability barrier, cells were made permeable by treatment with EDTA [15]. It can be seen from table 3 that deoxyriboaldolase activity is observed after EDTA treatment, and similar results were obtained with thymidine phosphorylase when the enzyme was assayed in reverse using dRib-1-P (and thymine) as substrates.

In summary, enzymes of deoxynucleoside catabolism (and ornithine transcarbamylase) are selectively released when the cells are subjected to an osmotic shock, albeit to a quantitatively lesser extent than alkaline phosphatase. However, these enzymes, in contrast to alkaline phosphatase and other surface enzymes, are not released by the conversion of whole cells to spheroplasts. This latter observation, together with the absence of activity in whole cells, strongly suggests that these enzymes are *not* periplasmic. Since they are selectively released by osmotic shock it is possible that they are associated in some unknown way with the inner surface of the cytoplasmic membrane.

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