AN E. COLI MUTANT WITH CRYPTIC UDP-SUGAR HYDROLASE AND ALTERED METABOLITE REGULATION[1]

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Received April 24, 1968

We have reported previously the isolation from E. coli of a UDP-sugar hydrolase, that cleaves UDP-sugars to the corresponding hexose-1-phosphate, uridine and P_i. E. coli also contains an ADP-sugar pyrophosphatase that cleaves ADP-sugars to sugar-1-P and 5'-AMP (Melo and Glaser, 1966; Glaser et al., 1967). The UDP-sugar hydrolase appears to be identical to the 5'-nucleotidase isolated by Neu (1967). These enzymes appear to be localized on the cell surface since they can be released by osmotic shock or spheroplast formation (Heppel, 1967), and intact cells will hydrolyze substrate provided in the medium essentially at the same rate as cell free extracts. We have suggested that although the enzyme appears to be located at the cell surface, it functions in the regulation of the nucleoside diphosphate sugar pool in the cell. We report preliminary observations on an E. coli mutant, in which the UDP-sugar hydrolase is no longer available to external substrate, and which shows an altered pattern of utilization of different carbon sources.

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[1] Supported by a Grant GB 6243 from the National Science Foundation.

[2] Recipient of a NATO fellowship of the Science Research Council of Great Britain.

Methods: UDP-sugar hydrolase was measured as described by Glaser et al. (1967), phosphodiesterase by the method of Neu and Heppel (1965), ADP-glucose breakdown by whole cells, was measured by measuring ADP-glucose with ADP-glucose pyrophosphatase, phosphoglucomutase and glucose-6-P dehydrogenase, or by measuring the breakdown of ADP-glucose ^{14}C, followed by isolation of residual ADP-glucose.

E. coli was grown in minimal medium of Davis and Mingioli (1950) with 0.4% glycerol as carbon source and 10^{-5} M $CoSO_4$, to allow maximal utilization of external UDP-glucose (Glaser et al., 1967). E. coli W3092C was obtained from Dr. W. Sly. Cells were subjected to osmotic shock by the method of Neu and Heppel (1965). Mutagenesis with N-methyl-N'-nitrosoguanidine was carried out by the method of Adelberg et al. (1965), followed by penicillin selection.

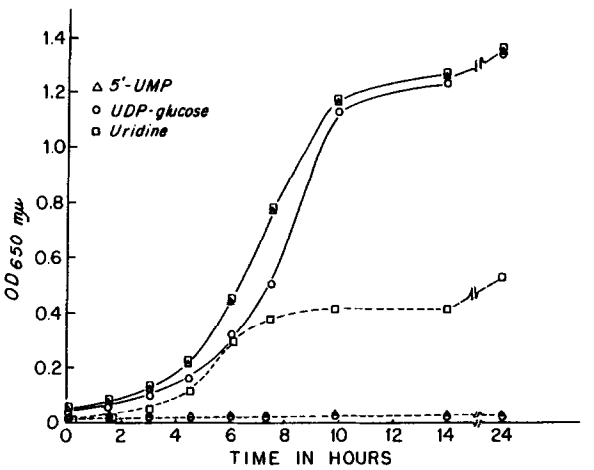

Fig. 1: Growth of E. coli N-3 and $UDPG^-$ in the presence of various nucleosides and nucleotides. Standard growth conditions were used. All nucleotides were added at a concentration of 10^{-3} M. ——— N-3, ------- $UDPG^-$ cells.

Results: An E. coli mutant (designated 3 N) was selected which can grow on glucose but not on glycerol or succinate, and is presumably blocked in fructose-1,6-diphosphate phosphatase (Fraenkel and Horecker, 1965). In the presence of glycerol this mutant required for growth the presence of small quantities of uridine, UDP-glucose or a variety of other nucleosides (see Fig. 2 below) as a supplementary carbon source. Mutants were then selected from this organism

which could grow on glycerol and uridine but not on glycerol and UDP-glucose. One of the mutants has been examined in some detail and is the subject of this report (designated UDPG$^-$).

TABLE 1

Surface enzymes in E. coli N-3 and UDPG$^-$

Enzyme Measured[+]	N-3				UDPG$^-$			
	Whole cells	Shock fluid	Sonic extract	Heated sonic extract	Whole cells	Shock fluid	Sonic extract	Heated sonic extract
I. UDP-glucose-hydrolase	0.77	0.75	--	--	0.08	0.8	--	--
Phospho-diesterase	1.44	0.88	--	--	2.2	2.2	--	--
II. UDP-glucose-hydrolase	0.36	--	0.07	0.34	0	--	0.053	0.40
II. UDP-glucose-hydrolase	0.316	0.34	--	--	0.10	0.42	--	--
Phospho-diesterase	0.68	0.48	--	--	0.56	0.60	--	--
Glucose-1-P-phosphatase	0.18	0.18	--	--	0.19	0.13	--	--
ADP-glucose pyrophosphatase	0.33	--	--	--	0.23	--	--	--

[+] All activities are expressed as μmoles of substrate utilized/hr/mg cell dry weight, except for the phosphodiesterase, where activity is expressed as OD at 410 mμ/10'/mg dry weight at 25°. Heated sonic extracts were prepared as described (Glaser et al., 1967) to destroy the UDP-glucose hydrolase inhibitor. Usually small UDP-glucose hydrolase activity was noted with intact cells of UDPG$^-$ strain, this could be accounted for by enzyme released into the medium during assay.

As seen in Fig. 1 the UDPG⁻ mutant will not grow on UDP-glucose or UMP, or other 5'-nucleotides as expected from the fact that the UDP-sugar hydrolase and the 5'-nucleotidase are the same enzyme. It should also be noted in Fig. 1 that at 10^{-3} M uridine, the cell yield is about 1/3 for the UDPG⁻ mutant as compared to the parent strain. This observation will be discussed in detail below.

A closer examination of the UDPG⁻ mutant (Table 1) showed that although the mutant was phenolypically UDPG⁻ and whole cells had essentially lost the ability to breakdown UDP-D-glucose, cell free extracts of the mutant contained normal quantities of enzyme. Furthermore active enzyme could also be released in normal amounts by osmotic shock, suggesting that the enzyme is still located near the cell surface, and has not become an "intracellular protein". Since active enzyme is released by osmotic shock it appears unlikely that the lack of activity with whole cells is due to the "intracellular protein" inhibitor of this enzyme described previously (Melo and Glaser, 1966).

The effect of the mutation is specific since other surface enzymes such as the phosphodiesterase and glucose-1-P phosphatase (acid phosphatase), and most significantly the ADP-sugar pyrophosphatase remain unchanged, and still accessible to externally supplied substrate.

Since the UDPG⁻ mutant has lost the ability to hydrolyze not only UDP-glucose, but also 5'-AMP and other 5'-nucleotides, it seems likely that the mutation involves the attachment of the enzyme to the cell surface, rather than the disappearance of a specific permease, required to make these substrates available to the UDP-sugar hydrolyase, since such a permease would have to share the same specificity requirements as the UDP-sugar hydrolase.

If there is a modified attachment of the enzyme and the cell surface, the mutation could affect either the enzyme or a surface structure of the cell. Preliminary tests of the enzyme obtained from the UDPG⁻ mutant have failed to detect any difference between this enzyme and the wild type in size (as determined on Sephadex G-100), its heat stability and its protection against heat in-

activation by metal ions. This obviously does not exclude a mutation in the enzyme, which is only reflected in its attachment to the cell surface.

We have been unsuccessful in trying to recombine UDP-hydrolase with osmotically shocked cells depleted of the enzyme. The UDPG$^-$ mutant cannot be distinguished from the parent strain by electron microscopy[3], and shows no difference from the parent strain in gross analysis (RNA, DNA, lipid and lipopolysaccharide).

In view of the differences in cell yield observed when cells were grown in 0.4% glycerol and 10^{-3} M uridine, the cell yield of the parent and mutant strains was examined under a variety of conditions. A few examples are shown in Fig. 2. Both the parent and the mutant grow equally well with glucose as a carbon source. In the presence of 0.4% glycerol 5 x 10^{-4} M uridine will give half maximal growth with the parent strain but 2 x 10^{-3} M uridine is required for the mutant. The UDPG$^-$ mutant will grow efficiently in uridine as a sole carbon source, but the parent strain will not.

When either of the strains is grown on glucose and glycerol the expected result is obtained, namely that the cells will grow until glucose is exhausted and then growth will cease.

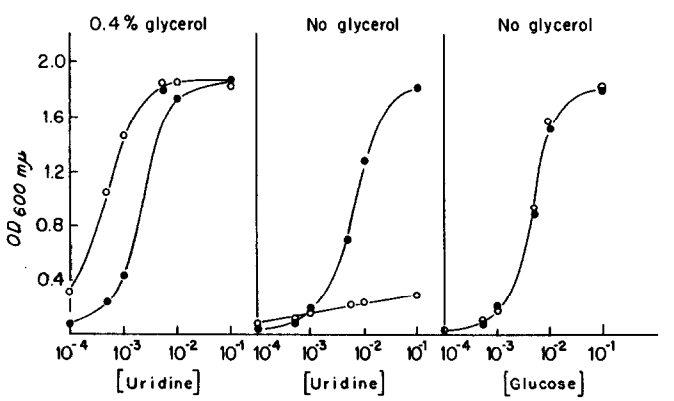

Fig. 2: Cell yield of E. coli N-3 and UDPG$^-$ on various carbon sources. O-O N-3, ●-● UDPG$^-$.

[3] We thank Dr. J. W. Williamson for examing sections of these cells in the electron microscope.

The results in Fig. 2 strongly suggest that under these conditions the UDPG⁻ mutant is using uridine as a main carbon source even in the presence of glycerol, this is confirmed by the data in Fig. 3 which show that very little glycerol is used by the UDPG⁻ mutant during growth.

These results are parallel by enzyme assays in cells grown under a variety of conditions. The level of glycerolkinase[4] is about 1/2 to 1/3 as high in the UDPG⁻ mutant as in the parent strain at several uridine levels. Both strains are however fully induced in stationary phase in the presence of glycerol after uridine has been exhausted and in both strains the glycerolkinase can be totally repressed by glucose. Conversely the ability of UDPG⁻ cells to release orcinol reactive material from uridine, presumably via uridine phosphorylase (Paege and Schlenk, 1952) is increased.

These results strongly suggest that the change in the UDP-sugar hydrolase

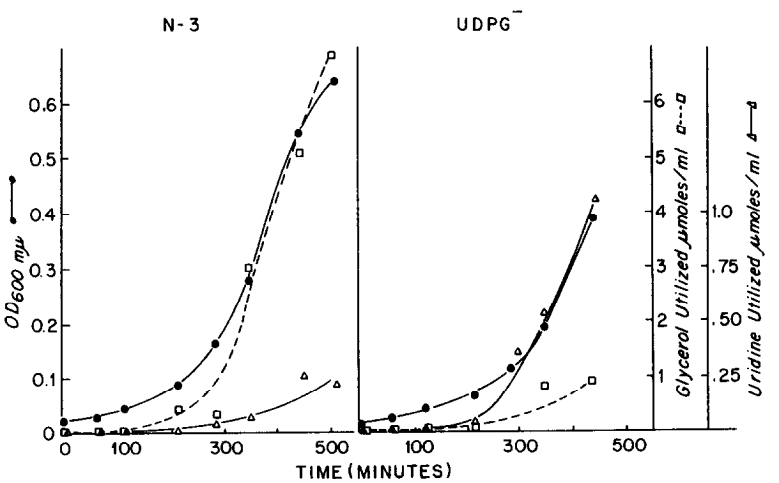

Fig. 3: Utilization of carbon sources for growth. Cells were grown on minimal medium with 0.4% glycerol. For N-3 the uridine concentration was 5×10^{-4} M, for UDPG⁻ 2×10^{-3} M. These concentrations of uridine give an equivalent cell yield for the two strains (see Fig. 2).

[4]Glycerolkinase is known to be repressed by glucose (Kock et al., 1964) and to be inhibited by fructose-1,6-diphosphate (Zwaig and Lin, 1966). The glycerolkinase level in UDPG⁻ strain was 0.4 μmoles/min/mg protein and in the parent strain 1.1 μmole/min/mg protein when grown on 5×10^{-4} M uridine or 2×10^{-3} M uridine.

has resulted in a change of intracellular metabolite concentrations, such that glycerolkinase is partially repressed, and that glycerol utilization in the presence of uridine is almost totally inhibited. This inhibition cannot be entirely accounted for by the decrease in glycerolkinase in these cells.

The results presented in this communication suggest that there are specific attachment sites for "surface enzymes" in E. coli, that this attachment site is subject to genetic modification, and that in the specific case of the UDP-sugar hydrolase, a subtle change in the enzyme localization, detected by the fact that it is no longer available to external substrate, results in significant changes in intracellular metabolite levels. Work is in progress trying to pinpoint the changed metabolites, and to isolate mutants which totally lack this enzyme.

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