BIOLOGIA PLANTARUM 37 (1): 15-19, 1995

Effects of 3-(2-alkoxyphenylcarbamoyloxy)chinuclidium chlorides on repair-deficient strains of *Chlamydomonas reinhardtii*

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Abstract

The effect of five 3-(2-alkoxyphenylcarbamoyloxy)chinuclidium chlorides (alkoxy = butoxy - octyloxy) on survival of a wild-type strain and repair-deficient strains of *Chlamydomonas reinhardtii* was studied. There was a direct relationship with increased toxic effects in the algal strains as a function of the elongation of the alkyl chain of the alkoxy substituents of the phenylcarbamate acid derivatives. Repair-deficient strains were more sensitive than the wild-type strain. The recombination-deficient strain uvs10 expressed the highest sensitivity to the test agents. This suggests that a gene responsible for recombination repair is involved in an important role in DNA repair of damages induced in *C. reinhardtii* by the phenylcarbamic esters.

Key words: alga, alkoxy substituents, alkyl chain, DNA repair, survival, toxic effect

Introduction

Esters of phenylcarbamic acid exhibit various biological effects, *e.g.* local anaesthetic (Gregáň *et al.* 1992, 1993a), antiarrhythmic (Csöllei *et al.* 1986), anti-ulceric (Beneš *et al.* 1972) and antimicrobial (Čizmárik *et al.* 1987), depending upon the number of carbon atoms in the alkyl chain of the alkoxy group as well as upon the position of the alkoxy group on the benzene ring of the molecule. However, one of the most important effect of phenylcarbamic acid esters is their high algicidal activity caused by the inhibition of chlorophyll synthesis (Mitterhauszerová *et al.* 1991a, b, Kráľová *et al.* 1992a, 1992b, Csöllei 1993). In addition, the esters of phenylcarbamic acid inhibited chlorophyll synthesis in spinach and wheat chloroplasts (Mitterhauszerová *et al.* 1991a, b, Kráľová *et al.* 1992a, b, Csöllei 1993).

The objectives of this research were to compare the toxic effect of five derivatives of phenylcarbamic acid (butoxy - octyloxy), differing in the length of the alkyl chain of the alkoxy substituent, in the wild-type strain and repair-deficient strains of *Chlamydomonas reinhardtii*.

Received 14 June 1994, *accepted* 1 August 1994.

Materials and methods

Algal strains: A wild-type strain of *Chlamydomonas reinhardtii* 137C (mt+) was obtained from Prof. R. Matagne (Department of Botany, University of Liège, Belgium). Repair-deficient mutants, uvs12 (excision-repair-deficient), uvs375 (unknown repair deficiency, but not excision or recombination repair), were isolated after N-methyl-N'-nitro-N-nitrosoguanidine treatment, and uvs10 (recombination-repair-deficient) after UV irradiation from a wild-type strain 137C in our laboratory.

Media and compounds tested: Liquid and solid minimal media were prepared according to Starr (1971). 3-(2-alkoxyphenylcarbamoyloxy)chinuclidinium chlorides (alkoxy = butoxy:C4, M_r = 354.9; pentyloxy:C5, M_r = 368.9; hexyloxy:C6, M_r = 382.9; heptyloxy:C7, M_r = 396.9; octyloxy:C8, M_r = 411.0) were synthetized at Faculty of Pharmacy, Comenius University, Bratislava, Slovak Republic according to Gregáň *et al.* (1993b). All chemicals were dissolved in dimethyl sulfoxide.

Survival curves: After 4 d of stationary cultivation and 2 d of cultivation with continuous shaking (at 25 °C and irradiance 15 W m^{-2} with a 12 h photoperiod) the algal cells were harvested by centrifugation at 2500 *g*. The cells were mixed with the test compounds and treated for 30 min in the dark while shaking. The cells were harvested by centrifugation suspended in distilled water and plated on minimal medium.

To assses a toxic effect of C4-C8 derivatives, survival curves were generated after microscopic survival evaluation (after 5 - 7 d of cultivation under light). For each treatment group more than 1000 cells and colonies were evaluated. This method allows the determination whether cells that failed to form a colony underwent any cell divisions (Vlček *et al.* 1987). Points shown in the figures represent mean values of five consecutive experiments (± 95 % confidence limits).

Results

Toxicity was observed of all test compounds in all strains as a function of the length of the alkoxy component and as a function of increased concentration (Fig. 1). Comparison of survival of single strains treated with butoxy derivative (Fig. 2A), pentyloxy derivative (Fig. 2B), hexyloxy derivative (Fig. 2C), heptyloxy derivative (Fig. 2D) and octyloxy derivative (Fig. 2E) showed that the wild-type strain was the most resistant to derivatives with a longer alkyl chain of the alkoxy group. The excision-repair-deficient strain uvs12 was the most resistant strain after C4 derivative treatment (Fig. 2A). However, after treatment with derivatives with the longer alkoxy substituent (Fig. 2C, 2D, 2E), uvs12 survival was lower than that in the wild type. The recombination-repair-deficient strain uvs10 was the most sensitive strain (Fig. 2A-2E). The strain uvs375 (with unknown repair deficiency) showed lower survival than the wild-type strain, but after treatment with derivatives having a longer substituent (C6-C8) it was less sensitive than the strain uvs12 (Fig. 2C-2E).

On the basis of the sensitivity to the test compounds the rank order of the strains was: uvs10 < uvs375 < uvs12 < 137C.

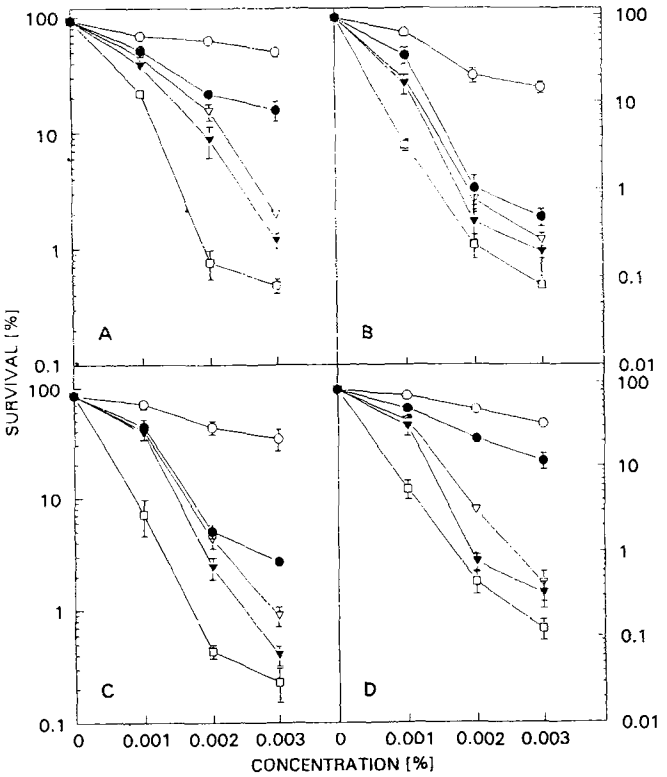

Fig. 1. Comparison of survival of the wild-type strain (137C) of *C. reinhardti* (*A*), the recombination-repair-deficient strain uvs10 (*B*), the excision-deficient strain uvs12 (*C*) the mutant strain uvs375 (*D*) after treatment with C4 (*open circles*), C5 (*closed circles*), C6 (*open triangles*), C7 (*closed triangles*) and C8 (*open squares*) derivatives of phenylcarbamic acid.

Discussion

In the algicidal activity evaluation of different derivatives of alkoxy-phenylcarbamic acid, attention has been mainly paid to their inhibitory effect on photosynthesis (Mitterhauszerová *et al.* 1991a,b, Kráľová *et al.* 1992a, b, Csöllei 1993, Gregáň *et al.* 1993b). As we have found in our toxicity experiments in *C. reinhardtii*, the inhibition of photosynthesis in spinach and wheat chloroplasts as well as of growth and synthesis of chlorophyll in *Chlorella vulgaris* increased with the prolongation of the alkyl chain of the alkoxy substituent (Mitterhauszerová *et al.* 1991a, b, Kráľová *et al.* 1992a, b, Csöllei 1993, Gregáň *et al.* 1993b).

Esters of phenylcarbamic acid interact with components of cell membranes - lipids and proteins - causing a perturbation of the membranes and impacting on biological functions. The extent of the inhibitory effects of alkoxy-phenylcarbamic acid

derivatives is strongly dependent on the alkyl chain length of the alkoxy substituent and partly on the benzene ring of the molecule (Kráľová *et al.* 1992a, Csöllei 1993).

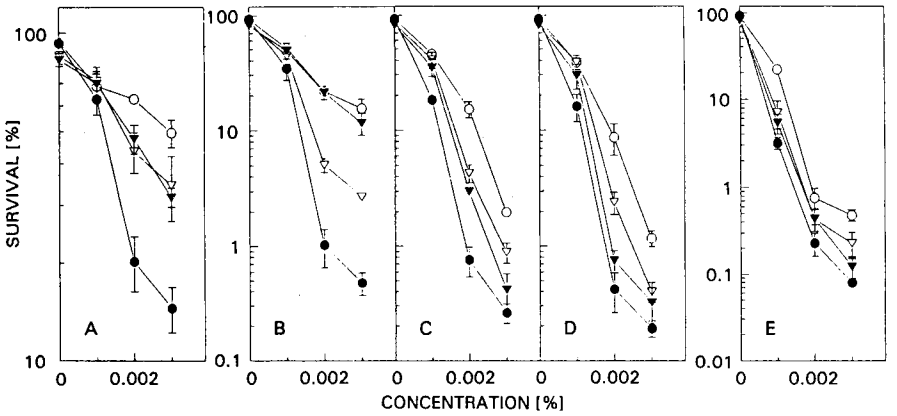

Fig. 2. Comparison of survival of the wild-type strain 137C (*open circles*) and repair-deficient strains uvs10 (*closed circles*), uvs12 (*open.triangles*) and uvs375 (*closed tringles*) of *C. reinhardtii* after application of C4 (*A*), C5 (*B*), C6 (*C*), C7 (*D*) and C8 (*E*) derivatives of phenylcarbamic acid.

In different systems, a correlation was found between induced toxicity and the presence of certain DNA lesions (Fleer and Brendel 1982, Brendel and Ruhland, 1984). The published data were not sufficient evidence to relate a biological effect to a specific type of damage. An argument for toxicity due to DNA lesions is the enhanced sensitivity of repair-deficient mutants (Brendel and Ruhland 1984). This approach was employed when "repair tests" were introduced for the genotoxicity evaluation of different chemicals using repair-deficient strains of bacteria *Salmonella typhimurium* and *Escherichia coli* (Ames *et al.* 1975, Green and Muriel 1976). Bacterial "repair tests" enable the assessment of specific repair pathways participating in repair of damages induced in DNA by the test compound.

Analogously to these "repair tests" in bacteria, we tried to compare survival of different repair-deficient strains of *C. reinhardtii* and a wild-type strain after treatment with five alkoxy derivatives of phenylcarbamic acid. Microscopic evaluation enabled us to distinguish which cells died as a result of cytotoxic effects of the test compounds, and which died as a result of lethal mutations. Differences in enhanced sensitivity of repair-deficient strains of *C. reinhardtii* in comparison with that of the wild-type strain indicated that DNA lesions participated in toxicity of the derivatives used. The recombination-repair-deficient strain uvs10 was the most sensitive strain to the phenylcarbamic acid esters tested. We can conclude that the gene product of uvs10+ gene is responsible for the repair of DNA damages induced in *C. reinhardtii* DNA by the phenylcarbamic acid esters.

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