Cation Fluxes Cause Plasma Membrane Depolarization Involved in β -Glucan Elicitor-Signaling in Soybean Roots

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Submitted 25 February 2005. Accepted 9 May 2005.

Inducible and specific ion fluxes on plasma membranes represent very early events during elicitation of plant cells. The hierarchy of such ion fluxes involved is still unknown. The effect of *Phytophthora sojae*-derived β -glucan elicitors on the plasma membrane potential as well as on surface K^+ , Ca^{2+} , and H^+ fluxes has been investigated on soybean roots using ion-selective microelectrodes. β -Glucans with different degrees of polymerization transiently depolarized the plasma membrane. The elicitor concentration necessary for half-maximal depolarization closely resembled the corresponding binding affinities of soybean root membranes toward the respective β -glucans. Upon repeated elicitor treatment, the root cells responded partially refractory, suggesting a complex responsiveness of the system. Within the root hair space, characteristic decreasing K^+ - and Ca^{2+} -free concentrations were induced by the elicitors, probably causing depolarization through the influx of positive charges. Whereas K^+ fluxes were inverted after passing the K^+ equilibrium (Nernst-) potential, Ca^{2+} influx continued. No anion fluxes sufficient to account for charge compensation were observed under the same experimental conditions. K^+ and Ca^{2+} fluxes as well as depolarization were inhibited by 100 μ M or less of the Ca^{2+} antagonist La^{3+} . Contrasting other systems, in soybean the main cause for elicitor-induced plasma membrane depolarization is the activation of cation instead of anion fluxes.

Additional keywords: β -Glucan receptor, *Glycine max*.

Plant roots take up water and a variety of nutrients from the soil. Thus, they are exposed to soilborne attacking or symbiotic microorganisms, whereby roots and root hairs function as highly sensitive microantennas for compounds released from such microorganisms. Root cells have developed a highly efficient and specific chemosensorial machinery to recognize chemical signals at very low concentrations (Boller 1995). If derived from pathogenic microorganisms, these signals (elicitors; e.g., oligosaccharides, glycoproteins, peptides, and fatty acids) induce specific signaling processes that activate defense reactions in the host plants (Boller 1995; Ebel and Scheel 1997; Nürnberger and Scheel 2001; Shibuya and Minami 2001). Moreover, a variety of “active” substances (e.g., cell-wall-degrading enzymes such as cellulases, xylanases, or pectinases) reaches the plant surface and elicits responses as well.

Soybean (*Glycine max* L.) is known to respond to elicitation with β -glucans—derived from cell walls of the phytopathogenic oomycete *Phytophthora sojae* and carrying the hepta-(1,3-1,6)- β -glucoside motif—in a manner comparable with soybean plants challenged with the pathogen (Ebel 1998). The (1,3-1,6)- β -glucan elicitors are very well characterized with regard to their biological activities in differentiated plants and cell cultures, and to the biochemical properties of the receptor–ligand interactions (Ebel 1998; Ebel and Mithöfer 1998). Recently, the cloned hepta- β -glucoside-binding protein cDNAs from soybean and French bean (*Phaseolus vulgaris* L.) have been identified as members of a putative receptor family in legumes (Mithöfer et al. 2000). Upon β -glucan binding to the receptor, various early signaling events are initiated within minutes, such as a transient increase of the free cytosolic Ca^{2+} concentration and the activation of protein kinases. Moreover, the synthesis of reactive oxygen species and, after a couple of hours, specific gene activations have been observed (Ebel and Mithöfer 1998). In soybean and other legumes, distinct cellular reactions finally lead to the accumulation of phytoalexins, representing one important part of a set of inducible defense reactions (Cosio et al. 1996; Ebel 1998).

Other typical early plant responses to elicitor signals are voltage changes at the plasma membrane, usually a depolarization (Felle et al. 2000; Kikuyama et al. 1997; Mathieu et al. 1991; Pugin et al. 1997; Thain et al. 1995), and different ion fluxes (Felle et al. 2000; Jabs et al. 1997; Kadota et al. 2004; Kuchitsu et al. 1997; Mathieu et al. 1991; Pugin et al. 1997; Wendehenne et al. 2002), of which the external alkalization may be the best known (Boller 1995). The meaning and function of these early events on the plasma membrane is still a matter of debate. The ion fluxes could be part of either the receptor-mediated signal transduction cascade or the plant defense reactions. However, a voltage change at the plant plasma membrane cannot represent a signal involved in the crosstalk of microorganisms and plants because it does not reach the causative microbe. On the other hand, external changes of ion activities might be, and probably are, signals directed to the attacking organisms. External alkalization, for example, could make transport capacities unfavorable with respect to the proton-motive force required for an effective transport of organic substrates. This, however, only holds true in cases of persistent alkalization, which is not always the case (Felix et al. 1993). Likewise, the hierarchy of ion fluxes that occur in response to an elicitor is still quite uncertain, although a general consensus

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seems to emerge that, upon the perception of an elicitor by the corresponding receptor, at first a Ca^{2+} channel might be activated. As a consequence, cytosolic Ca^{2+} activity increases that activates anion channels and, in turn, the anion efflux causes plasma membrane depolarization. This sequence has been suggested for elicitors such as the oligopeptide elicitor in parsley (Nürnberg et al. 1994), cryptogin and oligogalacturonides in tobacco (Kadota et al. 2004; Lecourieux et al. 2002; Wendehenne et al. 2002), and chitoooligosaccharides in rice (Kuchitsu et al. 1997) and alfalfa (Felle et al. 2000), as well as for symbiotic Nod factors in alfalfa (Felle et al. 1998, 2000). Nevertheless, other scenarios resulting in the induction of depolarization are feasible, as are deactivation of the plasma membrane H^+ -ATPase, activation of a K^+ channel, or an unspecific increase of membrane conductance.

In the present study using soybean root cells challenged by β -glucan elicitors, we show that the activation of cation fluxes involving K^+ and Ca^{2+} is the predominant event leading to membrane depolarization instead of anion fluxes. To demonstrate this, we analyzed and characterized the effects of β -glucan elicitors on both the plant plasma membrane potential and, using ion-selective microelectrodes, on ion fluxes within the root hair space before and during elicitor action.

RESULTS

The basic depolarization response.

Soybean root cortex cells as well as root hair cells were used to analyze the change of their plasma membrane potential in response to treatment with *Phytophthora sojae*-derived β -glucan elicitors. The addition of β -glucans with a degree of polymerization (DP) 7 to 15 (DP7-15) induced a concentration-dependent membrane depolarization (Fig. 1). A slight reaction was already detectable at approximately 10 nM DP7-15 elicitor (data not shown). Depolarization was saturated at approximately 10 μM and the effector concentration necessary for the induction of the half-maximal response (EC_{50}) was calculated to be approximately 280 nM (Fig. 1B; Table 1). The onset of the membrane potential change upon elicitor treatment was delayed, which was proven by the addition of 20 mM KCl which, upon encounter with the plasma membrane, caused an immediate depolarization without any delay (Fig. 1A). At half-maximal elicitor concentration, this lag-phase persisted for 1 to 2 min. At lower elicitor concentrations, the response delay increased (data not shown).

Specificity of plasma membrane depolarization.

In order to investigate the specificity of the depolarization response, a variety of β -glucan fractions were used as elicitors. All β -glucans with a DP greater than 5 were highly efficient stimulants of depolarization (Fig. 2). Depending on the DP of the β -glucans tested, this membrane response differed to some extent; as such, DP15 elicited stronger responses than equal concentrations of DP7-15 or DP16-24, the latter two being about equally active. No depolarization was observed in the presence of DP5 at any concentration tested between 0.1 and 5 μM . A comparison of these biological activities of the various β -glucans with the corresponding binding activities to the β -glucan receptor in soybean roots is presented in Table 1. The data suggest a direct correlation between the apparent binding affinity for a certain β -glucan fraction (expressed as IC_{50} , the required concentration for half-maximal displacement of the hepta- β -glucoside radioligand) (Cosio et al. 1990; Mithöfer et al. 2000) and the corresponding apparent depolarization activity (shown as EC_{50} , which was calculated from Fig. 2B by nonlinear regression analysis using logistic curve fitting).

Desensitization of the depolarization response.

Various specific perception systems for signaling molecules in plants have been described to show a desensitization effect when treated with the same elicitor for a second time (Felix et al. 1993, 1998; Felle et al. 1995; Granado et al. 1995). A second and third addition of DP15, following an initial treatment with the identical β -glucan elicitor and subsequently a washout and recovery phase, caused a depolarization response that became progressively weaker, although the response was not abolished altogether during the period investigated (Fig. 3A).

Table 1. Correlation between the apparent soybean root cell depolarization activity (EC_{50}) for β -glucan fractions with various degrees of polymerization (DP) and the corresponding apparent binding affinity (IC_{50}) of soybean root membranes^a

β -Glucans	EC_{50} (nM)	IC_{50} (nM)
DP5	ND	>6,000
DP7-15	280	250
DP15	120	180
DP16-24	190	40

^a Effector concentration necessary for the induction of the half-maximal response (EC_{50}) values were calculated by nonlinear regression analysis using logistic curve fitting. ND = not determined.

Fig. 1. Dose-response relationship of β -glucan elicitor (DP7-15) on the plasma membrane potential of soybean root cells. **A**, Kinetics of depolarization. At the end of each test, 20 mM KCl was added (only shown with 2.5 μM DP7-15) to determine the lag-phase. **B**, Maximal depolarization measured at the indicated DP7-15 concentrations. Each data point represents an independent determination.

Likewise, when DP7-15 was added following DP15 treatment, the response also was reduced by approximately 50% (Fig. 3B). No such effect was observed when the effective DP16-24 elicitor was tested after the addition of the apparently inactive DP5 fraction (Fig. 3C).

β -Glucan-induced ion fluxes in soybean root cells.

Ion-selective microelectrodes are valuable tools for studying ion fluxes across membranes (Felle 1993; Newman 2001). The

induction of such ion fluxes represent an integral part of early plant responses to elicitors (Felix et al. 1993; Kadota et al. 2004; Kuchitsu et al. 1997; Lecourieux et al. 2002; Mathieu et al. 1991; Mithöfer et al. 1999; Nürnberger et al. 1994; Wendehenne et al. 2002) or Nod factors (Felle et al. 1998; Kurkdjian et al. 2000). Although, in the former studies, fluxes of Ca^{2+} , Cl^- , or NO_3^- , H^+ , and K^+ are described to take place upon encounter with the respective first signaling molecules, their temporal sequence and physiological poise has not yet been cleared up

Fig. 2. Specificity of β -glucan elicitors differing in the degree of polymerization (DP5, DP15, and DP16-24) on the plasma membrane potential of soybean root cells. **A**, Kinetics of elicitor-induced changes in plasma membrane potential. **B**, Concentration-dependent maximal depolarization induced by DP15 and DP16-24.

Fig. 3. Desensitization and mutual interference of β -glucan elicitors with different degrees of polymerization. **A**, Plasma membrane potential of soybean root cells after repeated addition of DP15 at 1 μM . Time of washout procedure between the treatments as indicated. Effect of DP7-15 on membrane potential following a foregoing treatment with **B**, DP15 or **C**, DP5 and DP16-24.

in all cases. In order to analyze the hierarchy of the β -glucan-induced ion fluxes in soybean root cells, we used ion-specific microelectrodes. The extracellular root hair space suffered a loss of Ca^{2+} after the addition of $1\ \mu\text{M}$ DP7-15 or DP15 (Fig. 4). Simultaneously, measured on the same root, the external K^+ activity dropped within the first 2 min but recovered partially or completely. Also, the membrane depolarization has been recorded simultaneously with the changes in external K^+ and Ca^{2+} activities. Given a cytosolic root hair K^+ concentration of 70 to 90 mM (as determined by measuring the diffusion potential at different external K^+ concentrations) and measuring 50 to 60 μM K^+ in the root hair space, an equilibrium potential for K^+ (E_K) can be calculated (Fig. 4A, shaded bar). Moreover, external K^+ concentration decreased as long as the membrane voltage was negative to E_K , but recovered (increased) to again reach the initial concentration after getting positive to E_K . The Ca^{2+} equilibrium potential is far more positive; therefore, the Ca^{2+} fluxes were not affected that way (Fig. 4A).

Using the same technique, no or only minor anion movements within the root hair space were detected during these events, a finding which differed considerably from the massive transient Cl^- fluxes measured in alfalfa (Felle et al. 1998, 2000). This also counts for NO_3^- because the Cl^- electrode used in the present study senses NO_3^- as well. Consequently, no effect of the anion channel inhibitors anthracene-9-carboxylate (A9C) and 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB) was detectable on the β -glucan elicitor-induced depolarization (data not shown). On the other hand, La^{3+} (a well known inhibitor of Ca^{2+} and K^+ channels) strongly inhibited β -glucan-induced K^+ and Ca^{2+} fluxes (Fig. 5A and B) as well as the accompanying depolarization in a concentration-dependent manner (Fig. 6). Nifedipine, an inhibitor of voltage-gated Ca^{2+} channels, had no effect on either depolarization or on Ca^{2+} or K^+ fluxes (data not shown).

External alkalinization, a typical elicitor response in plant cells (Felix et al. 1993; Felle et al. 2000; Kadota et al. 2004; Kuchitsu et al. 1997), also could be demonstrated after addition of the β -glucan elicitor, albeit the response to $1\ \mu\text{M}$ elicitor was less than half a pH unit (Fig. 5C, inset). The addition of $100\ \mu\text{M}$ La^{3+} , which itself strongly acidified the root hair space, eliminated the elicitor-induced pH increase (Fig. 5C).

Fig. 5. Effect of La^{3+} (LaCl_3) on β -glucan-induced ion fluxes, measured within the root hair space of soybean. **A**, Elicitor-induced changes in K^+ activity (pK) followed by joint addition of $1\ \mu\text{M}$ DP15 and $100\ \mu\text{M}$ La^{3+} ; W = removal of elicitor. Inset: Addition of La^{3+} followed by joint addition of La^{3+} + DP15. **B**, Changes in external Ca^{2+} activity (pCa) after addition of La^{3+} followed by joint addition of La^{3+} + DP15. **C**, Changes in external pH after addition of La^{3+} followed by joint addition of La^{3+} + DP15.

Fig. 4. K^+ and Ca^{2+} fluxes within the root hair space of soybean following addition of β -glucan elicitors with different degrees of polymerization. **A**, Effect of $1\ \mu\text{M}$ DP15. Simultaneous measurement of membrane potential, Ca^{2+} activity (pCa), and K^+ activity (pK). E_K is the transmembrane equilibrium potential as determined from the cytosolic K^+ activity and measured external K^+ activity. Cytosolic K^+ activity of 70 to 90 mM was determined by measuring the diffusion potential (e.g., in the presence of cyanide) at different external K^+ activities (data not shown). **B**, K^+ and Ca^{2+} activities, measured after the addition of DP7-15.

DISCUSSION

Using sensitive electrophysiological methods that permit online analysis, we demonstrated that different β -glucan elicitor fractions from the mycelium of *P. sojae* induced concentration-dependent and specific responses at the plasma membrane of soybean root cells. As typical for elicitors, the first measurable cell physiological response occurred with an apparent lag-phase. This period probably is consumed by the perception of the signal molecules and the subsequent processes of transduction and transformation of the respective signal and its propagation to the corresponding cellular targets; a phenomenon typical for receptor-mediated signaling cascades and observed for several signaling processes.

In part, the β -glucan elicitor-induced depolarization is a refractory process (Fig. 3A). Although desensitization seems to be a common phenomenon for elicitor action, it is differently expressed in different test systems and it also depends on the type of elicitor used. Felix and associates (1993) demonstrated that, in tomato cell suspension cultures, the alkalization-inducing ability of elicitors dropped to the detection limit, lasting 2 h in the case of xylanase and 4 h with chitinase. Our data revealed that, in soybean roots, the β -glucan response de-

creased by 60% after approximately 1 h, but did not reach zero even after repeated addition of the stimulus (Fig. 3A and data not shown). The partially refractory state of the β -glucan-treated root cells might suggest some so far unknown complexity of signal transduction in this system, possibly consisting of a refractory response of the true receptor and an additional responsiveness mediated by another nonrefractory binding site. A spontaneous recovery from depolarization events has been interpreted in terms of a desensitization of the specific perception system (Felix et al. 1998). In our study, such a monocausal interpretation collides with the ability of the system to respond a second time to the same signal. Thus, other mechanisms might be involved in the depolarization process of the membrane potential back to its resting level. It is conceivable that either activation through a nonrefractory elicitor-binding site of the plasma membrane H^+ -ATPase or, more likely, a spontaneous inactivation of elicitor-activated ion channels occurs, as indicated by the results of the K^+ measurements (Fig. 4).

Although elicitor-induced depolarization may be regarded as a common and not necessarily specific defense response, it has been proven to be a valuable tool in the investigation of membrane transport and functional perception of signal molecules (Felle et al. 2000; Kuchitsu et al. 1997; Mathieu et al. 1991;

Fig. 6. Effect of La^{3+} on the plasma membrane potential of soybean root cells elicited by β -glucans. Depolarization induced by various concentrations of **A**, DP7-15 or **B**, DP15 measured in the presence of the respective La^{3+} concentrations.

Fig. 7. Simplified model of the transmembrane ion fluxes involved in the β -glucan-induced depolarization of soybean root cells. PL = plasma membrane and E = elicitor.

Pelessier et al. 1986; Pike et al. 1998; Thain et al. 1995; Zimmermann et al. 1998). It should be stressed that depolarization of the host plasma membrane cannot be a signal to the attacking microorganism, but voltage changes may serve in channel gating within that membrane. Note that ion fluxes across the plasma membrane cause rapid changes of free ion concentrations within the root apoplast, at the surface of the root, or in the root hair space, because the respective fluid volumes are rather small. Thus, already a small amount of ions that move across the membrane might rapidly change the conditions for the attacking organism.

Following elicitor treatment, calcium influx might be the earliest measurable response, subsequently increasing cytosolic free Ca^{2+} (Blume et al. 2000; Felle et al. 2000; Lecourieux et al. 2002). For soybean, this has been demonstrated using the aequorin technique (Mithöfer et al. 1999, 2001). Although, thus far, Ca^{2+} ion influx has been considered as being too small to depolarize the plasma membrane, there are reports on large elicitor-induced Ca^{2+} influxes that are discussed to contribute to the depolarizing event (Kadota et al. 2004; Pugin et al. 1997; Zimmermann et al. 1997). Thus, our data are in full agreement with the idea that Ca^{2+} ions can carry a substantial portion of the depolarizing current (Fig. 4). Apart from Ca^{2+} fluxes, a delayed K^{+} efflux has been observed that was regarded mainly as a charge-compensating and osmo-regulatory ion flux rather than related to a signaling cascade. Obviously, in soybean, this scenario is different. In root hair cells, after the usual lag-phase, β -glucan elicitors induced the onset of transient but substantial Ca^{2+} and K^{+} influx, both following different kinetics. According to their flux direction at the beginning, both ions contributed to depolarization; however, as soon as the membrane potential had passed, the E_K , the driving force for K^{+} , was inverted and K^{+} left the cell thereafter (Fig. 4). By reducing the amount of depolarizing charges, this finally led to a voltage recovery, fuelled by the H^{+} -ATPase. Ca^{2+} acts differently: despite the depolarization, an inwardly directed driving force remained active. Thus, the inversion of the Ca^{2+} flux did not mark a change in driving force, but more likely a spontaneous inactivation of the Ca^{2+} channel and, subsequently, the export of Ca^{2+} , probably driven by the primary active Ca^{2+} -ATPase. A comparison of the primary K^{+} and Ca^{2+} losses from the root hair space until the onset of K^{+} flux inversion yields 4.82 $\mu\text{moles K}^{+}$ and 2.05 $\mu\text{moles Ca}^{2+}$ (DP15), and 4.26 $\mu\text{moles K}^{+}$ and 2.32 $\mu\text{moles Ca}^{2+}$ (DP7-15), respectively, representing quite similar values. The numbers show that the poise of the initial depolarization current was on the side of K^{+} , a situation that changed after the inversion of the K^{2+} current, because the depolarization process continued for some time (Fig. 4). The elicitor-induced and La^{3+} -inhibited Ca^{2+} and K^{+} fluxes as well as the accompanying depolarization (Fig. 6) is in line with observations of Zimmermann and associates (1997), who identified a Ca^{2+} - and K^{+} -permeable, La^{3+} -sensitive plasma membrane channel of high conductance in parsley protoplasts. This channel was reversibly activated upon treatment with an oligopeptide elicitor and has been considered to contribute to the elicitor-induced macroscopic ion fluxes in parsley.

Recently, Ivashikina and associates (2001) reported on a $(\text{GlcNAc})_8$ elicitor-induced activation of a K^{+} channel (GORK) in *Arabidopsis* root hairs which finally led to membrane depolarization, presumably fuelled by anion efflux. However, whereas, in *Arabidopsis*, no primary K^{+} influx was recorded and K^{+} efflux started when the membrane potential had passed the E_K , in soybean, large K^{+} influx preceded K^{+} efflux.

The failure to detect anion efflux might have been caused by the elicitor sensitivity of the anion microelectrode, which could have masked the elicitor response. However, this idea is unlikely because of the absence of any inhibitory effect of NPPB or A9C

on both the elicited depolarization and the poor pH response (Fig. 5C). This latter notion is based on the finding that efflux of organic anions that occurs simultaneously with the depolarizing Cl^{-} efflux can be one of the reasons for external alkalinization (Felle et al. 1998, 2000). Thus, in the light of a seemingly weak anion channel involvement, the rather small β -glucan-elicited external alkalinization is evident. However, anion channel activation cannot be entirely excluded. We reported earlier that NPPB clearly inhibited the β -glucan-elicited increase of the cytosolic Ca^{2+} activity in soybean suspension-cultured cells treated with much greater elicitor concentrations (100 μM) (Mithöfer et al. 2001). Because NPPB is not an inhibitor of Ca^{2+} channels but of anion channels, we concluded that, upon elicitor perception, first anion channels and subsequently Ca^{2+} channels were activated. However, a comparison with the data obtained in the present work using submicromolar elicitor concentrations might point to signaling systems with high versus low responsiveness for the β -glucan elicitor: high responsiveness in differentiated tissues such as roots or cotyledons (Cheong and Hahn 1991; Cosio et al. 1990; this work) and low responsiveness in undifferentiated suspension cells (Mithöfer et al. 1999). Although this phenomenon has been known for some time, the molecular mechanism behind it still is not clear. Thus, at an apparent sensitivity in the submicromolar range (Figs. 1 and 2; Table 1), the importance of anion channels for depolarization or the events leading to it must be rated as minor.

A sequence of causal relationships for β -glucan-elicited de- and repolarization in soybean root cells is suggested: a cation (Ca^{2+} , K^{+}) influx causes depolarization which is followed by a repolarization mediated by a K^{+} efflux (Fig. 7). This efflux is initiated after passing the K^{+} equilibrium potential, while Ca^{2+} influx continues. Our proposal substantially differs to others in that no anion channel is included. To date, we have no definite information whether K^{+} and Ca^{2+} , in fact, move through the same channel, but the inhibition by submillimolar La^{3+} concentrations of both ion fluxes suggests this idea. Recently, for the plasma membranes of *Arabidopsis* as well as of wheat, root voltage-independent cation channels (VICs) have been described which are permeable to both K^{+} and Ca^{2+} (Demidchik et al. 2002a; White and Davenport 2002). Inward currents through VICs are known to be sensitive to La^{3+} but insensitive to the Ca^{2+} channel inhibitor nifedipine (Demidchik et al. 2002b), consistent with the properties we found for the β -glucan-elicited depolarization in soybean (Figs. 5 and 6; data not shown). Moreover, the patch-clamp studies on parsley plasma membranes, showing an elicitor-responsive large conductance Ca^{2+} inward channel exhibiting K^{+} inward conductance as well, supports our finding (Zimmermann et al. 1997). Thus, future work will focus on a detailed electrophysiological characterization of the β -glucan-activated cation channel in soybean root plasma membranes to elucidate its specific role in initiating defense mechanisms.

MATERIALS AND METHODS

Plant material, β -glucan elicitors, and preparation of the roots.

Soybean (*Glycine max* L. cv. 9007) seed were kindly provided by Pioneer, Buxtehude, Germany. Plants were grown for at least 10 days in a phytochamber as described elsewhere (Schmidt and Ebel 1987). β -Glucans with different DP were prepared from mycelia cell walls of the phytopathogenic oomycete *P. sojae* Kaufmann and Gerdemann and separated by gel chromatography to determine the apparent DP (Schmidt and Ebel 1987). In β -glucan mixtures, the average DP was used to calculate the β -glucan concentration on a molar basis (Cosio et al. 1990; Schmidt and Ebel 1987).

Root pieces, approximately 2 cm long, were excised and fixed with candle wax on the bottom of a chamber which was constantly perfused with a solution usually containing 0.5 mM Mes/Tris (pH 6.7 to 6.9), 0.01 to 1 mM KCl, 0.1 mM NaCl, and 0.01 to 0.1 mM CaCl₂; any conditions different from these are given in the figure captions or text. Desired β -glucan elicitor concentrations were obtained by diluting aqueous stock solutions of 100 mM DP5, 25 mM DP7-15, 0.3 mM DP16-24, and 30 mM DP15. Prior to all measurements, the seedlings were incubated in the basic test solution for at least 2 h.

Membrane potential measurements.

The electrical set-up for the impalement of the root or root hairs and for recording of membrane potential has been described previously (Felle et al. 1995). The micropipettes were pulled from filamented borosilicate glass tubing (Hilgenberg, Malsfeld, Germany) to tips of approximately 0.5 μ m, and were backfilled with 0.5 M KCl. The Ag/AgCl half-cells were connected with a high-impedance amplifier (S-7000; World Precision Instruments, Sarasota, FL, U.S.A.). Data were collected on a chart recorder (Linseis 22200, Selb, Germany). The response time to the elicitor (lag-phase) was determined by subtracting the response time to a KCl pulse from the one to the respective elicitor used (Fig. 1A).

Measurements

of K⁺, Ca²⁺, and H⁺ using ion-selective microelectrodes.

Both the fabrication of ion-selective microelectrodes and their extracellular application, in particular in the root hair space, have been described previously (Felle 1993; Felle et al. 1998). Briefly, pipettes with tips of approximately 2 μ m were pulled on a two-stage puller (List, Darmstadt, Germany) and heat polished. Internal silanization was carried out after heating the pipettes to 200°C for approximately 1 h by dipping the rear ends into a 0.2% tributyl-silane/chloroform solution. To obtain a covalent bonding of the silane with the glass surface, the pipettes were heated for another hour. Cool pipettes were backfilled with the sensor. To give the sensor in the tip enough firmness to stay in place for extended use, the respective sensor cocktail (Fluka, Darmstadt, Germany) was dissolved in a mixture of poly(vinylchloride)/tetrahydrofuran (40 mg ml⁻¹) at a ratio of 30/70 (vol/vol). After evaporation of the tetrahydrofuran, the remaining firm gel was topped up with the undiluted sensor cocktail, followed by the reference solution, required for the respective ion to be tested. After equilibration, these electrodes gave stable responses for at least 2 weeks, when stored in a dry chamber (silica gel). To measure ion activities and ion fluxes within the respective root hair zone, electrode tips were placed approximately 10 μ m from the root surface. Electrodes were connected to a high-impedance amplifier (FD 223; World Precision Instruments) which simultaneously measured and subtracted the signals obtained from the ion-selective electrode and the voltage reference.

Binding assays.

Preparation of the root membrane proteins was carried out according to Cosio and associates (1988). Protein content was measured according to Bradford (1976). Binding assays were performed with 100 to 150 μ g of membrane protein, which was incubated with increasing concentrations of β -glucans with a different DP in the presence of 3 nM ¹²⁵I-labeled 2-(4-aminophenyl)ethylamine conjugate of hepta- β -glucoside as radioligand for 2 h on ice (Cosio et al. 1988). Synthesis of the radioligand was described earlier (Cosio et al. 1990). Values for half-maximal displacement (IC₅₀) of the radioligand were calculated from the data by nonlinear regression using Sigmaplot 7.0 (Jandel Scientific, San Rafael, CA, U.S.A.).

ACKNOWLEDGMENTS

This work was supported in part by the European Community's Human Potential Program under contract HPRN-CT-2002-00251, SACC-SIG-NET, and by the Deutsche Forschungsgemeinschaft (SFB 369, Fe 213/12-1). We thank J. Fliegmann for critical comments on the manuscript.

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