GENETIC STUDIES OF *ESCHERICHIA COLI* K 12 MUTANTS WITH ALTERATIONS IN GLYCOGENESIS AND PROPERTIES OF AN ALTERED ADENOSINE DIPHOSPHATE GLUCOSE PYROPHOSPHORYLASE

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Received February 4, 1969

Genetical and biochemical analyse of *Escherichia coli* K 12 mutants altered in their ability to accumulate glycogen have recently been performed in our laboratory (Damotte *et al.*, 1968 ; Sigal and Puig, 1968). Three types of mutants (*glg* A, *glg* B and *glg* C) were selected by the appearance of colonies on synthetic nutrient agar flooded with iodine solution. In contrast with wild type colonies which accumulate glycogen and are stained brown by iodine, colonies of *glg* A mutants remain colorless because of the loss of glycogen synthetase activity. The *glg* B mutants have lost branching enzyme activity and give blue colonies. The *glg* C mutants are characterized by the formation of "super brown" colonies and differ from the wild type by accumulating larger amounts of glycogen not only during the stationary phase, but also during the log phase of growth. As the increased accumulation of glycogen by the *glg* C mutants is not correlated with an increased level of the enzymes involved in glycogen synthesis, it was concluded that regulation of glycogen synthesis in the wild type does not consist in enzyme repression, but in some other mechanism at the level of enzyme activity (Damotte *et al.*, 1968). In the present report, additional information on the mapping of the *glg* genes is given, and it is demonstrated that the *glg* C mutation affects the structural gene of adenosine diphosphate glucose (ADP-glucose) pyrophosphorylase.

Conjugation and transduction with phage P_1K_c had previously shown that the three glycogen genes are located near the *mal* A gene, the cotransduction frequencies with this gene being 51,59 and 2 per cent for *glg* A, B and C respectively (Sigal and Puig, 1968). Further precision in mapping has been achieved by the analysis of cotransduction with (*dap* + *hom*)$^+$ genes. These transductants have been scored for their ability to ferment maltose and for the staining of colonies with iodine.

TABLE 1

SEGREGATION OF *glg* AND *mal* A BETWEEN $(dap + hom)^+$ TRANSDUCTANTS

Phage lysate on strains	Genotypes of receptors strains	Number of $(dap + hom)^+$ selected transductants	Non selected markers	
			glg^-	mal^+
$(dap + hom)^+$ *mal* A^+ *glg* A^-	$(dap + hom)^-$ *mal* A^- *glg* A^+	215	140	116
$(dap + hom)^+$ *mal* A^+ *glg* B^-	$(dap + hom)^-$ *mal* A^-*glg* B^+	293	237	172
$(dap + hom)^+$ *mal* A^+ *glg* C^-	$(dap + hom)^-$ *mal* A^-*glg* C^+	126	100	50

Transduction was done by the method of Lennox (1955).

From the results reported on Table 1, frequencies of cotransduction with (*dap* + *hom*)$^+$ have been found to be 65.5, 80 and 79 per cent with the genes *glg* A, B and C respectively. Moreover nearly all the (*dap* + *hom*)$^+$ colonies that are also *mal*$^+$ have received the *glg* allele from the donor strain. This leads to the conclusion that the *glg* genes are located between *mal* A and (*dap* + *hom*) and excludes the possibility that they are located on the other side of (*dap* + *hom*).

Previous study of glycogen accumulation by various strains with known deletions of the *mal* A-*glp*D(*dap* + *hom*) region (Sigal and Puig, 1968) has shown that the *glg* genes are present in deletions of the *mal* A-*glp* D segment and absent in deletions including the *mal* A-(*dap* + *hom*) segment. Consequently the genes are in the following order :

mal A-*glp* D-*glg* (A, B or C)-*dap* + *hom*

The *glg* C mutants are of special interest, as these mutants are able to accumulate glycogen even during the log phase of growth. Therefore, one could expect these mutants to provide some information on the mechanism by which glycogenesis is regulated. For this purpose, the properties of their glycogenic enzymes have been analyzed and compared to those of the wild type.

A *glg* C mutant and its parent strain *E. coli* K 12 PA 601 are grown aerobically at 32° C in a synthetic medium supplemented with the necessary amino-acids, threonine being the limiting factor. Enzymatic determinations have been made on crude extracts obtained by sonication of cells harvested two hours after the end of log phase. The ADP-glucose pyrophosphorylase activity was determined in direction of pyrophosphorolysis by following the formation of ATP^{32}-P from ADP-glucose and ^{32}P-Pi as described by Preiss *et al.* (1966). The glycogen synthetase activity was determined by following the transfer of glucose ^{14}C from ADP-glucose ^{14}C to glycogen as described by Preiss and Greenberg (1965).

The study of heat inactivation curves has shown that the thermosensibility of the ADP-glucose-pyrophosphorylase in the *glg* C mutant is quite different from that of the wild type enzyme (Fig. 1). This last enzyme losses no activity by heating 15 min. at 55° C whereas the activity of the mutant enzyme decreases appreciably at as low a temperature as 40° C under the same conditions.

The half-life of this mutant enzyme is 19.5 minutes at 53° C (Fig. 2). At this temperature, the wild enzyme is quite stable for 30 minutes.

The glycogen-synthetase extracted from *glg* C mutant cells has

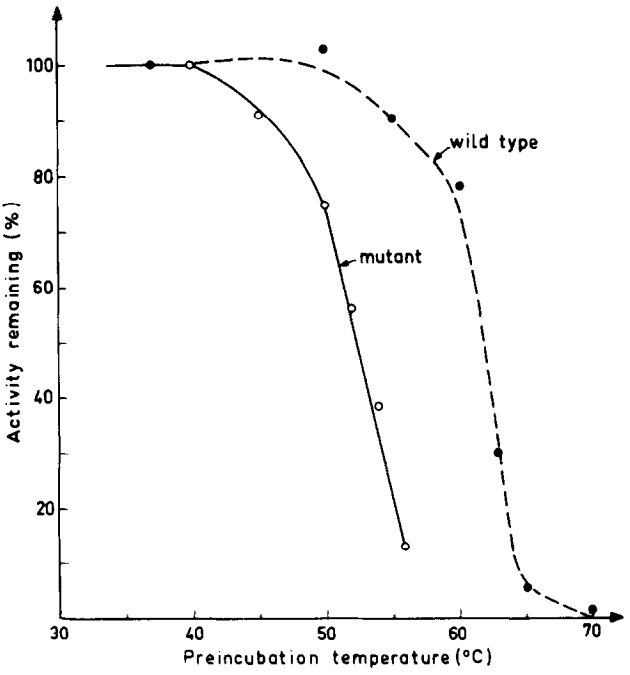

Figure 1 - Relative heat labilities of ADP-glucose pyrophosphorylase in cell free extracts from mutant *glg* C and from the wild type. The test tubes containing 2.5 ml of Tris-HCl pH 8.0 0.08 M were preincubated in a water bath at the indicated temperature prior to the introduction of the crude extract (0.1 ml). After 15 min. of thermal treatment, they were rapidly cooled to 0° C, and the activity was measured at 37° C according to Preiss *et al.* (1966).

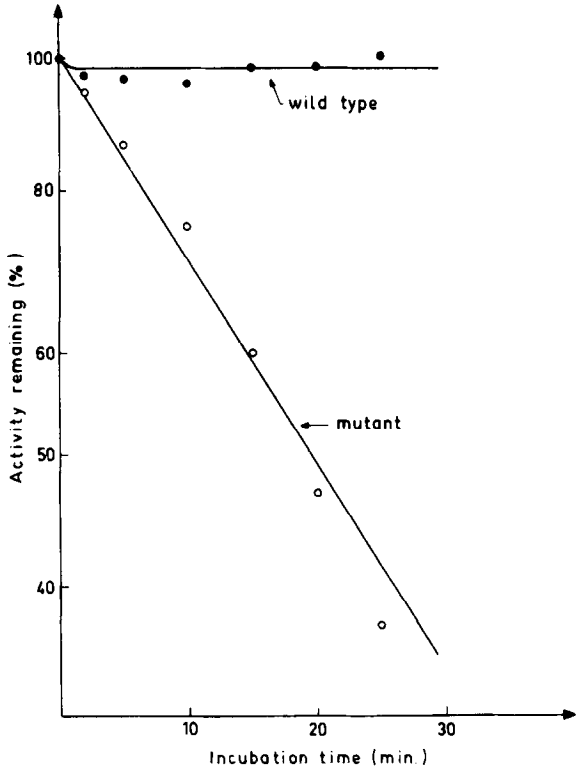

Figure 2 - Relative heat inactivation at 53° C of ADP glucose pyrophosphorylase in cell-free extracts from the wild type and from a *glg* C mutant.

about the same heat sensibility as wild type cells.

These experiments suggested that the C mutation has altered the structure of the ADP.glucose pyrophosphorylase synthesized, and could have also modified the sensitivity of this enzyme to its effectors. Preiss *et al.* (1966) have established that the ADP glucose pyrophosphorylase of *E. coli* B is inhibited by 5'-AMP, ADP and inorganic phosphate and activated by several glycolytic intermediates, especially fructose-1,6-diphosphate (FDP). This activator also modulates the sensitivity of the enzyme with respect to AMP (Gentner and Preiss, 1967).

We have studied comparatively kinetics of ATP formation with

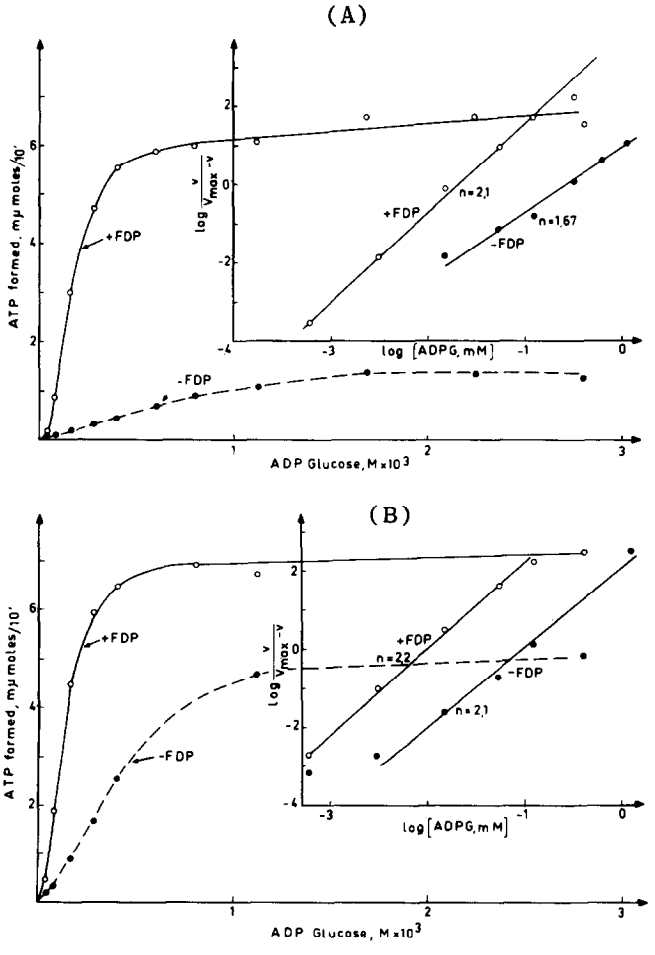

Figure 3 - Influence of ADP glucose concentration on the ADP glucose pyrophosphorylase activity of the wild type (Fig. A) and of the *glg* C mutant (Fig. B). Assays in the presence of fructose-1.6-diphosphate (1.2 mM) or in its absence. In the last case, the concentration of $MgCl_2$ was increased from 8 mM to 20 mM.

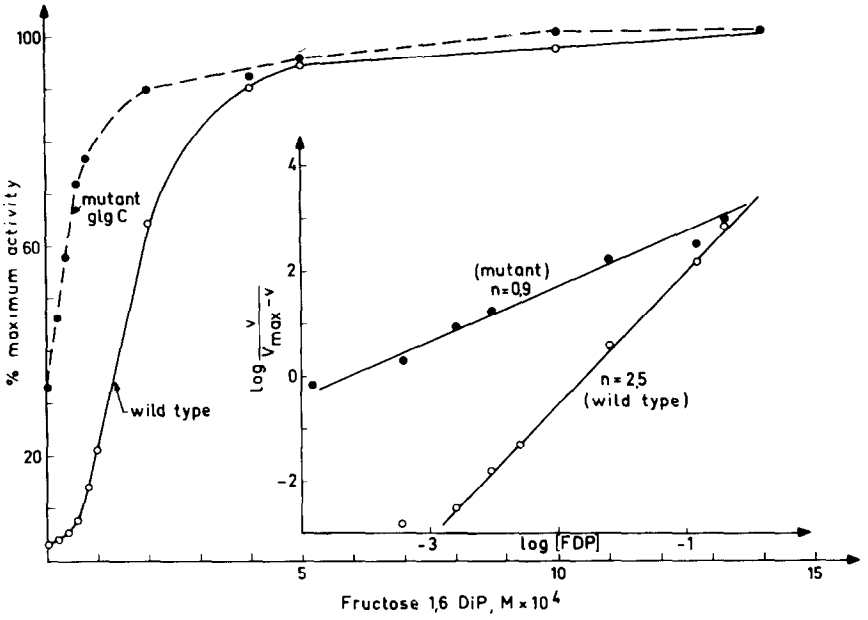

Figure 4 - Effect of fructose diphosphate concentration on the rate of ATP formation in the presence of ADP-glucose 0.4 mM.

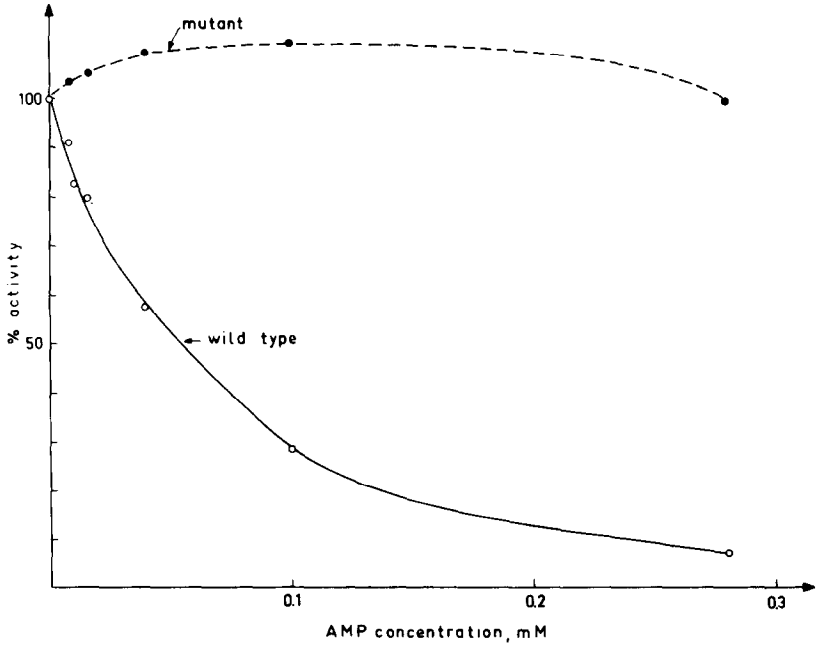

Figure 5 - Relative inhibition of ATP formation as a function of 5'-Adenylate concentration, in the presence of fructose-1.6-diphosphate (1.2 mM) by cell-free extracts of the wild type and the *glg* C mutant.

the wild type *E. coli* K 12 enzyme and with the *glg* C mutant. Our results with the wild type enzyme are similar to those reported by Preiss for *E. coli* B (Fig. 3 A). In the case of the mutant, the curves of enzyme activity as a function of substrate concentration still are sigmoïd in the presence or absence of FDP (Fig. 3 B), but the affinity of the mutant enzyme for its substrate, in the absence of FDP, is consistently increased.

If the percentage of enzyme activity, in the presence of saturing concentration of substrate, is plotted as a function of activator (FDP) concentration, one sees that in the case of the mutant enzyme, the curve is no longer sigmoïdal (Fig. 4). This corresponds to a change of n of the Hill equation from 2.5 to 0.9 (Changeux, 1963). Still more significant is the fact that the mutant enzyme has completely lost its sensitivity toward the inhibitory effect of AMP. This desensitization is observed in the presence of the FDP concentration that gives optimal enzyme activation (Fig. 5). It is also observed in the presence of the reduced FDP concentration (0.04 mM) which allows maximum inhibition of the wild type enzyme.

DISCUSSION

From these data, it is clear that the three structural genes for the enzymes involved in glycogen synthesis are closely linked. It is possible that they constitute a single unit of transcription although no evidence has yet been found that these genes are submitted to a coordinate process of induction or repression.

Our previous hypothesis that regulation of glycogenesis takes place at the level of enzyme activity is further substantiated by the demonstration of mutants in which one of the concerned enzymes (ADP-glucose pyrophosphorylase) is modified in its sensitivity to effectors. It is interesting that this modification consists in a total desensitization toward the allosteric inhibitor (AMP), and that the sensitivity to the activator (FDP) is modified but no suppressed. Obviously the *glg* C mutation of ADP-glucose pyrophosphorylase is at least partly responsible for the accumulation of glycogen by mutants during the log phase of growth. In this respect, our observations support the suggestion made by Preiss and its collaborators that the regulation of bacterial glycogen synthesis depends on the relative intracellular concentrations of AMP and of FDP (Gentner and Preiss, 1967; Govons *et al.*, 1968).

It will be necessary to determine the variations of intracellular concentrations of these effectors during the different growth phases in order to show that the facts observed *in vitro* are physiologically significant.

Acknowledgements

We wish to thank Dr. Maxime Schwartz for the (*dap* + *hom*)$^-$ mutant and for the deletion strains.

We also thank Miss A. Favard for her excellent technical assistance.

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