REDUCTION OF URIDINE DIPHOSPHATE IN *E. coli* 15T⁻*

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Received October 21, 1966

Biswas, Hardy, and Beck reported (1965) that no measurable reduction of uridine nucleotides was observed in extracts of *E. coli* 15T⁻, even under conditions of thymine starvation when the activity of ribonucleotide reductase (measured by reduction of cytidine diphosphate) was remarkably increased. This report suggested that infection of this strain by a T-even phage might be used to test for the virus-induced synthesis of a new ribonucleotide reductase. However, we were unable to confirm the original report, *i.e.*, extracts of various derivatives of strain 15 readily catalyze reduction of both UDP[1] and CDP.

E. coli strains B, 15, 15T⁻, TAU, and THU were grown at 37° in aerated mineral medium supplemented with glucose and with appropriate pyrimidines and amino acids, as previously described (Stern *et al.*, 1964). The bacteria were harvested by centrifugation and stored as frozen pellets. Each pellet (0.6 to 0.9 gm wet weight) was ground with 3 gm alumina per gm of bacteria and extracted with 5 ml 0.05 M Tris·Cl, pH 8, containing

*This investigation was supported by research grants CA 04464 from the National Cancer Institute and 7005 from the National Institute of Allergy and Infectious Diseases.

1. The following abbreviations are used: UDP, uridine diphosphate; CDP, cytidine diphosphate; ATP, adenosine triphosphate; EDTA, ethylene diamine tetracetate.

0.008 M dithioerythritol, per gram of bacteria. The extracts were assayed for reduction of UDP and of CDP in an incubation mixture (adapted from that used by Reichard [1965]) containing ATP 4.2 mM; magnesium acetate 10 mM; EDTA 0.7 mM; Tris·Cl, pH 8.5, 8 mM; dithioerythritol 6 mM; and C^{14} UDP (480 cpm/mμmole) or C^{14} CDP (600 cpm/mμmole) 0.4 mM. One hundred microliters extract was used in 120 μl total incubation volume. After incubating at 38° for 15 minutes, the reaction was stopped by heating. The nucleotides were dephosphorylated by incubating with potato apyrase and *E. coli* alkaline phosphatase, and the resulting nucleosides were separated by paper chromatography in borate buffer (Reichard, 1958). The deoxynucleoside spots were cut out and then counted in a scintillation counter.

The results are shown in Table I. Strains 15, 15T⁻, and TAU reduced UDP just as well as did strain B, while strain THU was somewhat more active.

TABLE I

COMPARISON OF REDUCTION OF UDP AND OF CDP BY EXTRACTS OF DIFFERENT STRAINS OF *E. coli*

E. coli strain	Nucleotide reduced		Ratio, $\frac{\text{UDP reduction}}{\text{CDP reduction}}$
	UDP mμmoles	CDP mμmoles	
B	0.22	1.34	.16
15	0.18	1.14	.16
15T⁻	0.19	1.21	.16
TAU	0.14	0.88	.16
THU	0.86	1.66	.52

For conditions see text

No attempt was made to prove conclusively that this was indeed nucleotide reduction rather than an exchange reaction, but no such exchange has

been demonstrated in *E. coli*. The results of Larsson and Reichard (1966) with purified ribonucleotide reductase from *E. coli* strain B indicate that the same enzyme is responsible for reducing all four common ribonucleoside diphosphates, and that the activators for reduction of UDP are the same as for reduction of CDP. Thus it seems probable that our results do indeed represent reduction of UDP.

Why Biswas *et al.* (1965) did not observe reduction of UDP remains a question. Although their procedure differed from ours in the substrains of bacteria used, in the extraction procedure, in the assay method, and in the incubation medium, none of these differences seems at first sight to be sufficient to explain the observations if indeed a single enzyme system is responsible for reducing both UDP and CDP. It is possible that the answer lies in the realm of regulatory effectors; perhaps an inhibitor relatively specific for UDP reduction was present in their sonic extracts but not in our alumina extracts.

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