

Fluorouracil and the Isolation of Mutants Lacking Uridine Phosphorylase in *Escherichia coli*: Location of the Gene

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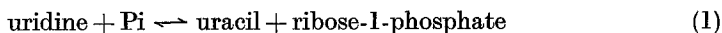
Summary. A selective technique is described for the isolation of mutants of *Escherichia coli* lacking uridine phosphorylase and the location of the gene specifying this enzyme on the bacterial chromosome is determined. Using strains with appropriate lesions it is shown that there are three routes via which 5-fluorouracil can be converted to compounds which inhibit cell growth.

Introduction

Four genes specifying enzymes involved in the catabolism of nucleosides have been shown to be very closely linked at position 89–90 on the linkage map of *Escherichia coli* (Ahmad and Pritchard, 1969). They are *tpp* (thymidine phosphorylase), *pup* (purine nucleoside phosphorylase), *drm* (deoxyribomutase) and *dra* (deoxyriboaldolase). The location of the gene specifying a fifth enzyme (uridine phosphorylase – EC.2.4.2.3) which also participates in nucleoside catabolism (Paegle and Schlenk, 1952) has not previously been reported, although mutants lacking this enzyme have been described (Neuhard and Ingraham, 1968). In this paper we show that this gene (*udp*) is not closely linked to the other four. It is close to *metE* at position 74–75 on the *E. coli* map (Taylor, 1970). We also describe a selective procedure for isolating *udp* mutants of *E. coli* using 5-fluorouracil (FU). Our data indicate that there are three, and probably only three, distinct routes via which FU can be converted to compounds which inhibit cell growth.

Method

Uridine phosphorylase catalyses the following reaction:



Other nucleosides, such as deoxyuridine and thymidine are also degraded by this enzyme (Krenitsky *et al.*, 1964; Beacham, I.R., personal communication).

The selective procedure used to isolate *udp*⁻ mutants was an extension of that already described for the isolation of mutants lacking the other nucleoside phosphorylases (Ahmad and Pritchard, 1969). The parent strain was first made resistant to 5-fluorouracil (2.5 µg/ml). Resistant mutants, which are also resistant to 6-azauracil (100 µg/ml) have previously been designated *azu*^r (Ahmad and Pritchard, 1969). They lack UMP pyrophosphorylase activity (data not shown),

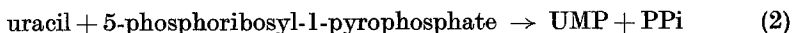


Table 1. *Uridine phosphorylase activity in udp^+ and udp^- strains*

Strain	Enzyme activity (nmoles uracil/min/mg protein)
SA6	175
SA53	not detectable

Cultures (5×10^8 cells/ml) growing exponentially in glucose synthetic medium were washed, resuspended in 1/10th volume of potassium phosphate buffer (0.05 M; pH 6.9) and a sonic extract made. Uridine (50 mM, 0.05 ml) was added to the extract (0.45 ml) at 37°. Samples (0.05 ml) were withdrawn at intervals and added to 0.1 N NaOH (0.45 ml; pH 13). The increase in absorption at 290 nm due to uracil formation was determined. (A difference in molar extinction coefficient of 5.41×10^3 between uridine and uracil was assumed (Razzell and Khorana, 1958)). Strain SA6 has the following genotype: Hfr Reeves 4 $tpp^- upp^- metB^-$. Strain SA53 is a udp^- mutant derived from SA6 as described in the text.

and following the proposal of O'Donovan and Neuhard (1970) we now designate them upp^- .

Such mutants can be sensitised again to FU (2.5 $\mu\text{g/ml}$) if adenosine (200 $\mu\text{g/ml}$) is added to the growth medium as a source of ribose-1-phosphate viz.



This suggests that FU can be converted to fluorouridine by uridine phosphorylase provided a supply of ribose-1-phosphate is made available.

If mutants of the upp^- strain resistant to the FU + adenosine mixture are now isolated some of them should lack uridine phosphorylase and hence be unable to grow on uridine as a sole carbon source.

Results and Discussion

In a preliminary test of this method it was found that some resistant mutants did grow poorly on uridine as sole carbon source but discrimination of this class was not easy. It seemed probable that these were udp^- mutants but that their ability to grow on uridine was due to conversion of this compound to uridine monophosphate which could then be used as a carbon source via the following route: uridine diphosphate; deoxyuridine diphosphate; deoxyuridine monophosphate; deoxyuridine; and thence via thymidine phosphorylase, deoxyribomutase and deoxyriboaldolase to glyceraldehyde-3-phosphate and acetaldehyde.

Consistent with this possibility, when the strain used to isolate udp^- mutants was both upp^- and tpp^- (using 10 $\mu\text{g/ml}$ FU and 100 $\mu\text{g/ml}$ adenosine) a class was obtained which showed negligible growth on uridine as carbon source. Out of 32 resistant mutants isolated from the $upp^- tpp^-$ strain, 22 had lost the ability to grow on uridine as carbon source and presumably lacked uridine phosphorylase. Absence of this enzyme activity was confirmed in one such mutant (Table 1). Some of the remaining 10 mutants were unable to grow on adenosine as sole carbon source. They presumably lacked purine nucleoside phosphorylase and their resistance to the FU + adenosine mixture would consequently be due to their failure to generate ribose-1-phosphate from adenosine (see also Ahmad and Pritchard, 1969).

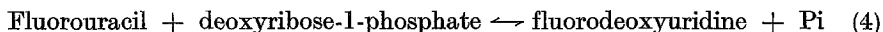
Table 2. *Sensitivity of various mutants to 5-fluorouracil*

Strain	Genotype				FU concentration ($\mu\text{g/ml}$)			
	<i>pup</i>	<i>tpp</i>	<i>udp</i>	<i>upp</i>	0.25	2.5	10	100
P226	+	+	+	+	—	—	—	—
P227	+	+	+	—	++++	+++	+	—
P228.2	—	+	+	—	++++	+++	+	—
SA6	+	—	+	—	++++	+++	++++	+
SA53	+	—	—	—	++++	++++	++++	++++
4K-4	+	+	—	—	++++	+++	++	—

Cells grown on solid synthetic medium were resuspended into 0.5 ml of phosphate buffer. The suspension was spotted on solid synthetic medium containing various concentrations of fluorouracil. Growth was recorded after 48 hrs of incubation at 37°. The number of +’s indicates the relative amount of growth at this time.

Strains SA53, SA6 and P227 were derived stepwise from P226. Strain P228.2 is a derivative of P227. Strain 4K-4 is a derivative of 4K (see Ahmad and Pritchard, 1969), and was kindly provided by Dr. I. R. Beacham.

An interesting outcome of these experiments was the observation that *upp*⁻ *tpp*⁻ strains are more resistant to FU alone than are *upp*⁻ strains. They are able to grow on medium containing up to 10 $\mu\text{g/ml}$ FU. This was surprising since it implies that *E. coli* can synthesise fluorodeoxyuridine from fluorouracil via thymidine phosphorylase.



At first sight this suggests that *E. coli* possesses a significant intracellular pool of deoxyribose-1-phosphate although previous studies have suggested that this is not so. Thus it has been repeatedly found (Bolton and Reynard, 1954; Siminovitch and Graham, 1955; Crawford, 1958; Munch-Petersen, 1968) that wild-type strains of *E. coli* incorporate only negligible amounts of thymine into their DNA even when very high concentrations are present in the growth medium and it has been shown that this lack of incorporation is due to the absence of an intracellular pool of dRib-1-P which is required for the conversion of thymine to thymidine (Boyce and Setlow, 1962; Fangman and Novick, 1966; Beacham, Barth and Pritchard, 1968).

In order to clarify this apparent paradox we have determined the sensitivity of a set of mutant strains lacking one or more of the three enzymes which catalyse the first step in each of the three routes at present implicated in inhibition of growth by fluorouracil (see reactions (1), (2) and (4)). The data (Table 2) suggest that these are the only routes by which FU can inhibit growth of *E. coli* since *upp*⁻ *tpp*⁻ *udp*⁻ strains are resistant to very high concentrations (100 $\mu\text{g/ml}$) of this compound. The contrasting sensitivity of *upp*⁻ *udp*⁻ strains shows that sufficient fluorodeoxyuridine, and from this fluorodeoxyuridine phosphate, can be synthesised via thymidine phosphorylase to inhibit thymidylate synthetase and cell growth (Cohen *et al.*, 1958). Clearly *thy*⁺ strains of *E. coli* have an intracellular pool of deoxyribose-1-phosphate but one which is presumably too small to have been detected by the methods described earlier. The failure to detect

Table 3. Linkage between *metE*, *rha* and *udp*

Selection	Selected transductants	Unselected marker		Total	
		<i>udp</i> ⁺	<i>udp</i> ⁻		
1	<i>met</i> ⁺	<i>rha</i> ⁺	1	4	288
		<i>rha</i> ⁻	98	185	
2	<i>rha</i> ⁺	<i>met</i> ⁺	0	3	96
		<i>met</i> ⁻	92	1	

Transduction was performed as previously described (Ahmad and Pritchard, 1969) using a virulent mutant of phage P1. The donor strain was SA53. The recipient (SA107) had the following genotype: *metE*⁻ *rha*⁻. Udp⁺ and Udp⁻ transductants were distinguished by scoring for growth on medium containing uridine as sole carbon source.

incorporation of thymine in wild-type strains may be due in part to competition from *de novo* synthesis of thymidylate. A more important reason is probably the fact that a very small pool of deoxyribose-1-phosphate is sufficient to render the cells sensitive to low concentrations of FU, because even trace amounts of fluorodeoxyuridine phosphate inhibit thymidylate synthetase sufficiently to lower the intracellular concentration of thymidine triphosphate, which in turn is known to be associated with enhanced breakdown of deoxynucleotides (Beacham *et al.*, 1968; Beacham and Pritchard, unpublished data). This breakdown will therefore increase the supply of deoxyribose-1-phosphate and hence of fluorodeoxyuridine phosphate. In other words, synthesis of this inhibitory compound will be self-amplifying.

With reference to the data given in Table 2, we should draw attention to an earlier study (Brockman *et al.*, 1960) in which it was found that a mutant lacking UMP pyrophosphorylase activity was resistant to very high concentrations of FU. There is no contradiction between this observation and ours, since the strain was isolated by serial transfer of a culture to medium containing increasing concentrations of FU, and is probably a multiple mutant. The evidence presented by Brockman *et al.* (1960) suggests that the strain they used has the genotype *upp*⁻ *tpp*⁻ *udk*⁻ (uridine kinase).

A preliminary mapping experiment indicated close linkage between *udp* and *argB*. The location of the *udp* gene was determined more accurately by P1 transduction (Table 3). The results show that the frequency of co-transduction between *udp* and *metE* is 66 per cent and that between *udp* and *rha* is about 4 per cent. The most probable order indicated by the three-point data shown is *metE* - *udp* - *rha*. The corresponding gene in *S. typhimurium* appears to have a similar location (Beck - reported in O'Donovan and Neuhard, 1970).

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