INFLUENCE OF SOME NEW AMINOPHOSPHONATES ON ELECTRICAL PROPERTIES AND STABILITY OF PLASMA MEMBRANES OF PLANT CELLS

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ABSTRACT

The effect of new aminophosphonates, synthesized for potential agricultural application, on membrane potential and electrical conductance of internodal cells of *Nitellopsis obtusa* and their efficiency in modifying the properties of cucumber (*Cucumis sativus* cv "Wisconsin") cotyledon membranes was studied. They differed in substituents at the carbon and phosphorus atoms. It was found that the organophosphorous compounds caused depolarization of the membrane resting potential and increased electrical conductance of alga membranes. They also influenced chlorophyll content and efflux of electrolytes from cucumber cotyledons.

The most significant effects were observed for the compound with iso-propyl substituents at the P atom. In contrast, the weakest modifier of the studied compounds was the one having phenolic substituents at that atom. The observed changes are most probably the result of direct interaction of aminophosphonates with the lipid phase of the plasma membrane and the induced structural changes in it.

KEY WORDS: aminophosphonates, plant membrane conductance, resting membrane potential.

INTRODUCTION

Many plant growth regulators belong to aminophosphonates, which makes this group of compounds agrochemically interesting. The interest began with first synthesized aminophosphonates by Piki (1943). Up to now, many aminophosphonates have been found to be also potent pesticides (Guenther and Loettge 1971; Baird et al. 1972; Perkow 1983/1988; Gancarz et al. 1985; Kochmann et al. 1986; Forlani et al. 1987). New compounds belonging to this rather large group are still being synthesized and their potential biological usefulness studied. The most important reason for that kind of research is the necessity to obtain new compounds able to replace those to which microorganisms developed resistance. This is also why the aminophosphonates studied in this work were synthesized. Moreover, the choice came from previously accumulated knowledge showing that some representatives of aminophosphonates yet studied exhibited very good biological activity (Wieczorek et al. 2000, 2001; Deron et al. 2001).

The good biological activity of aminophosphonates is usually postulated to be the result of their high lipophilicity (Gancarz and Dudek 1986) or their involvement in metabolic processes (Linsel et al. 1988). Generally, these observations point at both lipids and proteins of biological

membranes as sites where biological activity of organophosphorous compounds takes place (O'Brien 1979; Crowley 1980; Linsel et al. 1988; Di Tomaso 1993; Shimakuburo 1994; Sterling 1994; Marré et al. 1998). This is rather obvious since the first contact of a pesticide with an organism must occur at the cell membrane. This contact may lead to membrane injury and initiate a series of events that seriously impair cell function or, in extreme cases, lead to cell death.

In this paper we present the results of studies on physiological and electrophysiological properties of some aminophosphonates expected to correlate with their potential biological activity. Additionally, the role of different structural parts of aminophosphonates in that activity is discussed.

MATERIALS AND METHODS

The studied aminophosphonates were synthesized at the Department of Organic Chemistry, Biochemistry and Biotechnology, Technical University of Wrocław. Their general structure and particular substituents are given in Table 1. Synthesis details as well as spectral data are given elsewhere (Wieczorek et al. 2000, 2001).

TABLE 1. The general structure and substituent groups of the compounds studied.

R^4

C

R^3

HN

R^1

P

O

OR^2

OR^2

Compound no.	R ⁴	R ³	R ²	R ¹
1	CH ₃	CH ₃	C ₆ H ₅	n-C ₄ H ₉
2	n-C ₄ H ₉	n-C ₄ H ₉	C ₂ H ₅	n-C ₄ H ₉
3	CH ₃	n-C ₅ H ₁₁	C ₂ H ₅	n-C ₄ H ₉
4		-C ₅ H ₁₀ ⁻	i-C ₃ H ₇	n-C ₄ H ₉

Cucumber (*Cucumis sativus* cv “Wisconsin”) was grown under constant white light. Cotyledons from 7-day-old seedlings were used for experiments. Discs of 14 mm diameter were cut avoiding the midrib. They were rinsed in water and incubated 24 h on 0.25, 0.5 and 1 mmol·dm⁻³ aminophosphonate solutions under constant light (fluorescent lamp Pila LF 36 W “Daylight”, PAR 110 μE·m⁻²·s⁻¹). Conductivity of the treatment solution was assayed with a OK-102/1 conductometer (Radelkis, Hungary).

Chlorophylls were extracted in 80% acetone (Lichtenhaler 1987).

Fresh internodal cells of *Nitellopsis obtusa* were used in the investigation. The alga was taken from the natural environment (Zagłębcze Lake by Sosnowica, Lublin region, Poland).

Plant isolated, single internodal cells (of mean dimensions: 0.4 mm diameter and 3.1 cm length) were incubated for 24 h in darkness in measurements solution (artificial pond water – APW).

The control solution of pH 7.0 contained 1 mmol·dm⁻³ NaCl, 0.1 KCl and 0.1 CaCl₂. The incubation solutions containing the compounds studied were prepared by dissolving a compound in a small amount of ethanol (1 cm³/1 dm³ of solution) and then in APW. The range of used concentrations of aminophosphonates was 20-100 μmol·dm⁻³. The same amount of ethanol was added to the control

APW solution.

Membrane potential and electric conductance were measured in a routine way (Trela et al. 2001). The potential difference between vacuole and external medium was measured with one pair of microelectrodes filled with 3 mol·dm⁻³ KCl. In the current circuit Ag/AgCl electrodes were used.

The cells were placed in a three-compartment container. The compartments were filled with a liquid and partitioned by narrow empty spaces that insured electric isolation between the compartments. The middle compartment was perfused with control solution or solution containing a modifier at a rate of 1 cm³/min. In that part of the container current and membrane potential were measured. The middle compartment covered 3 mm of the alga cell. The experimental setup is shown in Fig. 1.

The electric conductance of the modified membrane fragment was determined on the basis of the voltage response to a constant signal of (ca. 20 nA of 200 ms duration and 10 s repetition time. All experiments were conducted in darkness at room temperature (22±1°C).

All reagents were of highest quality available.

RESULTS AND DISCUSSION

The studied aminophosphonates changed conductance of the plasma membrane of *Nitellopsis obtusa* and caused depolarization of the resting membrane potential (RMP). The results of these experiments are collected in Table 2.

All the compounds induced a slow depolarization of RMP of the alga membrane and increased the membrane conductance when used at concentrations higher than 20

TABLE 2. Effect of 0.05 mmol·dm⁻³ of aminophosphonates on the resting potential in cells of *Nitellopsis obtusa*. Data are means±SE of 3 experiments.

Compound	Relative changes of the resting potential (%)		Relative changes of the membrane conductance (%)	
	after 30 min	after 60 min	after 30 min	after 60 min
1	-2.6±0.5 ab	-9.8±1.8 a	-0.3±0.4 c	5.7±0.4 c
2	-1.9±0.6 b	-6.2±1.6 ab	-1.1±0.1 bc	3.8±0.3 c
3	-0.8±0.3 c	-3.8±2.5 b	-3.2±0.2 b	10.1±0.3 b
4	-3.1±0.5 a	-4.5±3.5 b	12.6±2.7 a	16.8±2.5 a

Values within column marked with the same letter are not significantly different (Duncan test, p = 0.05).

Fig. 1. Block diagram of measurement setup. V_H, V_L – voltage electrodes, I_H, I_L – current Ag/AgCl electrodes, V_m, I_m – analog signal of membrane voltage and current.

Fig. 2. The time course of membrane resting potential and specific conductance in the presence of 0.05 mmol dm⁻³ of compound 3.

Fig. 3. Effect of aminophosphonates on electrolyte efflux from cucumber (*Cucumis sativus* cv "Wisconsin") cotyledons, and chlorophyll content in cotyledons. The values are mean ($\pm$ SE) for 6 replicates.

$\mu\text{mol·dm}^{-3}$. On the other hand, concentrations equal to 100 $\mu\text{mol·dm}^{-3}$ caused irreversible destruction of the plasma membrane. Examples of time-courses of RMP and conductance, for compound 3 used at 50 $\mu\text{mol·dm}^{-3}$ concentration, are shown in Fig. 2.

As can be seen, transient decreases in both time-courses were also observed. The efficacy of the aminophosphates to change RMP increase, with incubation time and is highest for compound 1. Compounds 3 and 4 have similar modifying properties, which are significantly weaker than with compound 1. On the contrary, these compounds had the strongest influence on conductivity of algae membranes.

Qualitatively similar results were observed in physiological experiments (Fig. 3). The efficacy to influence the electrolyte efflux from cucumber cotyledons increased with the increasing compound concentration, the highest being observed for compound 4.

Compound 4 was also found to be most effective in influencing the chlorophyll content, while no significant differences (10-15%) were found in the case of other compounds studied.

High lipophilicity of biologically active compounds was shown previously to be a very important factor influencing the electrophysiology of the plasma membrane of *Nitellopsis obtusa* (Trela et al. 1990; Przestalski et al. 1991). However, the observed in this work differences in efficacy of the compounds studied did not seem to be the result of their lipophilicity alone. If one takes the number of methyl and methylene groups as a lipophilicity measure – a commonly applied approach to the lipophilicity problem (Kleszczyńska et al. 2001), then it is similar for compounds 2, 3 and 4. The number of lipophilic groups these compounds have differ by 14 to 16. The lipophilicity based mechanism of interaction with the cells studied seems to be

true in the case of compound 1 possessing only 6 such lipophilic groups. However, it also has two strongly lipophilic phenyl substituents at the phosphorus atom that increase the overall lipophilicity of this compound. In the case of other studied compounds it seems that their cell membrane modifying properties depend to a great extent on structural features too. It was already shown that iso-propyl substituents at the phosphorus atoms increased physiological activity of aminophosphonates (Bielecki et al. 2001). That may explain the higher activity of compound 4 in comparison with compounds 2 and 3. It also seems that a hydrocarbon substituent longer than the butyl one improves activity of such a compound in comparison with the one with shorter substituents, even when the latter is more lipophilic (compare compounds 2 and 3, Table 2).

The obtained results indicate that the studied aminophosphonates damage plant membranes to a different degree depending on their concentration, lipophilicity and structural features. These results may be useful while synthesizing new compounds for agrochemical application.

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WPLYW NIEKTÓRYCH NOWYCH AMINOFOSFONIANÓW NA WŁAŚCIWOŚCI BŁON KOMÓRKOWYCH U ROŚLIN

STRESZCZENIE

Zbadano wpływ nowych aminofosfonianów, przeznaczonych do zastosowania w rolnictwie, na potencjał membranowy i przewodnictwo komórek międzywęzłowych *Nitellopsis obtusa* oraz na modyfikację własności błon komórek liścieni ogórka (*Cucumis sativus* cv „Wisconsin”). Poszczególne związki różniły się podstawnikami na atomach węgla i fosforu. Stwierdzono, że powodowały one depolaryzację potencjału spoczynkowego błon komórek glonu oraz zwiększały przewodnictwo elektryczne przez te błony. Powodowały także mechaniczne uszkodzenia błon, czego skutkiem był wypływ elektrolitu z komórek liścieni ogórka oraz zmiany zawartości chlorofilu w tych komórkach.

Obserwowane efekty można powiązać z cechami budowy strukturalnej aminofosfonianów. Najbardziej znaczące obserwowano dla związku posiadającego podstawnik izopropylowy na atomie P. Przeciwnie, najłabszy wpływ na badane parametry błon wywierał związek w którym podstawnik propylowy na tym atomie został zastąpiony podstawnikiem fenylowym. Otrzymane wyniki wskazują na taki mechanizm oddziaływania aminofosfonianów z błonami roślinnymi, w którym wbudowują się one w fazę lipidową tych błon i wymuszają w niej zmian strukturalnych (defektów).

SŁOWA KLUCZOWE: aminofosfoniany, przewodnictwo błon komórkowych, potencjał spoczynkowy.