The Influence of Phytohormones on Zeta Potential and Electrokinetic Charges of Winter Wheat Cells

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The zeta potential measurements of protoplasts obtained from winter wheat cell culture and phospholipid liposomes were performed to determine the electrokinetic charge in a medium containing various phytohormones (kinetin, 2,4-D and zearalenone) in absence and in presence of $2 \cdot 10^{-5}$ M Ca^{2+}. Calli were induced from immature inflorescences (inf) and embryos (emb) and cultured to obtain non-embryogenic (NE) and embryogenic (E) cell tissues. All investigated phytohormones indicate ability to adsorb to the negatively charged surfaces (latex, L88 – model negative adsorption site) both in water solutions and at the presence of mannitol and buffer (MES). In biological systems (protoplasts and liposomes – prepared from phospholipids of protoplasts) the electrokinetic charges were dependent on the phospholipid and protein composition of cells. The influence of protein groups on electrokinetic charge was calculated from charge values of protoplasts and liposomes, assuming additivity of surface charges. The comparison of calculated charges for protoplasts and liposomes indicate that 2,4-D is better adsorbed to the phospholipid and proteins of NE cells whereas kinetin is bound to the phospholipid and protein sites of E calli. This effect may be connected with embryogenesis process, where non-embryogenic culture of wheat requires 2,4-D in the medium, and embryogenic culture requires cytokinin rather. Zearalenone binding is especially dependent on the kind of explant.

Introduction

It is now believed that some binding sites located on the plasmalemma play an essential role in the recognition of the information signals from the signalling molecules such as hormones (Rubery, 1981; Libbenga and Mennes, 1995). The existence of protein-carriers for auxin and cytokinin is now intensively studied (Lomax *et al*., 1995; Delbarre *et al*., 1996), but their activity/disactivity could depend on the lipid environment. Phytohormones seem to play an essential role in the embryogenesis process. Auxin (2,4-D) presence is required for callus induction from wheat, while its reduced concentration is necessary to stimulate embryogenesis process. Data obtained by Carnes and Wright (1996) suggest high endogenous hormone level in maize ovules during early embryogenesis and reduced embryogenic competence of immature embryos. The cytokinin concentration in tissue culture is also dependent on the explant. In winter wheat suspension cultures, cytokinin level derived from immature embryos was higher then from the suspension derived from inflorescences (Marcińska *et al*., 2001). However the mechanism of hormones action in the embryogenesis is still unclear.

In vitro cultures and protoplasts with their exposed plasmalemma have been used in the studies of the cell membrane properties, especially the interaction membrane-chemical factor. In particular, measurements of the zeta potential are very useful in analyses of the electric state of the plasmalemma of the cells from which the protoplasts were derived (Abe and Takeda, 1988; Obi *et al.,* 1989. Kinraide *et al*., 1998; Pillet, 1989). Such potential depends directly on the surface charges of the plasmalemma due to a combined ionisation of surface groups (proteins, lipids and saccharides) and can be modified by adsorption of ion and surface active molecules (Obi *et al*., 1989; Pillet, 1989)

In the present study, we have investigated the sensitivity of the plasmalemma derived from winter wheat cell culture to differently charged phyto-

hormones in the medium containing increasing amount of Ca^{2+} ions. Three substances were studied: 2,4-D, an auxin analogue with negative net charge, kinetin (cytokinin), positively charged and zearalenone without exposed ionogenic groups. Zearalenone is a substance of pathogenic character extracted from fungus *Gibberella zeae* that also was detected, although in trace amounts, in plants. Contribution of zearalenone to the processes of flowering and differentiation *in vitro* indicates that zearalenone could be regarded as plant hormone (Fu and Meng, 1993). Comparison of the zeta potential results obtained for plasmalemma protoplasts and phospholipid liposomes (prepared from plasmalemma) allows to speculate about phytohormone adsorption sites as proteins and/or phospholipids. Cell cultures were induced from two tissue sources, immature embryos and inflorescences, and protoplasts were isolated from embryo- and non-embryogenic calli. This system let us determine if the changes of zeta potential are tissue and/or embryogenic specific.

Material and Methods

Plant material

Seeds of winter wheat cv. Kamila were obtained from the Plant Breeding Station in Węgrzce Wielkie near Kraków. The seedlings were vernalized in perlite at 5° C with 8 h photoperiod for 9 weeks. Then the plants were planted in soil and grown in a glasshouse at 20/17° C (day/night) and at 16 h photoperiod. The immature embryos and inflorescence were taken from the plants and surface sterilised for 1 min in 70% ethanol, for 15 min in a 10% aqueous Domestos solution (commercial bleach), and finally rinsed 4 times with sterile distilled water (Marcińska *et al.*, 2001). The explants were cultured in dark on the modified MS (Murashige and Skoog, 1962) medium containing 2 $mg \cdot dm^{-3}$ 2,4-D (2,4-dichlorophenoxyacetic acid) as a source for the non-embryogenic callus. For the embryogenesis, 3-month-old calli were transferred into MS medium without 2,4-D and cultured at 16 h photoperiod in weak fluorescent light (4 W/m^2s^1) for 2 weeks.

Protoplast isolation

Embryo- (E) and non-embryogenic (NE) callus cultures induced from immature embryos (emb) and inflorescences (inf) used for zeta potential measurements were filtered through 125 μm nylon mesh and centrifuged at 100× *g* for 5 min. Samples of about 1 g of calli were added to 10 cm^3 of filter-sterilized enzyme solution composed of 1% Onozuka RS, 0.075% Pectolyase Y-23, 0.6 M mannitol at pH 5.6. After 3 to 4 h of incubation in the dark at 30° C, with agitation every hour, the protoplasts were filtered through a 70 and 30 μm nylon mesh and centrifuged at 100 × *g* for 5 min. The protoplasts were collected and washed twice with 0.6 M mannitol, pH 5.7. Washed protoplasts were stored in a refrigerator (4° C) until use.

Preparation of liposomes

Liposomes were prepared from phospholipids extracted from plasmalemma of cell cultures of embryo- (E) and non-embryogenic (NE) calli initiated from immature embryos (emb) and inflorescence (inf).

Highly purified plasmalemma was prepared by two-phase partitioning procedure, adapted from Sommarin *et al.* (1985) and Arz and Grambow (1994). Callus cells were filtered using 120 μm stainless mesh. Subsequently they were homogenized at 4° C with Sorval Omnimixer blender in 6 vol per g fresh weight of 10 mM tris(hydroxymethyl)aminomethane (Tris) buffered adjusted to pH 7.5 with HCl. The buffer contained also 250 mM sucrose, 1 mM ethylenediaminetetraacetic acid (EDTA), 2.5 mM dithiothreitol (DTT) and 1 mM phenylmethylsulfonyl fluoride (PMSF). The microsomal fraction was recovered after two successive centrifugation steps at 10,000 × *g* for 10 min and at 80,000 × *g* for 30 min, and re-suspended in 0.75 cm^3 of 5 mM potassium phosphate buffer (pH 7.8) containing 250 mM sucrose and 5 mM KCl. After homogenisation, 0.7 cm^3 of the microsomal fraction was added to a 9-g phase mixture to obtain a 9.7 g two-phase system, consisting of 6.5% (w/w) dextran T500, 6.5% (w/w) polyethyleneglycol (PEG) 4000, 5 mM K-phosphate (pH 7.8), 250 mM sucrose, and 4 mM KCl. After a three-step phase partitioning the resulting upper phase was purified by fivefold dilution with 10 mM Tris/2-(N-morpholino)ethanesulfonic acid (Tris/

Mes) buffer (pH 7.4), 250 mM sucrose and 1 mM EGTA and centrifuged at $100{,}000 \times g$ for 1 h.

The recovered plasma-membrane fraction was extracted with mixture of chloroform : isopropanol (1:1 v/v) and re-extracted with chloroform. The fraction of glycolipids, phospholipids and neutral lipids were isolated using the adsorptive and distributive column chromatography on silica gel under low nitrogen pressure, providing an inert atmosphere during lipid separation (Filek and Košcielniak, 1996; Uemura and Yoshida, 1984). Glycolipid and neutral lipid fractions were removed and the phospholipids were mixed with chloroform and evaporated at N_2 atmosphere. Multilameral liposomes were prepared by hydratation of the phospholipid film with deionized water and intensive vortexing (Caaverio *et al.*, 2001).

Zeta potential measurements

The measurements for protoplasts and liposomes were performed in media of defined ionic composition and ionic strength and of constant osmotic pressure at 20° C. The media contained 0.6 mM mannitol, 1 mM KCl, 0.3 mM NaCl and 1 mM MES-KOH buffer (pH 5.6). 2,4-D (2,4-dichlorophenoxyacetic acid), kinetin (6-furfurylaminopurine) or zearalenone (6-(10-hydroxy-6-okso-*trans*-1-undecenyl)-B risolic acid lactone) were introduced to the media in the constant concentration 25 $mg \cdot dm^{-3}$. For determination of Ca^{2+} influence the media were supplemented with 2×10^{-5} M $CaCl_2$.

The zeta potential values (ζ) were determined from electrophoretic mobility measurements with Zeta-PLUS apparatus (Brookhaven, USA). The palladium electrodes were used for electric field application. Electric conductivity was determined simultaneously for each individual measurement. Electrokinetic charge density (q) was calculated from the Gouy-Chapman equation (Obi *et al.*, 1989)

$$q^2 = 2\varepsilon\varepsilon_0 RT\Sigma([C_i](\exp(-z_i F\zeta/RT)-1))$$

where ε is the relative dielectric constant of medium (80.1, which is that of water at 20 C), ε_0 the dielectric constant of a vacuum, $[C_i]$ the bulk concentration of i-ions, z_i the number of charge of *i*-ion, F the Faraday constant, ζ zeta potential $\cong$ the potential of the diffuse part of double layer, R the gas constant, T- temperature.

All used chemicals were obtained from Sigma Chemical Co. (St. Louis, MO, USA). Polystyrene latex suspension (L88) was synthesised and purified by one of the authors. The purity of latex was checked by conductivity of supernatant and its UV-spectrum (Shimadzu UV-160 A, Japan).

Results

Zeta potential of protoplasts.

In the absence of phytohormones, the absolute values of zeta potential of protoplasts isolated from non-embryogenic calli (NE) of winter wheat were always higher in comparison to that of embryogenic (E) (Table I). The non-significant differences were observed between protoplasts prepared from cultures initiated from immature embryos (emb) and inflorescences (inf) within NE and E objects. The presence of Ca^{2+} ions in the medium generally caused a decrease in absolute values of zeta potential and this effect was especially visible in the case of protoplasts isolated from NE calli (Table I).

Phytohormones presence in the medium did not markedly change the zeta potential of both NE and E protoplasts. Only 2,4-D caused a significant decrease in the absolute value of ζ of NE protoplasts especially pronounced for protoplasts in medium containing 2×10^{-5} $mol \cdot dm^{-3}$ Ca^{2+} (Table I).

Zeta potential of liposomes.

Similarly like in the case of protoplasts, in the absence of Ca^{2+} ions and phytohormones, liposomes obtained from phospholipides extracted from E cultures were characterised by smaller absolute values of zeta potential in comparison to liposomes obtained from NE calli (Table I, data – hormone). The presence of Ca^{2+} ions caused generally a decrease in ζ absolute values for liposomes but greater changes were obtained for liposomes prepared from NE cultures.

In the case of NE objects (both inf and emb) (Table I), the effect of phytohormones in the absence and at the 2×10^{-5} M Ca^{2+} ions concentration increased in order kinetin $\approx$ zearalenone < 2,4-D.

PROTOPLASTS

Treatment	Non-embryogenic callus		Embryogenic callus	
	ζ inf. [mV]	ζ emb. [mV]	ζ inf. [mV]	ζ emb. [mV]
$0Ca^{2+}$ – hormone	−25.50±3.06	−28.22±1.96	−17.49±2.81	−19.37±2.51
$0Ca^{2+}$ + KIN	−26.02±4.12	−24.78±2.13	−17.72±2.44	−19.01±1.06
$0Ca^{2+}$ + 2,4-D	−21.61±3.85	−23.04±3.85	−18.95±2.65	−17.08±0.55
$0Ca^{2+}$ + ZEA	−24.04±1.93	−22.86±4.01	−17.91±1.70	−17.51±0.89
$2\times10^{-5}Ca^{2+}$ –hor.	−19.78±2.65	−17.56±0.79	−18.85±2.01	−17.23±1.90
$2\times10^{-5}Ca^{2+}$ + KIN	−16.64±2.17	−17.36±1.16	−15.05±1.72	−16.12±1.78
$2\times10^{-5}Ca^{2+}$ + 2,4-D	−14.53±3.10	−12.50±4.05	−15.04±1.33	−16.13±2.05
$2\times10^{-5}Ca^{2+}$ + ZEA	−21.01±2.31	−17.23±4.10	−13.97±1.04	−15.90±2.01

LIPOSOMES

Treatment	Non-embryogenic callus		Embryogenic callus	
	ζ inf. [mV]	ζ emb. [mV]	ζ inf. [mV]	ζ emb. [mV]
$0Ca^{2+}$ – hormone	−42.90±2.05	−41.23±1.98	−38.09±2.01	−37.12±1.25
$0Ca^{2+}$ + KIN	−41.04±2.71	−34.68±1.32	−35.23±1.85	−36.85±3.01
$0Ca^{2+}$ + 2,4-D	−34.68±2.96	−31.46±1.81	−37.34±2.02	−36.68±2.88
$0Ca^{2+}$ + ZEA	−42.47±2.53	−35.86±4.01	−30.96±1.74	−35.59±2.35
$2\times10^{-5}Ca^{2+}$ – hor.	−28.98±2.14	−28.55±2.75	−29.04±1.27	−36.64±1.19
$2\times10^{-5}Ca^{2+}$ + KIN	−25.65±1.98	−24.37±2.04	−19.75±2.53	−28.05±1.37
$2\times10^{-5}Ca^{2+}$ + 2,4-D	−22.51±2.01	−15.05±1.96	−29.88±1.21	−25.42±1.25
$2\times10^{-5}Ca^{2+}$ + ZEA	−25.02±2.03	−21.11±2.12	−25.05±2.14	−25.89±1.86

Table. I. Zeta potentials (ζ) of protoplasts and liposomes isolated from non- and embryogenic callus cells untreated (–) and treated with phytohormones in media containing MES, mannitol and 0 and 2×10^{-5}M Ca^{2+}. Calli were initiated from inflorescences (inf.) and embryos (emb.) of winter wheat. The ζ data represent the average of three separate experiments (± SD), counting at least 10 replicates for each experiment described in Material and methods.

KIN – kinetin, ZEA – zearalenone.

The phytohormone treatment of E liposomes generally does not cause significant changes of ζ potentials except of those for kinetin in medium containing 2×10^{-5}M calcium ions (inf).

Electrokinetic charge densities

The values of electrokinetic charge density (q), calculated by the Gouy-Chapman equation for protoplasts and liposomes are presented in Table II. The q values provide direct information about the electric state of the surface layer.

The surface charge of protoplasts is mainly due to ionogenic groups of phospholipids and integral proteins (proteins included into phospholipid membrane of cells), whereas the surface charge of liposomes comes from phospholipids only, thus (assuming at zero approximation additivity of surface charges) the charge differences Δq ($\Delta q = q_{protoplast} - q_{liposome}$, Table I) should provide information about protein ionic groups contribution to protoplast surface charge. The significant values of calculated Δq indicates an important role of proteins in establishing the electric state of protoplasts. In medium without Ca^{2+} ions and phytohormones, the higher values of Δq were obtained for cultures derived from inf in comparison to cultures derived from emb for both NE and E calli. These data are in agreement with the results of biochemical analysis indicating a higher amount of membrane proteins in protoplasts derived from inf than from emb (data not shown). These charge differences are also higher when comparing E to NE (Table II).

Both Ca^{2+} ions and phytohormones presence in medium influenced the charge of liposomes ($q_{liposome}$) and Δq values (characterising integral proteins in protoplasts).

In the absence of Ca^{2+} ions, the addition of kinetin and 2,4-D caused a decrease in Δq for cells from NE calli. For cells from E calli Δq changes depend on the explant origin: for inf all given phytohormones decrease Δq, while for emb they do not affect the Δq value.

Table. II. Effects of phytohormones on electrokinetic charge density (q) calculated from zeta potential measurements according to the Gouy-Chapman equation (Obi *et al*. 1989).

NON-EMBRYOGENIC CALLUS (NE)

Treatment	Inflorescence (inf)			Embryos (emb)		
	q protoplast [mC/m^2]	q liposome [mC/m^2]	Δq proteins (q protoplast $-q$ liposome)	q protoplasts [mC/m^2]	q liposomes [mC/m^2]	Δq proteins (q protoplast $-q$ liposome)
$0Ca^{2+}$ - hormone	−2.22±0.08	−4.02±0.06	1.80±0.01	−2.40±0.11	−3.71±0.12	1.32±0.03
$0Ca^{2+}$ + KIN	−2.24±0.11	−3.78±0.11	1.54±0.04	−2.12±0.11	−3.02±0.08	0.90±0.03
$0Ca^{2+}$ + 2,4-D	−1.82±0.08	−3.08±0.14	1.26±0.05	−1.97±0.15	−2.78±0.10	0.81±0.09
$0Ca^{2+}$ + ZEA	−2.02±0.12	−3.85±0.17	1.83±0.06	−1.93±0.10	−3.16±0.09	1.23±0.07
$2\times10^{-5}Ca^{2+}$ - hor.	−1.71±0.05	−2.63±0.07	0.92±0.06	−1.55±0.10	−2.52±0.10	0.97±0.05
$2\times10^{-5}Ca^{2+}$ + KIN	−1.47±0.08	−2.26±0.14	0.79±0.09	−1.53±0.12	−2.20±0.10	0.67±0.06
$2\times10^{-5}Ca^{2+}$ + 2,4-D	−1.22±0.10	−1.95±0.12	0.73±0.07	−1.04±0.15	−1.69±0.08	0.64±0.05
$2\times10^{-5}Ca^{2+}$ + ZEA	−1.82±0.10	−2.28±0.13	0.46±0.09	−1.55±0.10	−1.92±0.09	0.37±0.05

EMBRYOGENIC CALLUS (E)

Treatment	Inflorescence (inf)			Embryos (emb)		
	q protoplasts [mC/m^2]	q liposomes [mC/m^2]	Δq proteins (q protoplast $-q$ liposome)	q protoplasts [mC/m^2]	q liposomes [mC/m^2]	Δq proteins (q protoplast $-q$ liposome)
$0Ca^{2+}$ – hormone	−1.44±0.11	−3.43±0.10	2.00±0.05	−1.63±0.11	−3.27±0.09	1.64±0.08
$0Ca^{2+}$ + KIN	−1.47±0,12	−3.13±0.09	1.66±0.06	−1.57±0.10	−3.21±0.09	1.64±0.08
$0Ca^{2+}$ + 2,4-D	−1.57±0.10	−3.30±0.12	1.73±0.09	−1.43±0.10	−3.18±0.10	1.76±0.08
$0Ca^{2+}$ + ZEA	−1.48±0.10	−2.72±0.10	1.24±0.06	−1.46±0.10	−3.04±0.10	1.59±0.08
$2\times10^{-5}Ca^{2+}$ – hor.	−1.52±0.10	−2.58±0.08	1.05±0.08	−1.44±0.12	−3.35±0.10	1.91±0.10
$2\times10^{-5}Ca^{2+}$ + KIN	−1.30±0.09	−1.67±0.10	0.37±0.08	−1.41±0.10	−2.48±0.10	1.07±0.06
$2\times10^{-5}Ca^{2+}$ + 2,4-D	−1.30±0.12	−2.65±0.10	1.35±0.08	−1.41±0.10	−2.32±0.09	0.91±0,07
$2\times10^{-5}Ca^{2+}$ + ZEA	−1.15±0.10	−2.26±0.12	1.11±0.10	−1.33±0.10	−2.34±0.09	1.01±0.08

KIN – kinetin, ZEA – zearalenone.

In the absence of phytohormones, the presence of Ca^{2+} ions in medium caused a steady decrease in Δq values for NE objects. The presence of Ca^{2+} ions brings about a difference in phytohormone action. For NE calli at the 2×10^{-5}M Ca^{2+} ions Δq values obtained for plant hormone treated objects were lower in comparison to objects treated with media without hormones, especially significant in the case of zearalenone.

In the case of embryogenic calli at 2×10^{-5}M Ca^{2+} ions concentration all hormones studied caused a similar decrease in Δq values for emb objects (in comparison to non-hormone treated, Δq^0), whereas in the case of inf objects a noticeable decrease in Δq was observed for kinetin.

Discussion

The zeta potential changes of winter wheat protoplasts and liposomes indicate the possibility of phytohormones adsorption on the investigated surfaces. The Gouy-Chapman model used for electrokinetic charge calculation in the presented studies, has been usually adopted for the plant cell membranes responding to ionic solutes (Kinraide *et al*., 1998). Obi *et al*. (1989) studied zeta potential changes of barley cell protoplasts modified by organic groups. Earlier Pillet (1989) investigated the influence of auxin (IAA) on zeta potential of protoplasts obtained from the lower and the upper sides of the elongating part of gravireacting maize roots.

As most bio-surfaces of cells exhibit negative values of zeta potentials, the negatively charged polystyrene latex (L 88) was used to compare the

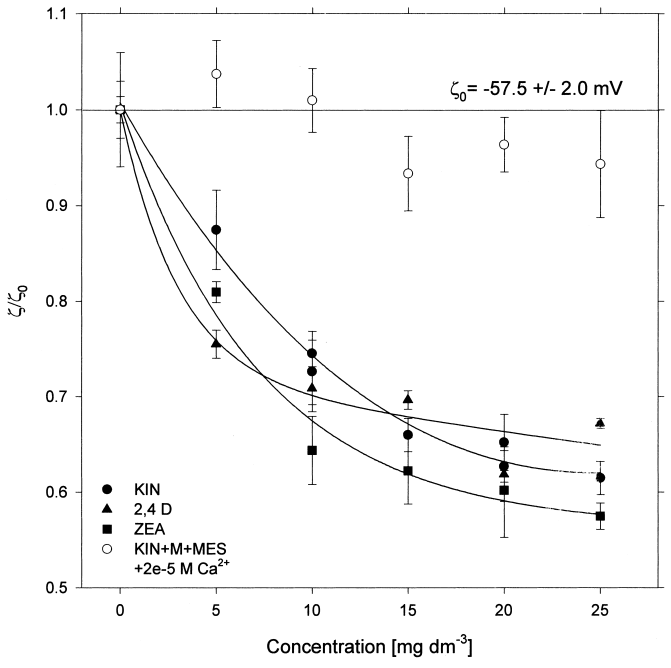

Fig. 1 A. The ratio of zeta potential of polystyrene latex (L 88) after treatment by phytohormone (KIN-kinetin, 2,4-D and ZEA-zearalenone) solutions (ζ) to its value measured in the absence of phytohormones (ζ_0). Full points correspond to aqueous phytohormone solution, empty points represent the data obtained for kinetin (KIN) in solution containing mannitol, MES and 2×10^{-5}M Ca^{+2}. The ζ / ζ_0 data represent the average of three separate experiments (± SD), counting at least 10 replicates for each experiment.

hormone adsorption on model negative centres. The relative changes of latex ζpotentials measured in water solutions (no buffer and no mannitol) as a function of phytohormone concentrations are presented in Fig. 1 by full points. Conductance of kinetin and zearalenone solutions indicates that these phytohormones did not contribute to an increase in ion content of water (data not indicated). However, 2,4-D dissociation led to significant change of pH value increasing amount of ions in solutions thus measured potentials corresponded to variable ionic strength. The medium composed of mannitol, calcium ions and the buffer (MES) levels off the changes of latex zeta potential that is caused by phytohormones determined in the water solutions (empty points presenting kinetin effect in Fig. 1).

The effect of kinetin and 2,4-D at constant and the same concentration and in the same medium as those used for biological objects on electrokinetic properties of latex was presented in Fig. 2. Values of zeta potential and surface charge (Fig. 2B) were more strongly dependent on the Ca^{2+} ion addition then on phytohormones. It is worth noticing that in solutions containing MES and mannitol of constant ionic strength, the adsorption of both kinetin and 2,4-D led to a de-

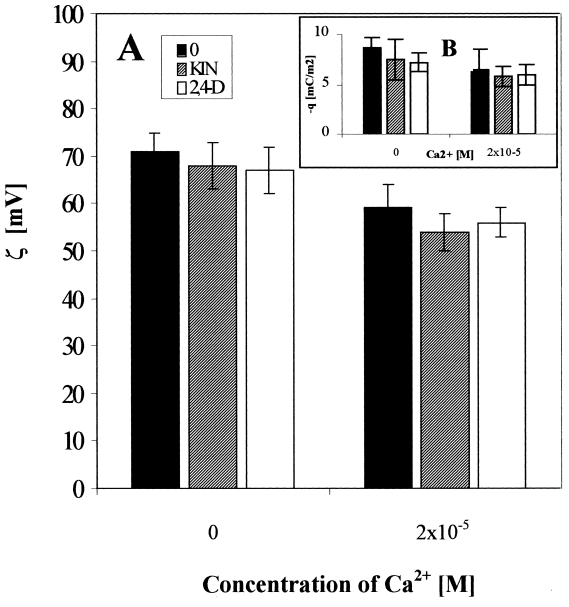

Fig. 2. Zeta potentials (A) and electrokinetic charge (inset B) of polystyrene latex in 25 mg·dm^{-3} phytohormones (KIN – kinetin, 2,4-D) solutions containing MES, mannitol and 0 an 2×10^{-5} M Ca^{+2}. The data represent the average of three separate experiments (± SD), counting at least 10 replicates for each experiment.

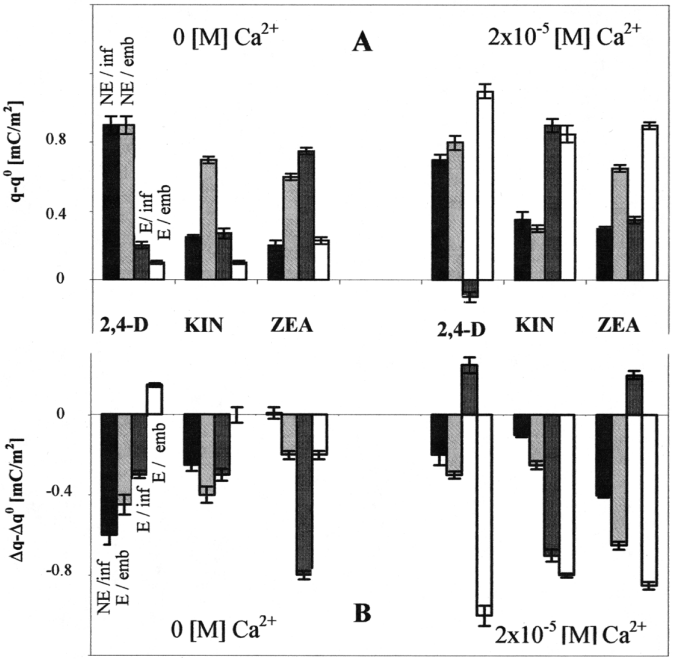

Fig. 3. The difference of charges of phytohormone-treated (q or Δq) and non-treated (q^0 and Δq^0) phospholipids (from liposomes) (A) and integral proteins (B) calculated from Table II. NE – non-embryogenic calli, E – embryogenic calli , inf – inflorescence, emb – embryos, KIN – kinetin, ZEA – zearalenone. The data represent the average of three separate experiments (± SD), counting at least 10 replicates for each experiment.

crease in charge absolute value. Similar changes were observed in biological systems studied.

Liposomes can be treated as model biological objects exhibiting only negative sites on their surface. Different values of zeta potential registered for studied types of liposomes could be connected with their various phospholipid composition. More negative values of charge density for liposomes (in medium without Ca^{2+} and phytohormones) prepared from NE cultures in comparison to liposomes obtained from phospholipides of E, are in accordance with the existence of higher amount of more negatively charged phospholipids (like phosphatidylinositol, phosphatidylserine, phosphatidylic acid – data not presented). In NE objects changes of zeta potential and surface charge of liposomes indicate that investigated phytohormones could be bound to negatively charged phospholipid surfaces leading to a decrease in their (ζ and q) absolute values (Table I).

However, it was suggested that in biological systems proteins located in the plant cell protoplasts play an important role in both auxin and cytokinin binding to cell surface and participate in cell transport as receptor proteins (Lomax *et al.*, 1995; Delbarre *et al.*, 1996) Perhaps such proteins could act as an adsorption site for studied phytohormones in the plasmalemma. Differences in electrokinetic charges prescribed to proteins (Δq) and liposomes ($q_{\text{liposomes}}$) could be useful in the answering the question whether investigated phytohormones are adsorbed on the specific protein receptors or on phospholipids situated in the plasmalemma. Comparing the values of charge differences for reference system (untreated with hormones) (Δq^0 – for proteins and $q^0_{\text{liposomes}}$ – for phospholipids; the values presented as – hormon in Table II) and system after hormone treatment (Δq and $q_{\text{liposomes}}$ for proteins and phospholipids, respectively) one can judge about the effect of various hormones on protein receptors (Fig. 3). In media containing no Ca^{2+} ions, binding of 2,4-D to protein-receptor could explain a decrease in $\Delta q - \Delta q^0$ values in both types of NE cells (inf and emb) (Fig. 3B). Similarly a noticeable decrease in $\Delta q - \Delta q^0$ indicates an existence of kinetin (cytokinin) binding sites in NE cells (Fig. 3B). No differences between $\Delta q - \Delta q^0$ values in the presence of zearalenone (except of E inf objects) (Fig. 3B) suggest that this phytohormone acts mainly on phospholipid layer (Fig. 3A). In the case of E objects the effect of hormones is generally smaller (inf) or none (emb) except the case of zearalenone which causes a meaningful change of protein charge for inf surfaces (Fig. 3B).

Ca^{2+} presence in the medium for both cells and protoplasts is usually necessary for the stabilisation of electric potential of cell surface. Ca^{2+} is added to culture medium as a standard component in MS nutrient. The presented experiments show that Ca^{2+} ions generally modified the effect of phytohormones on both phospholipids and proteins. The comparison of the action of studied phytohormones in media supplemented with calcium ions leads to the following conclusions:

1). Kinetin (Fig. 3). For NE objects kinetin has very little effect on both phospholipids and proteins. For E objects the kinetin influence increases in the presence of Ca^{2+} ions similarly for lipids and proteins.

2). 2,4-D (Fig. 3). In the case of NE (inf and emb) 2,4-D acted more strongly on the phospholipid charges in comparison to charges of protein. This phytohormone practically did not change the electrical state of E objects except of an especially great decrease in emb protein charge in medium containing 2×10^{-5} Ca^{2+} ions.

3). Zearalenone (Fig. 3). Phospholipids and proteins charge modification due to zearalenone treatment is similar for NE objects whereas in the case of E objects the effect depends on the tissue type (inf or emb) and especially strongly depends on the calcium ion content.

From these observations it is evident that phytohormones influence on the electrostatic state of cell surfaces is connected with both phospholipids and proteins composition characteristic for inf and emb as well as for NE and E. The model used for the calculation of proteins ionogenic groups contribution at the protoplasts surface should be only treated as the zero approximation because the effect of sterols and sucrose groups (present in biological surfaces) was not taken into consideration. However, the results obtained for kinetin and 2,4-D indicate that composition of phospholipids and proteins typical for NE cells are favourable for 2,4-D binding whereas E objects attract kinetin. This could explain why auxin presence is necessary for non-embryogenic cell growth and cytokinin presence is important for embryogenic cells to grow. The results obtained for zearalenone indicate that the phytohormone effect depends more strongly on the kind of explant than on the embryogenic competence of cells. Moreover the final effect of phytohormones influence on the negatively charged biological cells depended on both the ionic and organic environment of these surfaces.

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Abe S. and Takeda J. (1988), Effects of La^{3+} on surface charges, dielectrophoresis, and electrofusion of barley protoplasts. Plant Physiol. **87**, 389–394.

Arz M. C. and Grambow H. J. (1994), Polyphosphoinositide phosphatase C and evidence for inositol-phosphate-hydrolysing activities in the plasma-membrane fraction from light-grown wheat (*Triticum aestivicum* L.) leaves. Planta **195**, 57–62.

Caaverio J. M. M, Echabe I., Gutiérrez-Aguirre I., Arrondo J. L. R and González-MaHas J. M. (2001), Differential interaction of equinatoxin II with model membranes in response to lipid composition. Biophys. J. **80**, 1343–1353.

Carnes A. and Wright M. S. (1988), Endogenous hormone levels of immature corn kernels of A188, Missouri-17, and Dekalb XL-12. Plant Sci. **57**, 195–203.

Delbarre A., Muller P., Imhoff V. and Guern J. (1996), Comparison of mechanisms controlling uptake and accumulation of 2,4-dichlorophenoxy acetic acid, napthalene-1-acetic acid, and indole-3-acetic acid in suspension-cultured tobacco cells. Planta **198**, 532–541.

Filek M. and Kościelniak J. (1996), The effect of chilling maize shoots and roots on the electric potential, composition of fatty acids and the ATPase activity in the non-chilled organs of seedlings. J. Agron. Crop. Sci. **177**, 261–267

Fu Y. F. and Meng F. J. (1993), The effects of zearalenone on the growth and development of *Lemna gibba* G_3. Acta Phytophysiol. Sin. **9**, 359–398.

Kinraide T. B., Yermiyahu U. and Rytwo G. (1998), Computation of surface electrical potentials of plant cell membranes. Plant Physiol. **118**, 505–512.

Libbenga K. R. and Mennes A. M. (1995), Hormone binding and signal transduction. In: Plant Hormones: Physiology, Biochemistry and Molecular Biology (P. J. Davies, ed.). Kluwer Academic Publishers, Dordrecht, pp. 272–297.

Lomax T. L., R. J. Mehlhorn and Briggs W. R. (1995), Auxin transport. In: Plant Hormones: Physiology, Biochemistry and Molecular Biology. 2nd edn. (P. J. Davies, ed.). Kluwere Academic Publishers, Dordrecht, Boston, London, pp. 509–530.

Marcińska I., Filek M., Biesaga-Kościelniak J., Sagi F. and Bartok T. (2001), Cytokonin activities in cells offf wheat inflorescence in dependence of its developmental stage. Cell. Mol. Biol. Let. **6**, 313–318.

Marcińska I., Szechyńska-Hebda M., Biesaga-Kościelniak J. and Filek M. (2001), Applicability of polish winter wheat (*Triticum aestivum* L.) cultivars to long-term in vitro culture. Cereal Res. Commun. **29**, 127–134.

Murashige T. and Skoog F. (1962), A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. **15**, 173–197.

Obi I., Ichikawa Y., Kakutani T. and Senda M. (1989), Electrophoretic studies on plant protoplasts. I. pH dependence of zeta potentials of protoplasts from various sources. Plant Cell Physiol. **30**, 439–444.

Pillet P. E. (1989), Some physiological properties of protoplasts from gravireacting maize roots. In: The Physiological Properties of Plant Protoplasts (P. E. Pillet, ed.). Springer Publ. Berlin, pp. 227–235.

Rubery T. (1981), Auxin response. Annu Rev. Plant Physiol. **32**, 569–596

Sommarin M., Lundborg T. and Kylin A. (1985), Comparison of K, MgATPases in purified plasmalemma from wheat and oat. Substrate specificites and effects of pH, temperature and inhibitors. Physiol. Plant. **65**, 259–263.

Uemura M. and Yoshida S. (1984), Involvement of plasma membrane alternations in cold acclimation of winter rye seedlings (*Secale cereale* L. cv. Puma). Plant Physiol. **75**, 818–826.