Aust. J. Biol. Sci., 1974, **27**, 231–4

Mutagenesis by Photoactivation of Chlorpromazine, a Tranquilizer of the Phenothiazine Group

D. G. MacPhee and F. Paula Imray

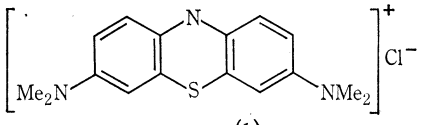
Department of Genetics, La Trobe University, Bundoora, Vic. 3083.

Abstract

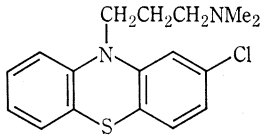
The tranquilizer chlorpromazine, in combination with visible light or near-u.v. radiation, can cause base-pair substitution mutations and frameshift mutations in *Salmonella typhimurium*. The mutagenic damage is subject to repair by the bacterial excision–repair system.

Introduction

During a study of photodynamic mutagenesis in bacteria, we found that mutations can be induced by visible light irradiation of cells of *Salmonella typhimurium* that had previously been sensitized by treatment with methylene blue (1) or toluidine blue (Imray and MacPhee, unpublished data). Structural similarities between these dyes and tranquilizers of the phenothiazine group led us to suspect that compounds of the latter group might also be capable of sensitizing bacteria to this type of mutagenesis. We now report that mutations can be induced by visible light or near-u.v. irradiation in cells of *S. typhimurium* that have previously been treated with chlorpromazine (CPZ) (2), a widely used tranquilizer of the phenothiazine group (Usdin 1971). The



(1)



(2)

mutations so induced can be either frameshift mutations (in which the reading frame of mRNA is shifted by the addition or deletion of a base from the corresponding DNA) or base-pair substitution mutations. Both types of mutation appear to result from DNA lesions which are susceptible to repair by the bacterial excision–repair system.

Methods

Two *S. typhimurium* strains that were originally developed by Dr B. N. Ames (Ames 1971) for detecting and classifying mutagens in a simple back-mutation test (reversion from histidine requirement to growth on minimal medium) were used in the study. One strain (LT2 *hisG46*) has a base-pair change in the *hisG* gene and is used to detect mutagens which cause base-pair substitutions, while the other (LT2 *hisD3052*) has a frameshift mutation in the *hisD* gene and is used for detecting frameshift mutagens (Ames 1971). Strains DG2604 and DG2594, which were selected as spontaneous chlorate-

resistant mutants of strains LT2 *hisG46* and LT2 *hisD3052* respectively, and which have deletions through the *chlA*, *uvrB*, *bio* and *gal* genes (Stouthamer 1969; Ames 1971), were also used. As a result of their *uvrB* deletions, these strains have a defective excision–repair system and therefore resemble similar strains described by Ames in being much more sensitive to mutagens which react with DNA to form repairable lesions (Ames 1971). Because the deletions in our strains extend through the *gal* genes, these bacteria have an incomplete, galactose-deficient lipopolysaccharide coat on their cell surface and show increased permeability to a number of substances, including antibiotics (Roantree *et al.* 1969).

The methods used for growing bacteria and for visible light irradiation with a 500-W Photoflood bulb have been previously described (Imray and MacPhee 1973). For near-u.v. irradiation, two General Electric F15 T8 BLB black light tubes were used. Prior to irradiation, cells suspended in high ionic strength buffer (Harm 1968) were sensitized by shaking with CPZ (100 μg/ml) for 30 min at 37°C in the dark. The sensitized cells were then irradiated in open Petri dishes and were agitated mechanically during irradiation. To determine the number of HIS^+ revertants, 0·1-ml samples of the irradiated suspensions were spread undiluted on minimal salts medium (Vogel and Bonner 1956) supplemented with a trace of nutrient broth (Oxoid, 2·5% v/v) and, when required for the strains with deletions through *bio*, an excess of biotin (0·025 μg/ml). Survival was determined by diluting the irradiated suspensions in saline to about 10^3 survivors/ml and plating 0·1-ml samples of this dilution on the same medium as was used to select revertants. (HIS^- cells form small colonies on this medium when plated at high dilution, permitting assay of survival and reversion on the same medium.) All plates were incubated at 37°C in the dark for three days before counting.

Results and Discussion

The results of experiments in which CPZ-sensitized cells of *S. typhimurium* were irradiated with visible or near-u.v. light are shown in Fig. 1. No significant increase in reversion frequency was caused by either visible or near-u.v. light in CPZ-sensitized cells of the base-pair substitution mutant strain *hisG46* (results not shown), but both types of irradiation increased the reversion frequency of its excision-deficient mutant strain DG2604 very significantly. In tests for frameshift mutagenesis both types of irradiation increased the reversion frequency of both strains tested, and again the effect was much greater in the excision-deficient strain DG2594 than in the excision-proficient parent strain *hisD3052*. In control experiments, a very slight (1·5–2-fold) increase in reversion frequency was observed following near-u.v. irradiation of unsensitized cells of strain DG2594, but no other significant increases in reversion frequency were found in any of the strains following treatment with CPZ alone, visible light alone, or near-u.v. light alone. The increased susceptibility of the deletion strains DG2594 and DG2604 to the mutagenic effects of CPZ-sensitized irradiation appears to result from their excision deficiency, and is not simply the result of an increase in cellular permeability to CPZ caused by their *gal* lesions, since it was found that strains with deletions of *uvrB* but not *gal* gave essentially the same reversion frequencies as the *gal–uvrB* deletion strains DG2594 and DG2604.

We therefore conclude that visible light or near-u.v. irradiation of CPZ-sensitized bacteria leads to the formation in DNA of lesions that can give rise to either base-pair substitution mutations or frameshift mutations. In repair-proficient bacteria a proportion of these lesions can be excised and the resulting gaps repaired by the excision–repair system, whereas in the absence of excision the lesions remain in the DNA and retain the potential to cause either base-pair substitution mutations or frameshift mutations at some later stage.

Many compounds known to be mutagenic for bacteria are carcinogenic, and recently a number of known carcinogens have been shown to be frameshift mutagens in bacteria (Hartman *et al.* 1971; Ames *et al.* 1972*a*, 1972*b*). Thus the fact that CPZ

in combination with visible light or near-u.v. radiation induces frameshift mutations as well as base-pair substitution mutations gives cause for concern, especially since it has recently been reported that near-u.v. irradiation of Chinese hamster cells sensitized with CPZ at 3·5 μg/ml causes chromosome abberations and an increase in the frequency of mutations to 8-azaguanine resistance and 6-mercaptopurine resistance (Kelly-Garvert and Legator 1973). In addition, it has been reported that prolonged exposure to CPZ produces changes in those cells of the body that can be readily affected by light. These changes are non-genetic (e.g. an unusual skin discoloration which has

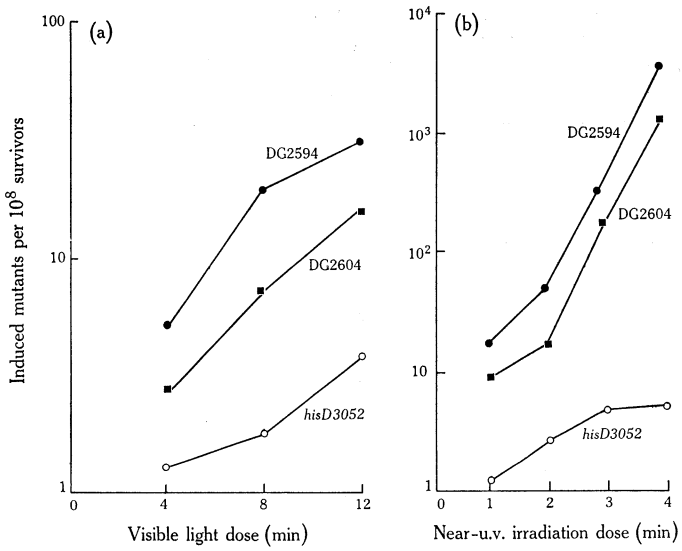


Fig. 1. Induction of HIS$^+$ revertants in chlorpromazine-sensitized *S. typhimurium* cells by visible light (*a*) and near-u.v. irradiation (*b*). Chlorpromazine was used at a concentration of 100 μg/ml. For (*b*), the strains were exposed to the black light source at a distance of 9 cm. Strain *hisG46* gave no induced revertants under the conditions used and is therefore omitted. Strain DG2604 is LT2 *hisG46* (*chlA uvrB bio gal*) (deletion), and reverts by base-pair substitution. Strain DG2594 is LT2 *hisD3052* (*chlA uvrB bio gal*) (deletion); both DG2594 and *hisD3052* revert by frameshifts.

been attributed to a melanin–phenothiazine complex formed when photo-induced free radicals of CPZ couple with melanin in the skin of susceptible individuals), but they do provide evidence that CPZ can actually be affected by visible light in human cells (Huang and Sands 1967; Carr 1968). It therefore seems important that the levels of CPZ that are found in skin cells of individuals undergoing prolonged therapy with high doses of CPZ be determined. In the meantime, we suggest that exposure to intense sunlight (which has a considerable near-u.v. component) or any other intense visible light source be considered a potential hazard for such individuals.

Acknowledgments

This work was supported by the Australian Research Grants Committee and by a Commonwealth Postgraduate Award to Paula Imray. We thank Dr B. N. Ames for providing strains, and Dr V. Krishnapillai, Professor B. W. Holloway and Professor A. J. Pittard for their comments on the manuscript.

References

Ames, B. N. (1971). In 'Chemical Mutagens: Principles and Methods for their Detection'. (Ed. A. Hollaender.) Vol. 1, p. 267. (Plenum: New York.)
Ames, B. N., Gurney, E. G., Miller, J. A., and Bartsch, H. (1972*a*). *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3129.
Ames, B. N., Sims, P., and Grover, P. L. (1972*b*). *Science* (*Wash. D.C.*) **176**, 49.
Carr, C. J. (1968). *Agressologie* **9**, 249.
Harm, W. (1968). *Biochem. Biophys. Res. Commun.* **32**, 350.
Hartman, P. E., Levine, K., Hartman, Z., and Berger, H. (1971). *Science* (*Wash. D.C.*) **172**, 1058.
Huang, C. L., and Sands, F. L. (1967). *J. Pharm. Sci.* **56**, 259.
Imray, F. P., and MacPhee, D. G. (1973). *Mol. Gen. Genet.* **123**, 289.
Kelly-Garvert, F., and Legator, M. S. (1973). *Mutat. Res.* **21**, 101.
Roantree, R. J., Kuo, T. T., MacPhee, D. G., and Stocker, B. A. D. (1969). *Clin. Res.* **17**, 157.
Stouthamer, A. H. (1969). *Antonie van Leeuwenhoek J. Microbiol. Serol.* **35**, 505.
Usdin, E. (1971). *Critical Rev. Clin. Lab. Sci.* **2**, 347.
Vogel, H. J., and Bonner, D. M. (1956). *J. Biol. Chem.* **218**, 97.

Manuscript received 5 November 1973