BIOLOGIA PLANTARUM 37 (1): 9-14, 1995

The influence of incorporated bromodeoxyuridine on mutagenicity testing by sister chromatid exchange induction in *Vicia faba* root tip cells

R. VESELSKÁ, P. KUGLÍK AND J. RELICHOVÁ

Department of Genetics and Molecular Biology, Faculty of Sciences, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

Abstract

The induction of sister chromatid exchanges (SCEs) in *Vicia faba* root-tip cells after short-term (2 h) and long-term (24 h) treatments with alkylating agents (N-methyl-N-nitrosourea, ethyl methanesulphonate) and maleic hydrazide was studied. The primary roots were treated with mutagens before or after 5-bromodeoxyuridine (BrdU) incorporation into DNA and the influence of mutagen application on SCE induction in the cells with non- and BrdU-substituted DNA were compared. The results showed that the presence of BrdU in DNA did not noticeably affect the SCE induction by alkylating agents when compared with non-substituted chromosomal DNA. On the contrary, application of maleic hydrazide after the incorporation of BrdU into DNA strongly increased the rate of SCEs. The lowest limit concentrations of mutagens capable of significantly increasing SCE frequency in the cells with non-substituted DNA after the long-term treatment were estimated.

Key words: ethyl methanesulphonate, faba bean, maleic hydrazide, N-methyl-N-nitrosourea

Introduction

The analysis of sister chromatid exchanges (SCEs) is believed to be one of the most sensitive cytological methods for detecting potential mutagenic and clastogenic agents (for review see Tucker *et al.* 1993). It is known that many DNA damaging chemicals induce SCEs at doses far below those that can induce chromosome aberrations (Scheglova and Chebotarev 1985). However, only a few chemical mutagens have been evaluated for their capability to induce SCEs in plant root

Received 23 Marz 1994, *accepted* 19 April 1994.

Abbreviations: BrdU - 5-bromodeoxyuridine; dThd - deoxythymidine; EMS - ethyl methanesulphonate; FdU - 5-fluorodeoxyuridine; MH - maleic hydrazide; MNU - N-methyl-N-nitrosourea; SCE - sister chromatid exchange; Urd - uridine.

Acknowledgments: This work was supported by grants from the Ministry of Health (Z/202-3) and Ministry of Education (G-465) of the Czech Republic.

meristems (Schvartzman 1987). In all cases, the treatments took place only for 2 h or less after the incorporation of BrdU into DNA, *i.e.* between the first and the second replication cycle (Uggla and Natarajan 1982).

Although *Vicia faba* is probably the most frequently used plant object for the study of the SCE induction by chemical mutagens, there is very little information on whether incorporation of the bromodeoxyuridine (BrdU) into DNA necessary for visualization of SCEs affects the sensitivity of the SCE test system to different chemical agents. In addition, there are insufficient data on SCE frequencies when low levels of various chemical mutagens are used.

For these reasons, we decided to test and compare the formation of SCEs after a short-term (2 h) mutagen treatment applied before or after the incorporation of BrdU into DNA. We also studied the effectiveness of the induction of SCEs after long-term (24 h) mutagen treatment in plant cells with non-substituted DNA. As model chemical mutagens, alkylating agents (EMS, MNU) and maleic hydrazide were used.

Materials and methods

The experimental material in this study consisted of primary root tip cells of *Vicia faba*, cv. Inovec (2n = 12). The method of bean cultivation has been described elsewhere (Kihlman 1975). Seedlings with primary roots 3 to 4 cm long were used for the experiments.

For the differentiation of sister chromatids, the roots were cultivated at 20 °C in the dark, at first for 17 h in a BrdU-medium (100 μM BrdU + 0.1 μM FdU + 5 μM Urd) and then for 21 h in a dThd-medium (100 μM dThd + 5 μM Urd). In order to obtain more cells in metaphase, the roots were exposed for 2 h to 0.05 % colchicine. After 24 h fixation in cold methanol - glacial acetic acid (3 : 1), the standard FPG (fluorescent plus Giemsa) technique as described by Kihlman and Andersson (1984) was performed.

The primary roots were exposed to aqueous solutions containing chemical mutagens N-methyl-N-nitrosourea (MNU) at concentrations 0.1 - 100 μM, ethyl methanesulphonate (EMS) at concentrations 100 - 10 000 μM, maleic hydrazide (MH) at concentrations 0.01 - 10 μM or fresh tap water for control. The mutagen treatment was performed for 2 h (short-term treatment) before or after the cultivation in BrdU-medium or 24 h (long-term treatment) before the cultivation in BrdU-medium.

The SCE frequency was expressed as a mean number of SCEs per S-chromosome of the standard karyotype of *Vicia faba*. After each treatment, 200 - 400 S-chromosomes were analysed for SCEs and the results were statistically evaluated by the comparison of 95 % confidence limits.

Results

Short-term mutagen treatment both before and after BrdU incorporation could significantly increase the yields of SCEs over the baseline level observed in controls.

Generally the SCE frequencies were slightly higher in the cells which were treated with the same concentration of MNU before BrdU-cultivation than in those observed after the cultivation in BrdU-medium (Table 1.). Similar data were obtained after

Table 1. The frequencies of SCEs per S-chromosome (means ± 95 % confidence limits) after short-term mutagen treatment before and after BrdU incorporation.

Mutagen	Concentrations [μM]	SCE/S-chromosome Non-substituted DNA number	increasing [%]	Substituted DNA number	increasing [%]
MNU	100	3.21 ± 0.22	255	3.25 ± 0.18	241
	10	1.99 ± 0.16	158	1.75 ± 0.15	130
	1	1.68 ± 0.14	133	1.26 ± 0.12	93
	control	1.26 ± 0.13	100	1.35 ± 0.10	100
EMS	10 000	2.86 ± 0.22	229	2.77 ± 0.39	207
	1 000	1.88 ± 0.20	150	1.87 ± 0.14	140
	control	1.25 ± 0.13	100	1.34 ± 0.14	100
MH	10	2.26 ± 0.28	153	3.63 ± 0.16	284
	1	1.83 ± 0.28	124	2.24 ± 0.22	175
	0.1	1.67 ± 0.27	113	1.49 ± 0.28	116
	control	1.48 ± 0.24	100	1.28 ± 0.14	100

EMS treatment. On the contrary, when the effect of MH was studied, we found out the yields of SCEs in the cells with BrdU-substituted DNA were mostly higher than in the cells treated before the cultivation in the presence of BrdU.

Long-term application of MNU (1 μM), EMS (1 000 mM), MH (0.1 μM) can increase significantly the SCE frequency in comparison to the control (Table 2.).

Table 2. The frequencies of SCEs per S-chromosome (means ± 95 % confidence limits) after long-term mutagen treatment before and after incorporation.

Mutagen	Concentrations [μM]	SCE/S-chromosome number	increasing [%]
MNU	10	2.53 ± 0.17	178
	1	1.80 ± 0.13	127
	0.1	1.19 ± 0.19	112
	control	1.06 ± 0.14	100
EMS	1 000	2.19 ± 0.24	194
	100	1.57 ± 0.21	139
	control	1.13 ± 0.28	100
MH	1	2.49 ± 0.30	190
	0.1	2.18 ± 0.22	166
	0.01	1.50 ± 0.28	114
	control	1.31 ± 0.20	100

These concentrations are the same (MNU, EMS) or lower (MH) as in the case of short-term treatments. When comparing long- and short-term mutagen treatments before BrdU incorporation was performed, there was approximately the same increase in SCE frequency as when using lower concentrations and longer treatment time.

Discussion

The incorporation of BrdU into DNA has long been known to sensitize cells to both non-ionizing and ionizing radiation (Schvartzman and Tice 1982). Moreover, there are some data indicating that incorporated BrdU affects the sensitivity of certain chemical mutagens in the formation of SCEs (Popescu *et al.* 1980, Morgan and Wolff 1984).

The primary purpose of our work was to determine the effectiveness of SCE induction in non- and BrdU-substituted DNA with the use of alkylating agents (EMS, MNU) and MH in *Vicia faba* root tip cells. Alkylating agents present very potent types of mutagens, which react with nucleophilic sites in DNA: especially the O^6 alkylations are the most prevalent of DNA lesions that lead to SCE formation (Morris *et al.* 1983, Samson and Linn 1987). Maleic hydrazide is a structural isomer of uracil, but its effect on the DNA molecule is not known. Nevertheless, experimental results suggest that MH induces apparently oxidative damage to DNA by the production of oxygen radicals (Heindorff *et al.* 1985).

A comparison of SCE induction after short-term treatment with MNU and EMS shows that, when both the mutagens are applied before the incorporation of BrdU into DNA, the total increase of SCEs is slightly higher than when the mutagen treatments are performed in BrdU-substituted cells. SCE are elicited in response to DNA synthetic activity on a damaged template (Wolff *et al.* 1974). Thus, our results with alkylating agents confirm that incorporated BrdU does not affect the sensitivity of DNA, and the number of SCEs visible in second generation metaphase cells depends on the number of DNA lesions present at the onset of both the first and the second S-phase.

On the contrary, the experiments with short-term application of MH show that the yields of SCEs are higher when mutagen is applied between the first and second cell cycles, *i.e.* after BrdU incorporation into DNA. Thus it seems probable that incorporated BrdU increases the sensitivity of DNA and consequently MH can interact with BrdU-substituted DNA to induce SCEs in *Vicia faba* root tip cells. Although very little information is known about the interactions between BrdU-substituted DNA and chemical agents, similar results have been obtained in animal cells after treatment with aphidicolin (Morgan and Wolff 1984), fluorochrome Hoechst 33258 (Stetka and Carrano 1977) and both cysteamine and cystamine (Speit *et al.* 1980).

When the effects of long-term mutagen treatment were studied, we found that the long-term application of a test compound before BrdU incorporation into DNA could effectively increase the yields of SCEs. These results may indicate that longer

mutagen treatments are more suitable for mutagenicity testing by SCE induction. It is known that the frequency of SCEs can be increased by a variety of environmental chemicals, sometimes at very low concentrations, at which no appreciable chromosome aberrations occur (Perry and Evans 1975). Indeed, when genotoxic activities of the same concentrations of EMS, MNU and MH were compared in our experiments, the SCE test system in *Vicia faba* cells had the same or higher sensitivity than most favorable plant genetic assays - *Tradescantia* or *Arabidopsis thaliana* test systems (in preparation). In conclusion, our results suggest that the formation of SCEs in root tip cells of *Vicia faba* enables one to use the system in screening possible effects of chemical mutagens in plants.

References

Heindorff, K., Aurich, O., Rieger, R., Michaelis, A.: Pretreatment of *Vicia faba* root-tip meristems with hydrazines results in "clastogenic adaptation" to maleic hydrazide. - Mutat. Res. **142**: 183-186, 1985.

Kihlman, B.A.: Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations. - Mutat. Res. **31**: 402-412, 1975.

Kihlman, B.A., Anderson, H.C.: Root tips of *Vicia faba* for the study of the induction of chromosomal aberrations and sister chromatid exchanges. - In: Kilbey, B.J., Legator, M., Nichols, W., Ramel, C.(ed.): Handbook of Mutagenicity Test Procedures. Pp. 531-554. Elsevier Science Publishers, Amsterdam 1984.

Morgan, W.F., Wolff, S.: Effect of 5-bromodeoxyuridine substitution on sister chromatid exchange induction by chemicals. - Chromosoma **89**: 285-289, 1984.

Morris, S.M., Beranek, D.T., Heflick, R.H.: The relationship between sister-chromatid induction and the formation of specific methylated DNA adducts in Chinese hamster ovary cells. - Mutat. Res. **121**: 261-266, 1983.

Perry, P., Evans, H.J.: Cytological detection of mutagen - carcinogen exposure by sister chromatid exchange. - Nature **258**: 121-125, 1975.

Popescu, N.C., Amsbaugh, S.A., DiPaolo, J.A.: Reduced N-methyl-N'-nitro-N-nitrosoguanidine sister chromatid exchange induction in Chinese hamster cells V79 pre-exposed to 5-bromodeoxyuridine. - Chromosoma **76**: 329-338, 1980.

Samson, L., Linn, S.: DNA alkylation repair and the induction of cell death and sister chromatid exchange in human cells. - Carcinogenesis **8**: 227-230, 1987.

Scheglova, E.G., Chebotarev, A.N.: [Comparison of the level of SCE and chromosome aberrations induced by chemical mutagens *in vitro* and *in vivo*.] In Russ.- Tsitolog. Genet. **19**: 89-93, 1985.

Schvartzman, J.B., Tice, R.R.: 5-bromodeoxyuridine and its role in the production of sister chromatid exchange. - In: Sandberg, A.A. (ed.): Sister Chromatid Exchange. Pp. 123-134. Alan R. Liss, New York 1982.

Schvartzman, J.B.: Sister-chromatid exchanges in higher plant cells: past and perspectives. - Mutat. Res. **181**: 127-145, 1987.

Speit, G., Wolf, M., Vogel, W.: Synergistic action of cysteamine and BrdU-substituted DNA in the induction of sister chromatid exchanges. - Chromosoma **81**: 461-471, 1980.

Stetka, D.G., Carrano, A.V.: The interaction of Hoechst 33258 and 5-BrdU-substituted DNA in the induction of sister chromatid exchanges. - Chromosoma **63**: 21-31, 1977.

Tucker, J.D., Auletta, A., Cimino, M.C., Dearfield, K.L., Jacobson-Kram, D., Tice, R.R., Carrano, A.V.: Sister-chromatid exchange: second report of the Gene-Tox program. - Mutat. Res. **297**: 101-180, 1993.

Uggla, A.H., Natarajan, A.T.: SCE in plants. - In: Sandberg, A.A. (ed.): Sister Chromatid Exchange. Pp. 217-233. Alan R. Liss, New York 1982.

Wolff, S., Bodycote, J., Painter, R.B.: Sister chromatid exchanges induced in Chinese hamster cells by UV-irradiation of different stages of the cell cycle: The necessity for cells to pass through S-phase. - Mutat. Res. **25**: 73-81, 1974.