GENETIC INSTABILITY OF AN ULTRAVIOLET-SENSITIVE MUTANT OF PROTEUS MIRABILIS

Helmut Böhme
Institut für Kulturpflanzenforschung der Deutschen Akademie der Wissenschaften, DDR-4325 Gatersleben

Received June 13, 1967

In the last years investigations of several radiation-sensitive bacterial mutants have brought to light the existence of mechanisms, which can repair inside the cell radiation- and chemical-induced structural lesions of the DNA.[1)] In case of inactivation of the repair process by mutation the mutant cell acquires an increased sensitivity to inactivation by irradiation or by some alkylating agents. Hill (1965) and Witkin (1966) showed the ability of the repair mechanisms to repair not only lethal but also mutative UV-induced DNA lesions. On the basis of these facts one could expect that at least a part of those changes in the DNA leading to potential spontaneous mutations may be repaired by the same mechanisms. A consequence would be, that a part of the radiation-sensitive mutants should exhibit an increased spontaneous mutability. This idea has already been discussed by Hanawalt and Haynes (1965) and Harm (1967). We have isolated several radiation-sensitive mutants of Proteus mirabilis and, in the following, report on one of these mutants in which the UV-sensitivity is accompanied by an increased genetic instability.

Material and Methods

Bacterial strains:

Strain	Marker	Origin
PG 273	$thr^-;uvr^+$	EMS induced mutant of wildtype PG VI
PG 667	$thr^-;uvr^-$	PG 273
PG Sb-3	str-d;uvr^+	PG VI

1)Abbreviations:DNA - deoxyribonucleic acid;UV - ultraviolet; EMS - ethyl methanesulphonate;marker designation: thr - threonine; uv-r - ultraviolet-resistance;str-d - streptomycin-dependence.

All cultural methods and media applied in experiments with P. mirabilis have been described elsewhere (Böhme 1961a); transduction experiments have been carried out according to Böhme (1963).

UV-irradiation: a low pressure mercury vapor lamp (Solimed) has been used; dose-rate: 50 ergs/mm^2 per second. Bacteria were irradiated in phosphate buffer (pH 7,0), diluted in buffer and plated immediately. Irradiation and plating was done in dim yellow light; incubation 24 h in the dark.

Mutagenic treatment: EMS treatment; 0,25 mol; 30 min.

Chemicals: Mitomycin C from Sigma Chemical Company, kindly supplied by Dr. T.H. Yost, Amherst, Mass. Streptomycin sulfate from Institut für Mikrobiologie und expt. Therapie, Jena; Oxytetracycline from VEB Jenapharm, Jena. EMS was synthesized and kindly supplied by Dr. Sieber, Jena.

Results

Isolation of the uvr$^-$ mutant PG 667: This mutant has been isolated after mutagenic EMS treatment of strain PG 273 by indirect selection for mitomycin sensitivity (Okubo and Romig 1965). EMS treated cells were washed, diluted in nutrient broth, grown for 4 h under aeration and plated on nutrient agar. After 18 h incubation the colonies were replicated onto a nutrient agar plate and a nutrient agar plate containing 0,15 γ/ml mitomycin. After further incubation (12 h) all colonies which failed to grow on mitomycin agar were picked up, purified by repeated single colony isolation and compared with PG 273 for their UV-sensitivity. By this method several UV-sensitive mutants have been isolated; all further experiments to be reported here have been carried out with mutant PG 667.

UV-sensitivity of PG 667. The UV survival curves of the uvr$^+$ strain PG 273 and the uvr$^-$ mutant PG 667 are shown in Fig. 1. As compared with PG 273 the sensitivity of PG 667 is increased by approximately a factor of 5. This is a lower degree of sensitization than that in typical excision-repair mutants of E. coli (Boyce and Howard-Flanders 1964) or B. subtilis (Okubo and Romig 1965). However, the reaction of PG 667 on treatment with alkylating agents shows that PG 667 represents a similar mutant type: the sensitivity to di- (2-chloroethyl)

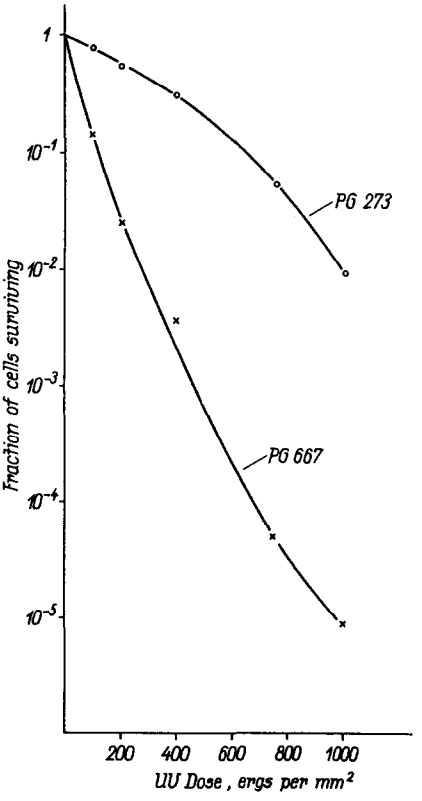

Fig. 1. The fraction of cells of PG 273 (uvr^+) and PG 667 (uvr^-) surviving UV irradiation

methylamine (HN 2) is increased to the same degree as UV sensitivity, whereas there is no difference in sensitivity to EMS between PG 273 and PG 667 (Böhme, in preparation). This result corresponds to the HN 2 sensitivity of UV-sensitive mutants of E. coli (Haynes, Patrick and Baptist 1964; Kohn, Steigbigel and Spears 1965) and to the differential reaction of an UV-sensitive mutant of B. subtilis on treatment with mono- and bifunctional alkylating agents (Reiter and Strauss 1965).

Spontaneous mutability of PG 273 and PG 667. Spontaneous mutability of the UV-resistant and UV-sensitive strains have been compared with respect to the following mutational changes:

mutation from streptomycin-sensitivity (str-s) to -resistance (str-r); from oxytetracycline-sensitivity (otc-s) to -resistance (otc-r); reversion from threonine-auxotrophy (thr^-) to -prototrophy (thr^+). The results are given in Table 1.

Table 1.

Frequency of spontaneous mutants in PG 273 and PG 667

Mutational change		Mutants per 10^8 plated bacteria	
		PG 273	PG 667
str-s	str-r	0,028	1,6
otc-s	otc-r	0,32	18,4
thr^-	thr^+	0,22	31,3

Bacteria in the logarithmic phase were washed once in saline (0,9 %) and plated onto each of the following media: nutrient agar containing 30 γ/ml streptomycin; minimal agar containing 20 /ml threonine plus 5 γ/ml oxytetracycline; minimal agar. Incubation 24 h, 37°C.

In a further experiment the frequency of various auxotrophic mutants has been determined in both strains. For this purpose colonies grown on nutrient agar were replicated onto each of a series of minimal agar plates containing threonin and of nutrient agar, respectively. No auxotrophic mutants have been detected among 6240 colonies of strain PG 273, whereas 8 mutants with different amino acid requirements have been found among 6960 colonies of strain PG 667.

These results offer proof for a drastically increased spontaneous genetic instability of the UV-sensitive mutant PG 667. This was confirmed by the analysis of the mutational behaviour within the genome of PG 667 of the allele str-d-3, which is well known with respect to its mutability (Böhme 1961a; Mates 1964). This allele has been transferred by transduction from the streptomycin-dependent strain PG-Sb-3 into PG 273 and PG 667. Three streptomycin-dependent transductants were isolated from each of the two crosses and the frequency of the spontaneous reversion to streptomycin-independence was determined. Table 2 shows the result of a typical experiment. Within the genome of PG 273

Table 2

Frequency of spontaneous revertants of streptomycin-dependent transductants of PG 273 and PG 667

Transductants from cross:	Isolate No.	spont. revertants/10^8 cells plated
PG 273 x PG Sb-3	19	12,7
	24	12,0
	30	17,6
PG 667 x PG-Sb-3	61	538,8
	70	2680,0
	87	821,4

The streptomycin-dependent transductant strains were grown overnight in nutrient broth with 50 γ /ml streptomycin, washed once in nutrient broth and plated on nutrient agar without streptomycin; incubation 72 h,

str-d-3 exhibits the normal frequency of revertants (10-20/10^8). The spontaneous mutability of this allele and/or the suppressor su-str (Böhme 1961b; Hofemeister and Böhme 1967), however, is greatly increased when transferred into the genome of the uvr^- mutant.

Discussion

The results reported above may be interpreted as follows: the mutation determining the increased UV sensitivity of PG 667 causes a defect in a repair system which in the non-mutated cell can repair a part of the UV-induced DNA damage. The same mechanism may repair also such changes which are induced in the normal environment of the cell, e.g. without mutagenic treatment, and which in case of missing repair cause spontaneous mutations. Thus the uvr^- mutation of PG 667 simulates as to its effects a mutator gene.

In addition to their significance for the repair of induced genetic lesions the biochemical mechanisms underlying these repair processes seem to be engaged in the process of genetic recombination (Clark and Margulies 1965). The possibility of repair of spontaneous changes of the genetic material reported above underlines further the biological significance of the repair mechanisms.

Note: After completion of the manuscript I was informed by

Dr. R. B. Chesin (Kurcatov Institute of Atomic Energy, Moscow), that in his laboratory Dr. Prosorov and coworkers isolated a rec⁻ mutant of B. subtilis which also shows increased spontaneous mutability. (Prosorov, A.A. et al. Dokl. Akad. Nauk, USSR, 1967 in press).

Acknowledgement. I am indebted to Miss B. Wuttky for valuable technical assistance.

References

Böhme, H. Biol. Zblt. 80, 5 (1961a).

– Z. Vererbungsl. 92, 197 (1961b).

– Biochem. Biophys. Res. Comm. 10, 60 (1963).

Boyce, R.P., and Howard-Flanders, P. Z. Vererbungsl. 95, 345 (1964).

Clark, A.J., and Margulies, A.D. Proc. Nat. Acad. Sci. (U.S.) 53, 451 (1964)

Hanawalt, Ph.C., and Haynes, R.H. Biochem. Biophys. Res. Comm. 19, 462 (1965).

Harm, W. Biol. Zblt. (1967) (in press).

Haynes, R.H., Patrick, M.H., and Baptist, J.E. Rad. Res. 22, 194 (1964).

Hill, R. Photochem. Photobiol. 4, 563 (1965).

Hofemeister, J. and Böhme, H. Molec. Gen. Genetics 1967 (in press).

Kohn, K.W., Steigbigel, N.H., and Spears, C.L. Proc. Nat. Acad. Sci. (U.S.) 53, 1154 (1965).

Mates, M., Z.f. Allgem. Mikrobiologie 4, 22 (1964).

Okubo, S., and Romig, W.R.J. Mol. Biol. 15, 440 (1965).

Reiter, H., and Strauss, B. J. Mol. Biol. 14, 179 (1965).

Witkin, E.M. Science 152, 1345 (1966).