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A CHEMORECEPTIVE BILAYER LIPID MEMBRANE BASED ON AN AUXIN-RECEPTOR ATPase ELECTROGENIC PUMP

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Auxin-binding proteins have been extracted from coleoptiles and primary leaves of maize and diffusion - reconstituted in phosphatidyl choline/partially-oxidized cholesterol membranes. Measurement of membrane ion flux at 25 mV external potential with buffered KCl electrolyte was performed for the receptor support matrix and various combinations of ATP, receptor and naphthalene-1-acetic acid. Addition of the three components in any order results in a substantial increase in current with a limit-of-detection for auxin of about 10^{-7} M. The pH-dependence of the response is consistent with previous suggestions that an ATPase pump acts to translocate protons in the presence of K^+ and Mg^{2+} and that the pump can be activated by auxin. This work provides the first direct link between the binding of a plant hormone to a putative receptor and the evocation of a biochemical response.

The proposition that the bilayer lipid membrane offers potential as a model for selective electrochemical sensing has been discussed in terms of transduction of binding events into an altered transmembrane ionic flux (1). Although a wide range of BLM-electrical work has been performed in recent years, relatively few successful studies with reconstituted receptor systems have been reported. One area that has been reproduced a number of times is the active cholinergic system, where reconstitution of extracted cholinergic material in BLM has resulted in oscillation of membrane conductance over short-time excursions on ligand excitation (2-5).

Abbreviations used: BLM, Bilayer lipid membrane; NAA, napthalene-1-acetic acid; C_6H_5COOH, benzoic acid.

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Maize coleoptile membranes contain high affinity auxin-binding sites that have been studied in several laboratories (6,7). Reported dissociation constants for naphthalene-1-acetic acid, are in the range 0.1-1 μM and the sites are thought to function as auxin receptors, though the evidence remains equivocal (7-9). Treatments that solubilise the auxin-binding sites from the membranes (10, 11) also solubilise an ATPase, but the two activities can be resolved by gel permeation chromatography (11, 12). It has been proposed that auxin-induced cell elongation involves activation of a proton-pumping ATPase in the cell membrane (13) and several reports of ATP-dependent H^+ transport into plant membrane vesicles have appeared recently (14-16). In the present paper, we report the partial purification of the coleoptile receptor, its reconstitution in a planar BLM and generation of an auxin/ATP-dependent electrochemical response.

METHODS AND MATERIALS

Membranes were prepared from coleoptiles and primary leaves (100 g) of maize (Zea mays, cv. Blizzard) by differential centrifugation between 4000g and 80000g (17). Auxin-binding proteins were solubilised from the membranes by a non-detergent method and partially purified by ion exchange chromatography (10). The pooled active fractions were precipitated with ammonium sulphate (80% saturation) and desalted on a 6cm x 1.5cm column of Sephadex (Pharmacia) G25 to yield 2.4ml of excluded protein in 50mM sucrose, 1mM $MgSO_4$, 2mM citrate-acetate, pH5.5. Aliquots were assayed for protein (18) (1.4mg/ml), for ATPase (19) (0.1μmol/mg protein/min at 30°C), and for NAA-^{14}C binding (10)(384 pmol/mg protein at 0.15 M NAA). Based on a receptor protein GMW of 40,000(7) and an average of published estimates for binding site number and affinities (17, 20), the active receptor concentration in the preparation was calculated as 126μg/ml or about 3μM. The preparation was lyophilised in 100μl aliquots and stored dry at 4°C until ready to use. When required, the aliquots were each reconstituted in 100μl of distilled water and the required volumes were introduced directly into the electrochemical cell electrolyte as were NAA (as a methanolic solution), ATP and the inactive auxin analogue, benzoic acid. Lipid membranes prepared from phosphatidyl choline and partially oxidized cholesterol (both 2 percent w/v in n-decane) were supported in a circular aperture situated in a teflon sheet (1). An external +25mV direct potential was applied across the membrane between Ag/AgCl single junction electrodes using in each stirred solution compartment 5 ml of 0.1M KCl electrolyte adjusted to the required pH with buffer. In most cases, reactants were injected on the positive potential side of the membrane.

RESULTS AND DISCUSSION

First, a wide range of background effects with the individual chemical species including the receptor support matrix (sucrose, citrate, $MgSO_4$ and CH_3COOH) were studied. The most important membrane perturbation was caused

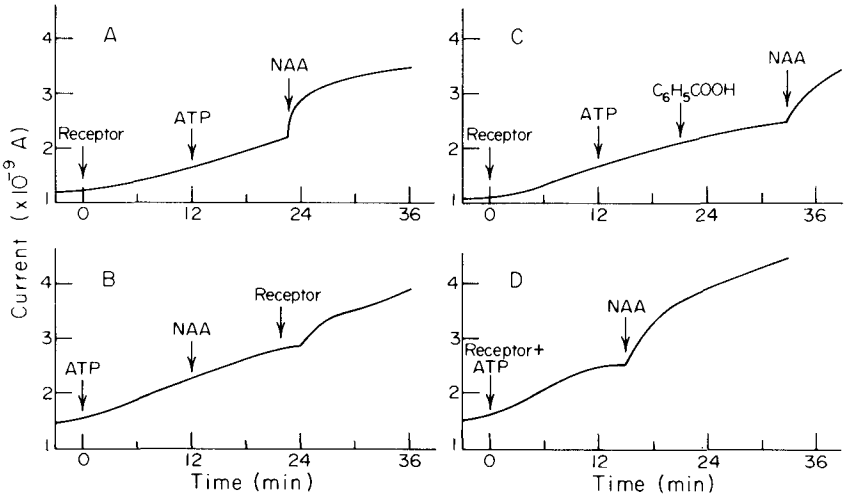

Figure 1. Electrochemical response of auxin receptor to NAA, ATP and C_6H_5COOH for various orders of addition to positive-potential side of membrane (A,B and C). Response for transmembrane addition of receptor/ATP and NAA (D). Final concentrations are -- receptor 6 x 10^{-9} M, ATP 4 x 10^{-6} M, NAA 2 x 10^{-5} M and C_6H_5COOH 2 x 10^{-5} M. The receptor support solution contained sucrose (0.05 M), Na citrate (0.002 M), $MgSO_4$ (0.001 M) and CH_3COOH (adjustment to pH 5.5).

by the receptor protein fraction. After a lag of 2 to 5 minutes, a small but rapid current increase was observed, followed by membrane rupture within 20 minutes for receptor concentrations greater than 2 x 10^{-8}M. In the presence of NAA (but not benzoic acid) rupture occurs at concentrations greater than 1 x 10^{-8}M. This increase in destabilization is likely caused by greater membrane incorporation of the receptor-NAA complex relative to free receptor or by conformational effects. Combinations of ATP and receptor solution or ATP and NAA produce minor changes in membrane ion flux. However, addition of the three components NAA, receptor and ATP, in any order, results in a substantial increase of current (Fig. 1, A,B). Approximately ninety percent of the total current increase is achieved over a period of two minutes except when the receptor is the final added reactant and membrane incorporation must first occur. With benzoic acid in place of NAA there is no response, but an ion flux increase is almost instantly generated by further addition of NAA (Fig. 1 C). This implies that the two separate protein entities, the auxin receptor and the ATPase, must be loosely associated and functionally dependent since the ion flux increase due to the ATPase pumping

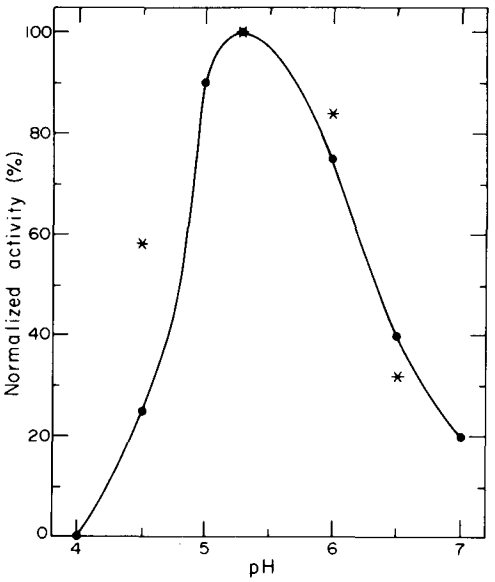

Figure 2. Comparison of normalized receptor activity (•) and electrochemical response (*) with pH. Conditions as for Fig. 1.

action is not observed in the absence of auxin. A response for receptor/ATP added to one membrane face can be rapidly generated by NAA addition to either solution compartment as shown in Fig. 1 D.

Addition of Mg^{2+} produced no significant effect on ion flux, presumably because this ion is present in the receptor stock solution. Replacement of the KCl electrolyte with NaCl containing 10^{-5}M KCl reduced the signal generated on receptor stimulation, indicating that potassium ion may be a component of the electrochemical action. Adjustment of hydrogen ion concentration was used to examine the possibility that proton transport is involved in this system. Maximum receptor binding activity occurs at a pH of 5.3. At higher pH values, both decreased ion availability and binding activity should result in a concerted reduction of ion current, whereas in the lower pH range the latter will be offset by a greater concentration of ion available for transport. Normalization of electrochemical responses (current integral over standard time interval) at various pH values using 100% activity arbitrarily set at pH 5.3 results in the comparison of response and known receptor activity with H^+ concentration shown in Fig. 2. These results are consistent

with the previous suggestions that an ATPase pump acts to translocate protons in the presence of potassium and magnesium ions (14-16) and that such a pump can be activated by auxin (13).

From the analytical point of view it is interesting to note that the limit-of-detection for NAA in this work was approximately 10^{-7} M, which compares favourably with the 10^{-5}-10^{-6} range for enzyme electrodes. Further purification of the receptor fraction from contaminant proteins should allow greater membrane loading for increased electrochemical sensitivity.

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