MUTATION IN *ESCHERICHIA COLI* DURING PHOTODYNAMIC INACTIVATION AND SUBSEQUENT HOLDING IN BUFFER

Sreeradha BASU and B. BAGCHI

Department of Physics, Calcutta University College of Science, 92, Acharya Prafulla Chandra Road, Calcutta-700 009, India

Received 21 September 1978

1. Introduction

Acridine dyes interact or intercalate with DNA and can cause frameshift mutation or insertion mutation during replication or recombination [1]. In the presence of light guanine is destroyed producing a nick in the single strand; when the nicks appear in both the strands almost opposite each other double strand breaks are produced [2–4]. These damages can cause an enhancement of the mutations observed in the dark. Results similar to expectation were obtained by many workers in phage-dye or cell-dye systems [5–10]. However, the mechanism of such mutation is still not fully understood. Mutation induction in *Escherichia coli* in response to ultraviolet radiation and a number of other mutagens, that damage DNA or interrupt its replication is currently explained in terms of an errorprone repair mechanism. Two hypotheses, one based on 'inducibility' of error-prone repair (SOS repair), and another on 'immunity' of newly synthesised DNA to constitutive error-prone repair are current to explain the control of the error-prone activity [11–13]. No attempt was made to interpret the results of mutation study during photodynamic inactivation in the light of the above hypotheses. Such an error-prone pathway was suggested [14] to be operative in the repair of proflavine-sensitised, visible light-irradiated ϕX 174 DNA.

Induction of mutation from histidine requirement to histidine independence in an auxotrophic strain of *E. coli* B/r during acriflavine-sensitised visible-light irradiation are reported here. The effect of post-irradiation liquid holding on the frequency of mutation are investigated. The results are finally interpreted on the basis of low ultraviolet enhancement of mutation.

Address correspondence to B. Bagchi

2. Materials and methods

2.1. *Strain, media and buffer*

The cell strain used was *E. coli* B/r (*hcr*$^+$ *fil*$^-$ *exr*$^+$ *thy*$^-$ *his*$^-$). The cells were collected from an overnight culture grown from an isolated single colony in nutrient broth medium (peptone 1%, Lab Lemco 1%, NaCl 0.5%, pH 7.4). Both for survival and mutation assay, semi-enriched agar plates were used. These contained per litre: 10.5 g K_2HPO_4; 4.5 g KH_2PO_4; 1 g $(NH_4)_2SO_4$; 0.5 g sodium citrate, 5 mg thiamine; 2 mg thymine; 0.2 g $MgSO_4$; 0.4% glucose; 4%, v/v, nutrient broth; and 1% Difco minimal agar. For cell washing and dilution, M9 buffer was used.

2.2. *Dye sensitisation and irradiation*

The procedure for dye sensitisation and irradiation with visible light has been described as in [15]. The cells were sensitised with 15 μg/ml acriflavine in the dark for 45 min at 37°C. The irradiation was done with an electronic flash gun used for ordinary photography. Each flash corresponded to an average fluence of 9.5×10^2 J.m^{-2} under the experimental condition used in the present study. The dose of visible radiation are represented in terms of the number of flashes. For ultraviolet irradiation a GE 15 W germicidal lamp having peak emission at 253.7 nm was used. The dose was reduced by inserting suitable

Elsevier/North-Holland Biomedical Press

absorbers in the light path and the resulting fluence was determined by actinometry. All irradiations were performed at 0°C. The cell suspension was continuously stirred during irradiation.

2.3. *Scoring of mutation from histidine requirement to histidine independence*

In experiments to measure induced reversion to amino acid independence in the auxotrophic strain of bacteria, the cells were treated with the damaging agent, concentrated to an appropriate value and plated on minimal agar partially enriched with 4% nutrient broth. When a highly diluted culture was plated for counting survivors, the few *his*$^-$ cells on the plate were capable of multiplying sufficiently to produce visible colonies. For detecting *his*$^+$ revertants, a low dilution was used. The very large number of *his*$^-$ cells present exhaust the nutrient before any cell can produce a visible colony. The *his*$^+$ revertants being allowed a period of growth on the plate could express the mutation in a functional gene product and grew to large colonies on a barely visible lawn of *his*$^-$ cells.

3. Results

3.1. *Induction of mutation during irradiation with visible light and subsequent holding in buffer in acriflavine-sensitised* E. coli *B/r cells*

E. coli B/r cells were sensitised with 15 μg/ml acriflavine in the dark and were irradiated with visible light. The cells at each dose were concentrated to ~10^7 viable cells/ml (this could be done from the knowledge of photodynamic inactivation rate of the cells), and were plated directly for mutation scoring. For survival assay the irradiated cells were plated after proper dilution. The number of revertants (*his*$^-$ to *his*$^+$) per survivor are plotted in fig.1 as a function of the dose expressed as the number of flashes. The spontantaneous level of mutation was subtracted from the number of mutants at each level. The OF point gives the number of mutations (12.5 mutants/10^7 survivors) caused by acriflavine alone in the dark. Figure 1 is a linear plot. The slope of the log of mutation frequency versus log of the dose gives the nature of the dependence of mutation frequency on the dose of visible light. It has a value very near to unity (= 0.95).

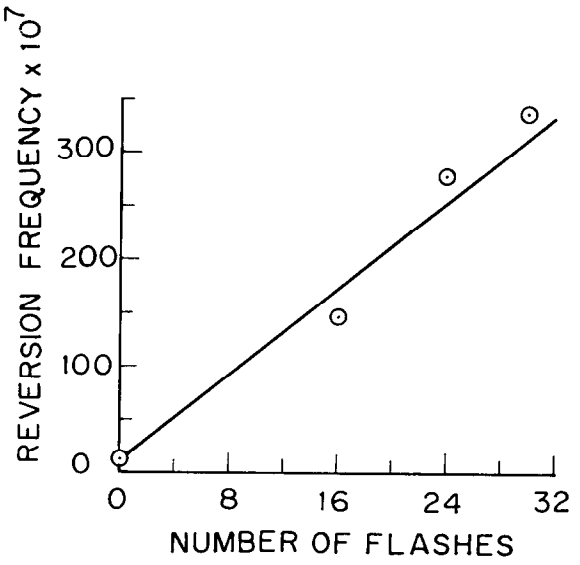

Fig.1. Induction of mutation from *his*$^-$ to *his*$^+$ during visible light irradiation of acriflavine-sensitised *E. coli* B/r cells. At each dose, the cells were concentrated to ~10^7 viable cells/ml and the number of revertants and survivors scored on semi-enriched agar plates. Each point is the mean of ⩾ 3 readings. The level of spontaneous mutation (7 revertants/10^7 cells) was subtracted from each reading.

In another experiment, the cells inactivated to different levels were diluted 100 times with M9 buffer and kept in the dark at 37°C. At various times portions of the culture were withdrawn, washed, concentrated to ~10^7 survivors/ml, and mutation and survival assayed as usual. Reversion frequency increased as a function of the holding time. Figure 2 gives a typical result for cells inactivated with a visible light fluence corresponding to 16 flashes.

3.2. *Effect of low dose of ultraviolet light on the mutation frequency of photodynamically-treated cells*

Cells, inactivated to the 16 F level of survival were held in buffer for 2 h in the dark at 37°C. These were then harvested, washed and concentrated to 10^7 cells/ml and irradiated with low doses of ultraviolet light. The frequency of *his*$^+$ revertants were determined as usual on semi-enriched agar plates. Mutation frequency as a function of ultraviolet fluence is represented in fig.3. The logarithmic slope of the curve was again

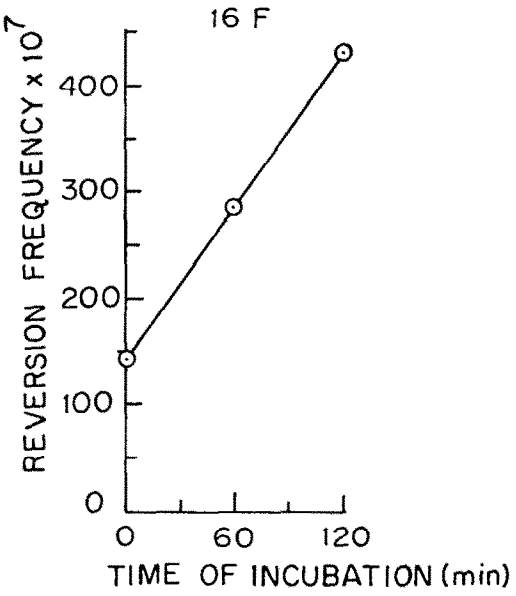

Fig.2. Increase of the mutation frequency of cells, photodynamically inactivated to 16 F level and diluted 100 × in buffer, as a function of the time of holding at 37°C in the dark. The procedure for the scoring of mutations is the same as in fig.1.

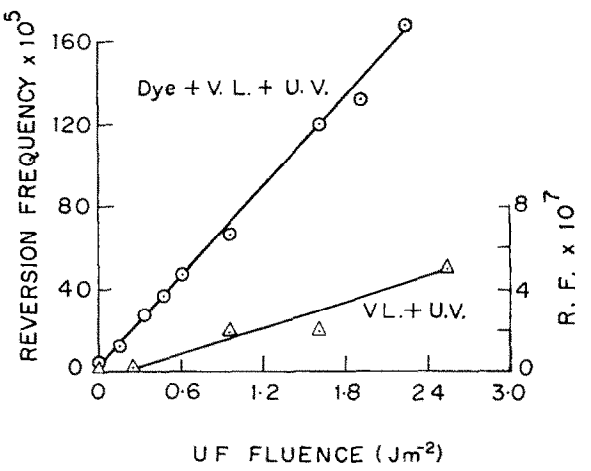

Fig.3. Mutagenic effect of ultraviolet radiation on the photodynamically-treated cells. Cells in fig.2 after 2 h of holding were concentrated to 10^7 survivors/ml and were irradiated with low doses of ultraviolet light. For mutation scoring 10^2 times dilution was necessary. Number of mutants/10^5 survivors are plotted against the ultraviolet fluence. Lower curve in this figure gives the increase in the reversion frequency (R.F.) as a function of the ultraviolet dose of cells which were not dyesensitised but were pre-irradiated with 16 flashes of visible light.

almost equal to unity (= 0.93) signifying a linear dependence between the dose and mutation frequency.

The lower curve in fig.3 represents a control experiment done with undyed cells. The cell culture was irradiated with 16 flashes of visible light prior to ultraviolet irradiation. The reversion frequency (R.F.) is shown in the right hand scale of the abscissa. It is seen that the effect of visible light alone on low ultraviolet mutagenesis is almost negligible compared to that by dye plus visible light.

4. Discussion

In *E. coli* B/r cells a high level of mutation takes place both during dye-sensitised irradiation and subsequent holding in buffer at 37°C in the dark. The high level of mutation induction in this excision repair-proficient strain possibly implicates that most of the damages in photodynamically-treated cells are refractory to repair by an error-free excision repair pathway. An extreme high level of mutation in *E. coli* irrespective of whether these are wild-type, *polA*, *recA* or *exrA* has been observed in [17] by growing the thymine-requiring cells in the presence of a trace amount of thymidine or thymine. This high level of mutation was possibly due to the introduction of replication errors. However, for unexcisable lesions, as are produced by MMS or dichlorovos, there was no difference observed in mutation induction between WP2 wild-type or WP2 *uvrA* cells [18]. For phage *Kappa* of *Serratia*, photodynamic production of mutation has been found [19] to be independent of the presence of *hcr*$^+$ or *hcr*$^-$ phenotypes.

The slope of log of mutation frequency versus log of fluence calculated from fig.1 has a slope very near to unity indicating a linear dependence. Such a linear dependence can be found if this slope is computed from the results of other workers (see [10], fig.2). Normally ultraviolet mutagenesis follows a dose squared response; but for X-rays increase of induced lysogen follows linear kinetics [16]. On the basis of SOS hypothesis [11,12,16], the linear increase in mutation with dose should mean that the damage

produced by a single photon during dye-sensitised irradiation serves both as 'SOS-inducing' and 'SOS-mutable' lesion. If after irradiation, the cells are held in liquid before plating, the mutation frequency increases. In [15] we have shown that during holding in buffer many dye molecules come out from inside the cell. This might open up new regions of DNA which were previously refractory to any kind of repair (S.B., B.B., unpublished results). The possibility that dye molecules might stand in the way of repair of premutational lesions was also speculated [20]. Enhancement of mutation from auxotrophy to prototrophy during liquid holding was observed from all levels of survival. The results from one level only are shown in fig.2. An increase of mutation during liquid holding (at 25°C) following ultraviolet irradiation was observed in *Aspergillus nidulans* [21] and in the *E. coli uvr*$^+$ strain [22]. However, on the basis of expulsion of the dye molecules from inside the cell one would expect to observe saturation after some time. This was not observed in the present case.

Experiments with low dose of ultraviolet light are shown in fig.3. Since the starting material are the photodynamically-inactivated cells held in buffer for 2 h at 37°C, these contain very little or no dye, hence the increase of mutation frequency cannot be explained on the basis of enhancement of ultraviolet mutation by dye molecules as was observed in [23]. Visible light itself might have some mutagenic effect [24] and it can produce some lesions in the cell which are mutable and can contribute to overt mutagenesis during subsequent ultraviolet irradiation [16]. However, within the dose range used in the present study such an effect was not very pronounced (lower curve in fig.3). Thus the low ultraviolet mutagenesis is possibly due to the fact that most of the damages caused by photodynamic inactivation and the sites freed from bound dye during liquid holding are mutable. The photoproducts introduced by ultraviolet light in the damaged DNA are not excised. And the two types of damage together might contribute to the high level of reversion frequency.

The slope of log frequency versus log dose is again very near to unity. In a cell culture, where SOS induction is already there, such a response is expected. However, the conditions chosen here are not the known SOS-inducing treatments. Also, the inducing error-prone repair cannot account for such an enormous increase in the number of mutants in an excision proficient cell by such a low dose of ultraviolet light, nor can it account for the rapid enhancement of mutation during holding in buffer.

Results showing deviation from a single mode of mutagenesis, linked with other SOS functions, are gradually accumulating [13,25–30]. Most remarkable among these are the results in [22,29] who observed that many post-ultraviolet mutations to *str*^{r} arises during liquid holding and that in a WP2 *uvrA tif* 1 *sfi*$^-$ strain of *E. coli* mutation after γ-ray irradiation increases many times even after 2 h of post-irradiation incubation when the cells are almost completely repaired, and have undergone division for at least two cycles. Bridges [13,29] and Green (see appendix to [22]) have tried to explain these results in particular, the high level of mutation in his last experiment as a 'loss of immunity' of the newly synthesised DNA to error-prone repair. We suspect that some similar phenomena might be operative here also.

Acknowledgements

The authors thank Dr S. B. Bhattacharya of the Saha Institute of Nuclear Physics, Calcutta for the gift of the *E. coli* B/r strain. Particular thanks are due to the Librarian and other staff members of the Bose Institute, Calcutta for providing excellent library facilities. Technical assistance of Mr B. Banerjee is also acknowledged. The work was supported by a grant from the University Grants Commission, Govt. of India.

References

[1] Lochmann, E-R. and Micheler, A. (1973) in: Physico-chemical Properties of Nucleic Acids (Duchesne, J. ed) vol. 1, pp. 223–267, Academic Press, London, New York.
[2] Freifelder, D. and Uretz, R. B. (1966) Virology 30, 97–103.
[3] Sastry, K. S. and Gordon, M. P. (1966) Biochim. Biophys. Acta 129, 32–41.
[4] Jacob, H-E. (1971) Photochem. Photobiol. 14, 743–745.
[5] Kaplan, R. W. (1949) Nature 163, 573–574.
[6] Webb, R. B. and Kubitschek, H. E. (1963) Biochem. Biophys. Res. Commun. 13, 90–94.
[7] Nakai, S. and Saeki, T. (1964) Genet. Res. 5, 158–161.

[8] Zampieri, A. and Greenberg, J. (1965) Mut. Res. 2, 552–556.
[9] Calberg-Bacq, C. M., Delmelle, M. and Duchesne, J. (1968) Mut. Res. 6, 15–24.
[10] Ito, T. (1973) Mut. Res. 20, 201–206.
[11] Radman, M. (1975) in: Molecular Mechanisms for Repair of DNA (Hannawalt, P. C. and Setlow, R. B. eds) pt A, pp 355–367, Plenum Press, London, New York.
[12] Witkin, E. M. (1975) in: Molecular Mechanisms for Repair of DNA (Hannawalt, P. C. and Setlow, R. B. eds) pt A, pp. 369–378, Plenum Press, London, New York.
[13] Bridges, B. A. (1977) Mut. Res. 44, 149–164.
[14] Calberg-Bacq, C. M., Siquet-Descans, F. and Piette, J. (1977) Photochem. Photobiol. 26, 573–579.
[15] Bagchi, B. and Basu, S. (1978) Photochem. Photobiol. in press.
[16] Witkin, E. M. (1976) Bacteriol. Rev. 40, 869–907.
[17] Bressler, S. E., Mosevitsky, M. I. and Vyacheslavov, L. G. (1973) Mut. Res. 19, 281–293.
[18] Bridges, B. A., Mottershead, R. P., Green, M. H. L. and Gray, W. J. H. (1973) Mut. Res. 19, 295–303.
[19] Brendel, M. (1973) Mol. Gen. Genet. 120, 171–180.
[20] Brendel, M. (1970) Mol. Gen. Genet. 108, 303–311.
[21] Wohlrab, G. and Tuveson, R. W. (1969) Mut. Res. 8, 265–275.
[22] Bridges, B. A. and Mottershead, R. (1961) Mut. Res. 13, 1–8.
[23] Witkin, E. M. (1961) J. Comp. Cell. Physiol. 58 (suppl.) 135–150.
[24] Webb, R. B. and Malina, M. M. (1967) Science 156, 1104–1105.
[25] Kada, T., Doudney, C. O. and Haas, F. L. (1961) Genetics 46, 683–702.
[26] Eitner, G. (1977) Mut. Res 44, 299–304.
[27] Witkin, E. M. and Wermundsen, I. E. (1977) Mol. Gen. Genet. 156, 35–39.
[28] Jeggo, P., Defais, M., Samson, L. and Schendel, P. (1977) Mol. Gen. Genet. 157, 1–9.
[29] Bridges, B. A. (1977) Mol. Gen. Genet. 151, 115–120.
[30] West, S. C. and Emmerson, P. T. (1977) Mol. Gen. Genet. 151, 57–67.