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A new assay for phosphodeoxyribomutase: Surface localisation of the enzyme

Phosphodeoxyribomutase, which catalyses the interconversion of deoxyribose 1-phosphate (dRib-1-*P*) and deoxyribose 5-phosphate (dRib-5-*P*) (together with thymidine phosphorylase, purine phosphorylase and deoxyriboaldolase) participates in the degradation of deoxynucleosides[1,2]. The latter three enzymes are all localised near the cell surface of *Escherichia coli*[3–5]. No continuous assay has been reported for phosphodeoxyribomutase; sampling assays based on chemical differences between dRib-1-*P* and dRib-5-*P* have so far been used[6–8]. In this paper a sensitive continuous assay for phosphodeoxyribomutase is described and used to show that this enzyme also is localised near the cell surface of *E. coli*.

The assay is based on the formation of a hydrazone of dRib-5-*P* with phenylhydrazine, a principle used in assays involving ketoacids[9]. When dRib-5-*P* is mixed with phenylhydrazine a compound is formed with a characteristic absorption spectrum which has a peak at 269 nm (Fig. 1). However 300 nm was chosen for the assay due to the high absorption of phenylhydrazine itself, and the bacterial extract, at lower wavelengths. The absorption at 300 nm is proportional to the quantity of dRib-5-*P* used (Fig. 2). dRib-1-*P* and ribose 1-phosphate were found not to react with phenyl-

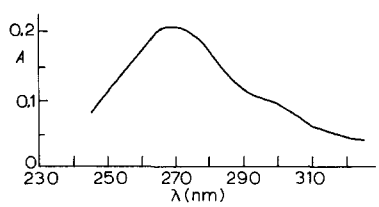

Fig. 1. Absorption spectrum of the hydrazone of dRib-5-*P*. dRib-5-*P* (0.05 μmole) and phenylhydrazine (0.5 μmole) were mixed in a total volume of 1.0 ml of 0.1 M cacodylate buffer (pH 6.8) and the absorption spectrum recorded with an SP800 spectrophotometer. The reference cell was identical but dRib-5-*P* was omitted.

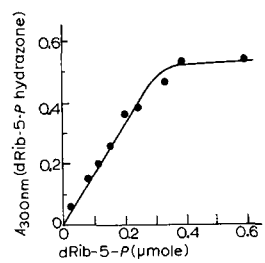

Fig. 2. Reaction of dRib-5-*P* with phenylhydrazine. dRib-5-*P* and phenylhydrazine (0.5 μmole) were mixed in a total of 1.0 ml of 0.1 M cacodylate buffer (pH 6.8) and the absorbance at 300 nm measured after 2–3 min against phenylhydrazine (0.5 μmole). From this curve a molar extinction coefficient of $1.69 \cdot 10^3$ was determined .

hydrazine since the aldehyde group is esterified. Ribose 5-phosphate also did not react under the assay conditions. In strains of *E. coli* containing deoxyriboaldolase dRib-5-*P* is cleaved to give two other aldehydes, acetaldehyde and glyceraldehyde 3-phosphate[10], which also form hydrazones with phenylhydrazine and could possibly be formed under the conditions of mutase assay. However, identical results are obtained both in the presence and absence of deoxyriboaldolase in the reaction mixture (osmotic shock extracts from a phosphodeoxyribomutase negative (*drm*⁻) strain of *Salmonella typhimurium* induced with deoxyribose were used as a source of deoxyriboaldolase[11]). This indicates that even in the presence of aldolase the dRib-5-*P* is trapped to form the

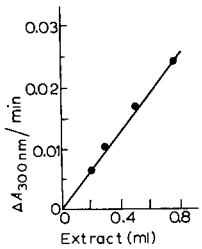

Fig. 3. Proportionality of rate of reaction and enzyme concentration. Cells of *E. coli* CR34 were grown in supplemented glucose minimal medium containing 30 μM thymine, harvested in exponential phase, and washed with 0.01 M Tris buffer (pH 7.2). The cells were resuspended in 0.1 M cacodylate buffer (pH 6.8) and disrupted by sonication. The sonicate was spun at 20 000 $\times$ *g* for 15 min before use and contained 1.5 mg/ml of protein. The reaction was followed for at least 6 min at 37° in an SP800 spectrophotometer.

hydrazone, and acetaldehyde and glyceraldehyde-3-*P* are not formed. This is presumably due to the large excess of phenylhydrazine present.

The assay mixture contains 0.5 μmole of phenylhydrazine, 2.0 μmoles of dRib-1-*P*, 75 μmoles of buffer such as cacodylate (pH 6.8) and 1–2 mg of bacterial protein in a total volume of 1.0 ml. All assays were performed at 37°. The use of phosphate buffer was avoided since phosphate inhibits the enzyme[12]. pH 6.8 was chosen, although the reported pH optimum for the enzyme is about pH 8.5 (ref. 2), since the rate of the nonenzymic formation of the hydrazone of dRib-5-*P* rapidly decreases as the pH is raised. Fig. 3 shows proportionality of initial velocity with enzyme concentration. Table I shows the results of assays performed on a variety of strains of *E. coli* and *S. typhimurium*. It can be seen that in strains known to be *drm*$^-$ no phosphodeoxyribomutase activity can be detected.

The results in Table II show that phosphodeoxyribomutase is released by the osmotic shock procedure of NEU AND HEPPEL[14] indicating that it is localised near the

TABLE I

PHOSPHODEOXYRIBOMUTASE ACTIVITIES IN SONIC EXTRACTS OF *E. coli* AND *S. typhimurium*

Strain 2006 and its derivatives are *S. typhimurium* KSU. Strain C600, CR34 and P152 are *E. coli* strains requiring threonine, leucine and thiamine for growth. All these strains have previously been assayed for phosphodeoxyribomutase[8,11] and were grown in supplemented glucose minimal medium. 30 μM thymine was used for *drm*$^-$ or *dra*$^-$ strains and 300 μM thymine for strain 2006. Cells were grown, harvested and sonicated as described in Fig. 3. Note that *thy*$^-$ (thymidylate synthetase negative) *dra*$^-$ (deoxyriboaldolase negative) strains have higher mutase activities than nonmutant strains. This is due to endogenous induction by dRib-5-*P*[7,8,13]. n.d. = not detectable.

Strain	*Genotype*			*Specific activity (nmoles/min per mg)*
	thy	*drm*	*dra*	
2006	−	+	+	4.0
2006-1	−	+	−	11.0
2006-6	−	−	+	n.d.
C600	+	+	+	1.31
CR34	−	+	−	9.84
P152	−	−	+	n.d.

TABLE II

RELEASE OF PHOSPHODEOXYRIBOMUTASE BY OSMOTIC SHOCK

Exponential cells at 10^{10}/ml were washed, resuspended in 5 ml of cacodylate buffer pH 6.8 (0.1 M) and split into two portions of 2.5 ml. One was sonicated (as described in Fig. 3) to give Fraction 4, and the other was subjected to osmotic shock by the procedure of NEU AND HEPPEL[14] to give Fractions 1–3. Osmotic shock involved treatment of the cells with 20% sucrose–0.03 M Tris (pH 8.0)–1 mM EDTA followed by rapid dispersion in cold water. The supernatants from both these treatments were retained for assay. The cell pellet left after the cold-water treatment was resuspended in 2.5 ml of 0.1 M cacodylate buffer (pH 6.8) and a sonic extract was prepared. Thymidine phosphorylase and β-galactosidase assays were used as controls, the former being a known surface enzyme[3,4], and the latter an intracellular enzyme[15].

Fraction	*Phosphodeoxyribomutase*		*Thymidine phosphorylase*		*β-Galactosidase*	
	Units/ml	*% Total*	*Units/ml*	*% Total*	*Units/ml*	*% Total*
1. Sucrose–Tris–EDTA	1.89	5.1	81.4	5.3	5.6	0.2
2. Cold water	27.6	74.4	1144	73.8	17.4	0.7
3. Cell pellet	7.6	20.4	325	20.9	2400	99.1
4. Sonicate	41	—	1372	—	—	—

cell surface (see also ref. 5). It has been found that whole cells of *E. coli* have a greater capacity to catabolise the deoxyribose moiety of thymidine than sonic extracts, and it was suggested that phosphodeoxyribomutase was sensitive to sonic disruption of the cells[5]. It can be seen from Table II that the yield of enzyme released by osmotic shock is similar to that obtained by sonication. Furthermore, the specific activity of phosphodeoxyribomutase when the cells are mechanically disrupted is almost identical to that obtained in sonic extracts. Thus it appears that phosphodeoxyribomutase itself is not sensitive to the sonication procedure used here.

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Induction of tyrosine aminotransferase in isolated liver cells

Isolated liver cells are an attractive system for studying liver functions *in vitro* provided they exhibit normal cellular functions. Previous studies have shown that tyrosine aminotransferase (L-tyrosine–2-oxoglutarate aminotransferase, EC 2.6.1.5) was not induced by cortisol in isolated rat-liver cells and the lack of enzyme induction was attributed to the fact that such cells had damaged cell membranes. Recently, HOWARD *et al.*[2] have prepared isolated liver cells by incubating rat livers with a mixture of collagenase (EC 3.4.4.19) and hyaluronidase (EC 3.2.1.35 and 3.2.1.36). Such cells appear to have normal intact cellular membranes and have a high endogenous respiration rate[3]. Indeed, BURTON *et al.*[4] have shown that cells prepared by this method incorporate [^{14}C]leucine into the fatty acid synthetase complex. RAPPAPORT AND HOWZE[5,6] have prepared cell suspensions from mouse liver using sodium tetraphenylboron, a potassium chelating agent, as the dispersing agent. Recently, GERSCHENSON AND CASANELLO[7] have prepared isolated rat-liver cells by this method and have shown that such cells respond to insulin and glucagon. These data show that cells prepared by either method exhibit normal liver function. The purpose of this report is to demonstrate that isolated rat-liver cells prepared by either method are able to induce tyrosine aminotransferase in the presence of dexamethasone phosphate, a synthetic glucocorticoid. Previous studies by THOMPSON *et al.*[8] have shown that minimum deviation hepatoma cells may be induced by dexamethasone phosphate to form tyrosine aminotransferase.

Liver cells were prepared from rats obtained from the Holtzman Co. (Madison, Wisc.). Two procedures were used for the preparation of the liver cells. The first procedure was essentially the one described by HOWARD *et al.*[2,3] where the liver from 100–200-g rats was dispersed with a freshly prepared solution of 0.05% collagenase (Schwarz) and 0.10% hyaluronidase (Sigma) in Ca^{2+}-free Hanks salt solution. About 50% of the cells did not take up the vital stains, eosin Y and trypan blue, which is less than that reported by HOWARD *et al.*[2,3]. These cells had a good endogenous O_2 uptake which was linear for at least 2 h.

In the second procedure, sodium tetraphenylboron (Sigma) was used to disperse cells from the livers of 7–14-day-old rats according to the procedure described by GERSCHENSON AND CASANELLO[7] except that a Ca^{2+}- and glucose-free Hanks salt solution was used as the washing and incubation solution. In general, this procedure provided a better yield of isolated liver cells, and these cells had less tendency to reaggregate in solution than the cells prepared by the enzymatic method. However,