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BRIEF COMMUNICATION

# Effects of Alkyl Chain Length of Membrane-active, Polysubstituted Ammonium Compounds on Photosynthesis and Growth*

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**Abstract**

[1-/2-Alkoxycarbaniloyloxy/cyclohexylmethyl] dimethylammonium chlorides with local anaesthetic properties inhibited both photosynthesis of plant chloroplasts and growth of vetch (*Vicia sativa* L.) and green algae *Chlorella vulgaris* Beijer. The inhibitory efficiency depended on alkyl chain length of the alkoxy-substituent of the studied compounds.

Amphiphilic molecules having local anaesthetic properties interact strongly with the hydrophobic lipidic parts of the membrane — they can penetrate between the lipidic molecules causing expansion of the bilayer, decreasing the phase transition temperature of membrane (Račanský *et al.* 1988) and changing both the electric properties and fluidity of model membranes (Seelig *et al.* 1988).

The synthesis of seven [1-/2-alkoxycarbaniloyloxy/cyclohexylmethyl] dimethylammonium chlorides (alkoxy = methoxy-n-heptyloxy) (AC) is described by Gregáň *et al.* (1987) together with the corresponding characteristics of their physico-chemical properties and local anaesthetic activities.

For electron paramagnetic resonance (EPR) studies chloroplast preparations obtained from spinach and containing approx. 4—5 mg of chlorophyll (Chl) a in 1 $cm^3$ of the measured sample were used. The concentration of the studied ACs was constant — 0.05 M — and the measurements were carried out with an instrument *ERS-230/ZWG AdW*, Berlin, Germany (at microwave outputs 5 mW, modulation $5 \times 10^{-4}$T), which operated in the X band.

After irradiation of the chloroplasts, the intensities of superposed signals I and II of non-influenced chloroplasts that corresponded to photosystems (PS) 1 and 2, respectively, were increased 1.5 to 2-times. Because of interaction of ACs with plant chloroplasts, the electron flow from PS 2 to PS 1 was damaged, which resulted in lower intensities of the signal II, and therefore in a higher ratio of ESR signals intensities measured in the light and in the dark. The measure of the photosynthesis inhibition can be expressed by parameter $P$

$$P = [(I_{light}/I_{dark})]_{sample}/[(I_{light}/I_{dark})]_{control} \times [mg(Chl\ a)]^{-1}$$

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where $I$ are intensities of the EPR signals in the light and in the dark, respectively. The inhibitory efficiency of the studied ACs depended strongly on the alkyl chain length of the alkoxy-substituent and it was maximum with the n-pentyloxy-derivative (Fig. 1*A*). When using the most effective compounds (n-butoxy, n-pentyloxy- and n-hexyloxy-derivatives), a release of $Mn^{2+}$ ions in the EPR spectra was detected.

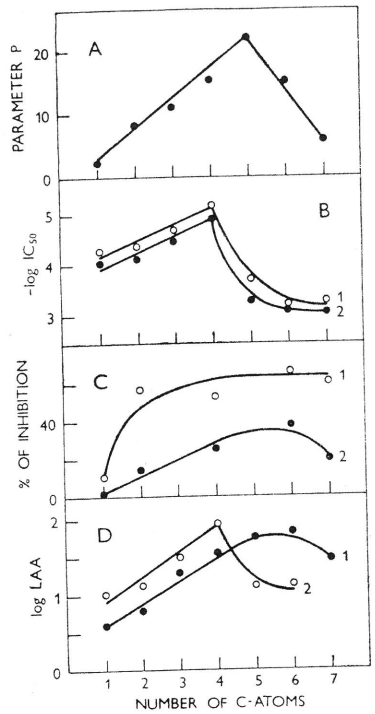


Fig. 1. The dependence of various biological parameters upon the number of C atoms in alkoxy--substituent of [1-/2-alkoxycarbaniloyloxy/cyclohexylmethyl]dimethylammonium chloride: *A* — Parameter $P/C_{AC}$ = constant = 0.05 M; *B* — $IC_{50}$ values of synthesized chlorophyll content (*1*) and growth (*2*) of green algae *Chlorella vulgaris*; *C* — % of inhibition of root (*1*) and stem growth (*2*) of vetch (*Vicia sativa* L.) at 1 mM $C_{AC}$ (confidence intervals for $\alpha = 0.05$ were $\leqq \pm 5.3$ % (roots) and $\leqq \pm 4.0$ % (stems); $n = 100$); *D* = surface (*1*) and infiltration (*2*) local anaesthetic activity (corresponding values taken from Gregáň *et al.* 1987).

The studied AC derivatives inhibited also the growth and Chl synthesis of green algae (*Chlorella vulgaris* Beijer.) when stacionary-cultivated in the cultivating medium of Šetlík (1968) during 7 d with the photoperiod 16/8 h light/dark. The growth of algae was evaluated from the absorbance values of the algal suspension at 660 nm, the synthesized Chl content was determined spectrophotometrically (*Specord UV VIS Zeiss*, Jena, Germany) after its extraction with *N*,*N*-dimethylformamide from the centrifuged algal cells (Inskeep and Bloom 1985). Fig. 1*B* shows the dependence of $IC_{50}$ (concentration of ACs derivative causing 50 % inhibition of algal growth or Chl synthesis, evaluated from the linear dependence of the corresponding inhibition on the logarithm of AC con-

centration) on the alkyl chain length of alkoxy-substituent. For the calculation of $IC_{50}$ values experimental results obtained at 4 various AC concentrations (3 repetitions for each concentration) were used. The correlation coefficients were in the range of 0.98—0.99. The most efficient was the n-butoxy-derivative with an algicidal effect at 3.9 kg $m^{-3}$.

The influence of ACs on growth of roots and stems of vetch (*Vicia sativa* L.) was determined at constant (1 mM) concentration (Fig. 1*C*). The grains of vetch were swelled in aqueous solutions of AC for 24 h and then pregerminated at room temperature for 72 h in the dark on rolled filter paper wetted with the same AC solution. Subsequently, the length of the roots and stems of the plant was measured, and the % of the inhibition (related to control samples) was evaluated. The inhibitory effect of AC on root growth was more pronounced than the corresponding effect upon the stem growth. For the applied 1 mM concentration the differences in the root growth inhibitory efficiency among derivatives with 2—7 carbon atoms in the alkoxy-substituent were relatively smaller than in the cases mentioned above (Fig. 1*A*, *B*).

Hence the mechanism of action of the studied compounds is similar for photosynthesis inhibition in chloroplasts and algae, it can be suggested that ACs interact with lipid parts of membranes causing their perturbation. The increase of the biological activity with the increasing length of the alkoxysubstituent is probably connected with hydrophobic interactions of ACs with the membrane.

The further decrease of activity of derivatives with a longer alkoxy-substituent is probably caused by steric effects-predominantly intramolecular interaction may take place between the alkyl chain of alkoxy-substituent in ortho-position and the cyclohexyl ring of AC molecule resulting in a decrease of penetration ability of the AC hydrophobic parts into the membrane.

The dependence of the local anaesthetic efficiency on the alkyl chain length of the alkoxy-substituent (Fig. 1*D*) was in good agreement with the determined inhibitory effects of the studied compounds (Fig. 1*A*, *B*, *C*). This effect showed also the important role of hydrophobic interactions in various biological activities of amphiphilic compounds having local anaesthetic properties, based on interactions with biological membranes.

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