THE SENSITIVITY OF THE GALACTOSE OPERON OF E.COLI TO UV-LIGHT

Peter Starlinger and Eckehart Kölsch

Institut für Genetik der Universität zu Köln
Germany

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The capacity of bacterial cells to synthesize enzymes can be inactivated by uv-light (Bowne and Rogers, 1962; Pardee and Prestidge, 1963). It seemed to be of interest to extend such studies to several enzymes of a single operon. The galactose operon was chosen for such a study.

Materials and Methods: Strain W8 is a prototrophic reisolation of E.coli K12; strain B_{s-1} is a radiation sensitive mutant of E.coli B (Hill, 1958). Synthetic medium M9 with 2 g/l succinate as a carbon source was used throughout the experiments. The cells were grown in this medium at 37° with aeration to a titer of 2×10^8 per ml. Samples of 15 ml were irradiated in Petri dishes of 8 cm ∅ with an Osram HNS 12 Hg lamp with magnetic stirring. The samples were then again incubated as above in the presence of 5×10^{-3}M d-fucose as an inducer of galactose enzymes. Samples were withdrawn at 0 minutes and after induction period of 40 minutes. The cells were concentrated by centrifugation, lysed with lysozyme and EDTA, and again centrifuged for removal of debris. Galactokinase (Sherman, 1963), galactose-1-phosphate uridyl transferase (Buttin, 1963a) and galactose-4-epimerase (Kalckar, Kurahashi, and Jordan, 1959) were assayed from the supernatants, as described in the papers quoted.

Results: The cellular capacity of E.coli B_{s-1} to synthesize the three galactose enzymes is inactivated at different rates, as shown in fig. 1. All three inactivation curves show a shoulder, and the final

slopes extrapolate to a value of 3. The reason of this "multihit" behaviour is not known.

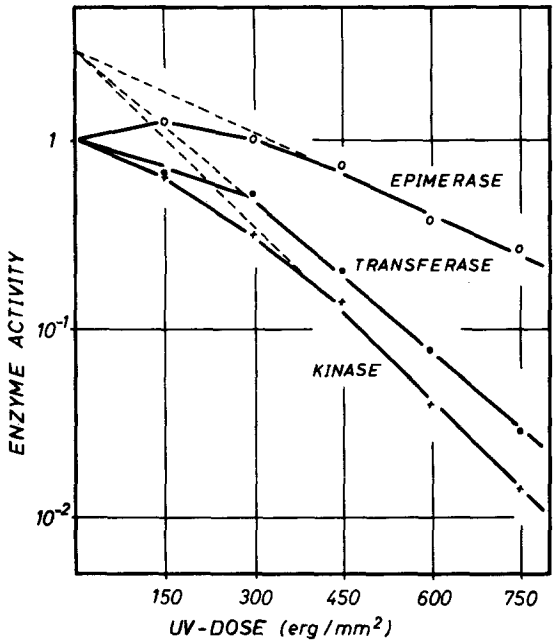

Fig. 1: Inactivation of enzyme forming capacities of B_{s-1} by uv.
Enzyme activities are expressed as fractions of the zero dose value. The points of the curves are the averages of three independent experiments.

The curves obtained for strain W8 were similar, except for the fact that the final slopes in the case of W8 were less steep by a factor of about 1.5. This is in sharp contrast to the difference in survival after uv between these two strains. Under our conditions the final slopes of the bacterial survival curves differed by a factor of 75. The reactivation mechanisms, which are operative in K12 strains to restore survival damage, may not be operative in the case of damage to the enzyme forming capacity.

The curves of figure 1 are obtained from measurements of the enzyme formed only after a single time interval after induction of enzyme formation. It was veryfied in separate experiments that these values really represent

the rate of enzyme formation and are not influenced by a delayed onset of enzyme formation.

Discussion: The bacterial capacity to synthesize galactose enzymes is inactivated by uv-light. Such an inactivation could be caused (a) by direct damage to the genetic region, which determines the structure of the galactose enzymes, or (b) by general cell damage, which influences indirectly the synthesis of these enzymes. The genes for the galactose enzymes form a unit of coordinate expression (Yarmolinsky, Jordan, and Wiesmeyer, 1961). Indirect damage should therefore influence them to the same extent. Since the capacity for the synthesis of the single enzymes is affected to different degrees, we prefer the assumption that direct damage is, at least partially, involved.

Direct damage might influence the activity of the operon in either of three ways: (a) The activity of the whole operon is abolished. This type of damage cannot be the only one, since it would not explain the differential inactivation of the three enzyme forming capacities. (b) The damage is localized to one of the structural genes only, leaving the others unaffected. In this case the slopes of the inactivation curves would give a measure of the amount of uv-sensitive material in each gene, which itself should be a function of the size of the gene and its base sequence. (c) Recent experiments suggest that the messenger RNA for a whole operon may be synthesized in one large piece (Martin, 1963). If this were the case for the galactose operon also, where pertinent data are not available, a third possibility for direct uv-damage to the operon should be considered. The synthesis of m-RNA could be allowed up to the point of damage and might be prevented beyond it. In this case the genes near the point of origin of m-RNA synthesis should be relatively insensitive and the sensitivities of all genes should fall into a gradient in the order of the genetic map.

The sensitivities of the three enzyme forming capacities increase in the order: epimerase, transferase and kinase.

This is the relative order of the genetic map, where the operator region lies at the epimerase end (Buttin, 1963b).

Whether the sensitivities found are compatible with assumption (b), cannot be evaluated because of lack of sufficient data on the structural genes. Assumption (c) could be valid, if the synthesis of m-RNA would start at the operator end of the operon. Further experiments are required to clarify this situation and are in progress in this laboratory.

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