ACCUMULATION OF URIDINE DIPHOSPHOGALACTOSE IN A BACTERIAL MUTANT DEFECTIVE IN EPIMERASE*

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Received August 29, 1960

It has previously been described that certain "galactose negative" strains of Escherichia coli, C7M, if grown in broth lyse in the presence of even minute amounts of galactose (Fukasawa and Nikaido, 1959a; Yarmolinsky, Wiesmeyer, Kalckar, Jordan, 1959). It was shown that these mutants have a single defect in the enzyme UDPGal** 4-epimerase (Kalckar, Kurahashi, Jordan, 1959; Fukasawa and Nikaido, 1959b). Addition of glucose at the onset of lysis is able to salvage the main part of the bacteria and growth is again resumed (Yarmolinsky et al., 1959). Cells grown in glycerol ammonia mineral medium in the presence of galactose do not undergo lysis immediately. A static phase of 1 to 3 hours precedes the onset of lysis. There is an accumulation of Gal-1-P. More remarkable, however, is a very striking accumulation of UDPGal (Spyrides and Kalckar, 1960).

Wild type galactose positive E. coli or galactose negative E. coli mutants with defects in enzymes other than 4-epimerase contain on an average 0.15 μmoles of UDPG and about 0.05 μmoles of UDPGal per gram cells (wet weight). In this epimerase-less strain, however, UDPGal predominates. Instead of the usual UDPGal/UDPG ratio of 0.25 to 0.3 one finds a ratio of 10 to 20 or even higher. Moreover the absolute

*Contribution No. 288 supported by grants from the National Science Foundation (#G9605, #G6477) and National Institutes of Health (#A2498).

**The following abbreviations were used: UDPGal - Uridinediphospho galactose; UDPG - Uridinediphospho glucose; Gal-1-P - Galactose-1-phosphate; 4-epimerase - Uridinediphospho galactose 4-epimerase; OD_{260} - Optical density at 260 mu; TMG - Thiomethylgalactoside.

amount of UDPGal is greatly increased as compared with the other strains. Increases of UDPGal concentrations as high as 50 fold can be seen in the presence of galactose under conditions in which the onset of lysis is delayed or prevented.

Materials and Methods

Strain C7M (Fukasawa and Nikaido, 1959a) was grown in glycerol ammonia mineral medium (Hartman, 1956) at 37° or at 25°. The water soluble component was extracted from the harvested cells by means of boiling 70% ethanol (25 ml 70% ethanol per gram wet weight) and the clarified supernatant after centrifugation was concentrated about 100 fold in a flash evaporator. Specific enzymic determinations of UDPG and UDPGal (Kalckar, Anderson, Isselbacher, 1956; Maxwell, 1957) were performed either directly on the concentrate or preferably on eluates from spots obtained by paper chromatography of concentrates. The solvent system used for chromatography was ethanolic neutral ammonium acetate (Paladini and Leloir, 1952). The UV quenching area with an R_f corresponding to a UDPG standard was excised and eluted with water. In somewhat larger scale isolations from extracts of C7M the nucleotides were first absorbed and eluted from Norite (Crane and Lipmann, 1953; Kalckar and Cutolo, 1952) and then subjected to paper chromatography as described. UDPG was determined by the specific UDPG dehydrogenase (Kalckar *et al.*, 1956), UDPGal was determined likewise with the subsequent addition of 4-epimerase (Maxwell, 1957). A second enzymatic assay using purified Gal-1-P uridyl transferase (Kurahashi and Sugimura, 1960), glucose-1-phosphate and UDPG dehydrogenase was used as an independent method for determination of UDPGal.

Results

If C7M cells are grown in glycerol ammonia mineral medium in the presence of galactose, the development of lysis is protracted. After galactose induction of the two enzymes, galactokinase and Gal-1-P uridyl transferase, a cessation of growth ensues (see Fig. 1). The lack of

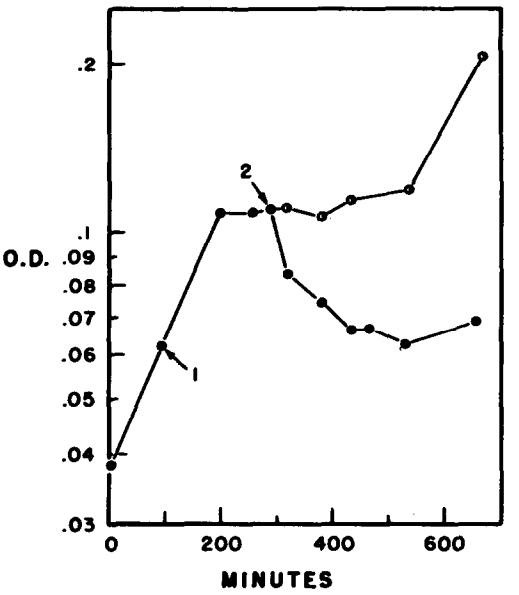

Figure 1. Growth curve (logarithmic scale) of a culture of E. coli C7M. Temperature 37°C (experiment 1, see also Table 1). At arrow "1" galactose is added. At arrow "2" the culture is divided in two portions and glucose is added to half of the culture. The medium was a mineral, ammonia, glycerol medium. For further details see text (Methods) and Table 1.

growth represents a true stasis and not a dynamic equilibrium between growing and lysing cells as appears from determination of β-galactosidase in cells and in medium (induction with TMG and galactose). The medium contained only negligible amounts of this enzyme whereas the cells contained large amounts of the enzyme. Hence no lysis of significance could have occurred during this period.

During the static phase the concentration of UDPGal increases 10 to 30 fold over that of cells grown in the absence of galactose (cells collected at 300 minutes [Fig. 1]). Also, the UDPGal/UDPG ratio becomes as high as 10 to 20 instead of the normal ratio of 0.3. A further addition of glucose during the static phase not only prevents the onset of lysis but also permits resumption of growth. In spite of this the UDPGal accumulation persists and the UDPGal/UDPG likewise remains abnormally high (cells collected at 600 minutes [Fig. 1]).

It seems, therefore, clear that the accumulation of the large amounts of UDPGal cannot be singled out as a major cause in the development of unbalanced growth and lysis. The absolute increase of UDPGal formation could well be a sign of the development of a compensating mechanism. Thus, if C7M cells are grown at 25° instead of 37° in ammonia glycerol mineral medium and galactose is added, growth continues (although the generation time increases gradually to about 4 hrs). Accumulation of UDPGal can reach concentrations as high as 2 µmoles per gram wet weight or almost 50 fold the normal cellular content of UDPGal. Also in this case the UDPGal/UDPG ranges as high as 10 to 20.

Quite aside from the problems of physiological interest the epimerase-less mutant can be used for isolation of UDPGal which normally occurs only in minute amounts in cells. This nucleotide was isolated by Norite fractionation and identified by two enzymatic methods referred

Table 1

Addition to Mineral Ammonia Medium		µmoles per g. wet weight UDPG	UDPGal	Uridine Nucleotide
Glycerol 10^{-1} M		0.15	0.04	
Glycerol 10^{-1} M Galactose 10^{-3} M	Exp. 1	0.05	0.50	
	Exp. 2	0.05	0.95	1.20
Glycerol 10^{-1} M Galactose 10^{-3} M Glucose 10^{-2} M	Exp. 1	0.03	0.45	

Experiment 1 was incubated at 37° C (see also Fig. 1) whereas experiment 2 was incubated at 25°C.

UDPG and UDPGal were identified by enzymatic assays (see Methods) and by the R_f of paper chromatography spots (Paladini and Leloir, 1952).

Uridine nucleotide was further identified in experiment 2 by the ultraviolet absorption spectra at pH 2, 7 and 11 of water eluates of UDPGal-UDPG spots from paper chromatography. Ratio OD_{260} pH 2 to OD_{260} pH 11 : 1.3. Ratio OD_{246} pH 2 to OD_{246} pH 11 : 1.0. Hydrolysis in 0.02 M HCl gave nucleotide spot with R_f equal to that of UDP (Paladini and Leloir, 1952).

to under "Methods" as well as optical and chromatographic methods (see footnotes to Table 1).

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Acknowledgment

The valuable technical assistance of Mr. Randolph Frye and Miss Hanne Troedsson (trainee of the Scandinavian American Foundation) is acknowledged with thanks. For gifts of E. coli strain C7M purified 4-epimerase and transferase we want to thank Drs. Fukasawa and Nikaido, E.S. Maxwell and K. Kurahashi respectively.