Mechanism of Inhibitory Action of the Local Anaesthetic Trimecaine on the Growth of Algae (*Chlorella vulgaris*)

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Abstract. Using the model compound trimecaine, it was found that algicidal effects exhibited by the local anaesthetics of the acetanilide type were caused by two different mechanisms. The first inhibitory mechanism occurring at low concentrations of the anaesthetic is connected with the uncoupling of the photophosphorylations in algal chloroplasts and is accompanied by the enhancement of the oxygen evolving rate in algal photosynthesis. The second mechanism of inhibition of the photosynthesis in algae, taking place at higher concentrations of the anaesthetic, is connected with the damaging of the manganese containing protein on the donor side of photosystem 2 and is accompanied by a decrease of the oxygen evolving rate in algal photosynthesis.

Key words: Photosynthesis inhibition — Growth of algae — Local anaesthetics — Photophosphorylation — Trimecaine

Introduction

Many local anaesthetic compounds exhibit amphiphilic properties and their biological action is caused by interactions with biological membranes (Seeman 1972, Trudell 1991, Ueda 1991). A great number of local anaesthetics contain the biologically active group -NH-CO- showing inhibitory effects on the photosynthetic apparatus of chloroplasts (Trebst 1980). It has been found that local anaesthetics inhibit electron transport in photosynthesis (Semin et al. 1987, 1988, 1989; Šeršeň et al. 1990; Mitterhauszerová et al. 1991a,b; Kráľová et al. 1991, 1992a,b,c; Gregáň and Polášek 1992; Csöllei 1993) and they inhibit growth and chlorophyll synthesis in algae as well (Mitterhauszerová et al. 1991a,b; Kráľová et al. 1991, 1992a,b,c; Gregáň and Polášek 1992; Gregáň et al. 1993; Csöllei 1993). Semin et al. (1987, 1988, 1989) have found that some local anaesthetics exhibit stimulating effects at concentrations lower than the inhibitory ones.

The aim of this work was to explain the mechanism of inhibitory action of local anaesthetics of the acetanilide type on growth and chlorophyll synthesis in

the alga *Chlorella vulgaris*. Trimecaine was selected as a representative of this group of local anaesthetic compounds.

Materials and Methods

Trimecaine (2-diethylamino-2′,4′,6′-trimethylacetanilidinium chloride) was obtained from Slovakofarma (Hlohovec, Slovakia) and was used without further purification.

The oxygen evolving rate (OER) in algal suspension was measured at 24 °C by a Clark type electrode (SOPS 31 atp. Chemoprojekt, Prague) in a chamber constructed according to Bartoš et al. (1975). Illumination was carried out with a 250 W halogen lamp (about 100 W/m^2) and the composition of the algal suspension corresponded to that of Šetlík (1968). Before the OER measurements the suspension was accomodated in the dark during 4 h.

The experiments concerning growth and chlorophyll (Chl) synthesis in the algae were performed by the stationary cultivation method according to Kráľová et al. (1991). The starting Chl concentration in the suspension of *Chlorella vulgaris* was about 6 mg Chl/l. The suspension was then cultured with an illumination regime of 16 h light and 8 h dark at 24 °C for 14 days. The Chl content in the suspension was evaluated after its extraction into N,N-dimethylformamide according to Inskeep and Bloom (1985).

The electron paramagnetic resonance (EPR) spectra were registered by an ERS 230 apparatus (ZWG AdW, Berlin, Germany) which operates in the X-band at 24 °C. The algal concentration was about 1.3 mg Chl/ml, the used microwave power was 5 mW, the modulation amplitude was 10^{-3} or 5×10^{-4} T. The samples were illuminated directly in the resonator cavity with a 250 W halogen lamp and they were protected against warming by a water filter.

The emission fluorescence spectra were recorded by a fluorescence spectrophotometer F-2000 (Hitachi, Japan) at room temperature (24 °C). The samples of *Chlorella vulgaris* (10 mg Chl/l) were excited at 436 nm using a slit width of 10 nm.

Results

The local anaesthetic trimecaine decreased algal chlorophyll content reflecting the growth of *Chlorella vulgaris* (Fig. 1, curve *A*). The minimum algicidal concentration (MAC) of trimecaine causing total inhibition of Chl production in *Chlorella vulgaris* was about 7×10^{-5} mol/mg Chl.

The immediate effect of trimecaine on algae was observed by measuring the oxygen production with a Clark type electrode. The dependence of OER upon the concentration of trimecaine is presented in Fig. 1, curve *B*. Depending on the concentration of trimecaine, two different effects on OER were observed – at low trimecaine concentrations the effect was stimulating, at higher concentrations this effect was inhibitory. The inhibitory effects on OER in spinach chloroplasts exhibited by structurally similar local anaesthetics used at sufficiently high concentrations were observed by Mitterhauszerová et al. (1991a,b) and Kráľová et al. (1991, 1992a,b,c). It was found that local anaesthetics caused inhibition of OER

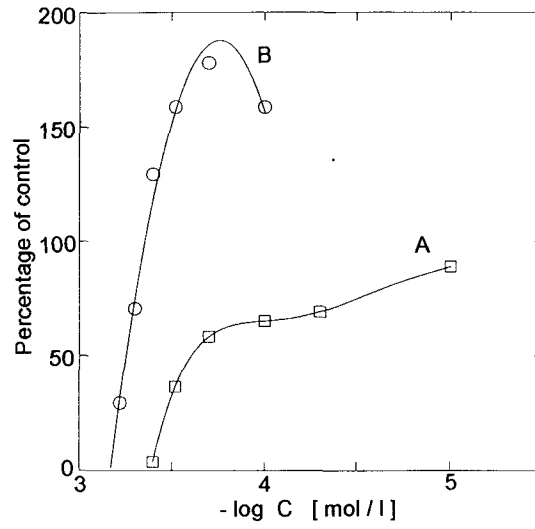

Figure 1. The dependences of the chlorophyll synthesis (curve *A*) and the OER (curve *B*) in *Chlorella vulgaris* on the concentration of trimecaine (the studied parameters are expressed as the percentage of the control samples). The Chl content in the algal suspension was 6.3 mg Chl/l for OER and 6.1 mg Chl/l (starting concentration) for the growth experiments, respectively.

in plant chloroplasts by damaging the manganese containing protein in the photosystem 2 (PS 2) with subsequent release of Mn^{2+} ions into the interior of thylakoid membranes.

It is very probable that the inhibition of OER in algae is caused by the same mechanism as in plant chloroplasts, i.e. by the damaging of the manganese containing protein. To support this idea, an experiment using EPR spectroscopy was performed. The EPR spectra of algae demonstrating the damaging of the manganese containing protein are presented in Fig. 2, curve *B*. EPR spectrum of algae treated with trimecaine consists of six lines which belong to Mn^{2+} ions released from the manganese cluster into the interior of thylakoid membranes. Besides these 6 lines a further EPR signal at $g = 2.0042$ with approximately 2×10^{-3} T width

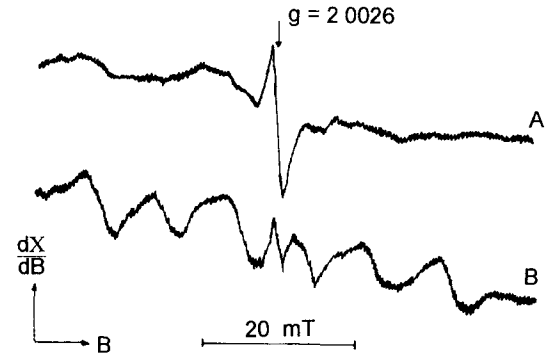

Figure 2. The EPR spectra of *Chlorella vulgaris*: untreated (curve *A*) and treated with 0.1 mol/l of trimecaine (curve *B*). The modulation amplitude was 10^{-3} T, for further experimental conditions see Materials and Methods.

was observed. The effect of trimecaine on this signal consisting of the so-called EPR signal I and signal II, composed of signal II_{slow} and signal $II_{very\ fast}$ (for details see Šeršeň et al. 1993, Kráľová et al. 1993), is manifested by a diminution of the signal II_{slow} which belongs to the D^+ intermediate on the donor side of PS 2 (Fig 3*B*) On the other hand, the signal I belonging to the PS 1 remains practically unchanged (Fig 3*B*, dotted line) However, at very high concentrations of trimecaine an intensity decrease of this signal was also observed

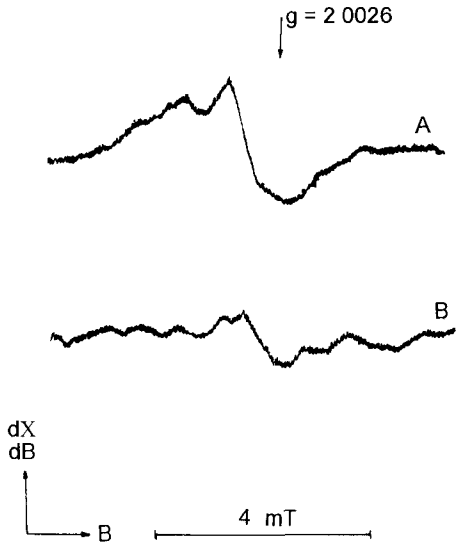

Figure 3. The EPR spectra of *Chlorella vulgaris* untreated (curves *A*) and treated with 0 01 mol/l of trimecaine (curves *B*) in the dark (full curve) and in the light (dotted curve) The modulation amplitude was $5\ 10^{-4}$ T, for further conditions see Materials and Methods

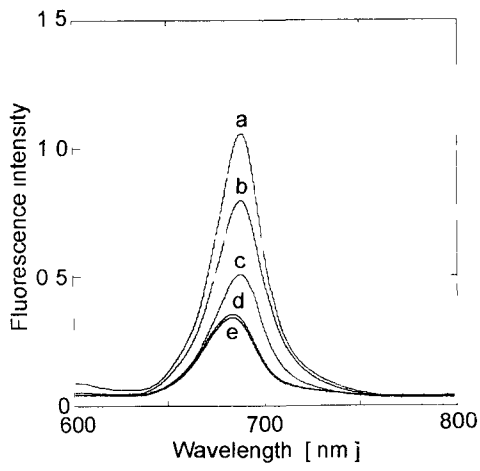

Figure 4. The fluorescence spectra of *Chlorella vulgaris* untreated (curve *a*) and treated with 0 1 mol/l of trimecaine (curves *b*/ 3 min *c*/ 10 min *d*/ 20 min and *e*/ 30 min after addition of trimecaine), for further conditions see Materials and Methods

The effect of trimecaine on algal PS 2 was demonstrated also by the fluorescence spectra of *Chlorella vulgaris* in the presence of the effector (Fig. 4). From these spectra it is evident that the intensity of the peak ($\lambda = 685$ nm) belonging to the pigment protein complex of PS 2 (Goedheer 1968; Atal et al. 1991) shows a time-dependent decrease due to interactions of trimecaine with algal PS 2.

Discussion

The stimulating effect of trimecaine, i.e. the enhancement of OER in algae can be connected with the uncoupling of phosphorylations in algal chloroplasts or with changes in the arrangement of the thylakoid membranes.

In the first case, trimecaine is a good protonophore which can lose the proton after diffusion through the envelope membranes into the algal chloroplasts due to different pH values of the stroma chloroplast region in the dark (approximately 7) and in the light (approximately 8) (Heldt 1980). In comparison with the ionic form of trimecaine its uncharged form (owing to its higher lipophilicity) diffuses more easily through the membranes into thylakoids. There it is combined with the protons evolved during the photosynthetic water photolysis. This process results in the dissipation of the proton gradient between the inside and the outside of thylakoids, thus the photosynthetic electron transport can proceed faster, and photophosphorylations are uncoupled.

For the second case it can be assumed that due to the incorporation of trimecaine into thylakoid membranes changes occur in their arrangement leading to the enhancement of the photosynthetic activity. This explanation is supported by the results of Gallová et al. (1992) who found that carbisocaine at low concentrations decreases the microviscosity of phosphatidylcholine model membranes. On the basis of this finding it can be assumed that the migration of the plastoquinone pool between the photosynthetic centres is easier which enables a faster photosynthetic electron transport (Yamamoto et al. 1981).

The comparison of the concentration dependences concerning OER and growth of algae shows that the concentrations of trimecaine causing the total inhibition of both studied parameters are different (Fig. 1). It is evident that the concentration of trimecaine causing death of *Chlorella vulgaris* algae in a long-term action causes enhancement of OER immediately after the treatment.

On the basis of these findings it can be suggested that trimecaine, due to its protonophore properties, at low concentrations causes uncoupling of photophosphorylations which results in the loss of the ability to form ATP. On the other hand, the lack of ATP hinders the formation of sugar as the final product of photosynthesis. Therefore during long-term action of low trimecaine concentrations on *Chlorella vulgaris* consumption of the own starch stores of the algae occurs. If all starch stores in the algal cells are completely consumed, the cells die because

of the absence of further ATP formation. Besides the above-mentioned uncoupling mechanism the inhibition of the algal growth at higher concentrations of trimecaine is caused by the damage of the manganese containing protein in PS 2 as well.

It is not probable that the rearrangement of thylakoid membranes leading to the enhancement of OER is the reason of the inhibition of algal growth. The membrane rearrangement alone, which does not limit the other biological functions, will not inhibit the growth of algae.

It can be concluded that these two mechanisms of action (i.e. the uncoupling mechanism at low effector concentrations and the damaging of PS 2 at higher effector concentrations) can be generalized also for other structurally similar amphiphilic compounds showing local anaesthetic activity, e.g. alkoxyphenylcarbamates or aryloxyaminopropanols.

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Final version accepted August 26, 1994