



HAL
open science

Conformational dynamics underlie the activity of the auxin-binding protein, Nt-abp1.

K. David, E. Carnero-Diaz, N. Leblanc, M. Monestiez, J. Grosclaude, C. Perrot-Rechenmann

► **To cite this version:**

K. David, E. Carnero-Diaz, N. Leblanc, M. Monestiez, J. Grosclaude, et al.. Conformational dynamics underlie the activity of the auxin-binding protein, Nt-abp1.. *Journal of Biological Chemistry*, 2001, 276 (37), pp.34517-23. 10.1074/jbc.M102783200 . hal-00150140

HAL Id: hal-00150140

<https://hal.science/hal-00150140>

Submitted on 31 May 2020

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

Copyright

Conformational Dynamics Underlie the Activity of the Auxin-binding Protein, Nt-abp1*

Received for publication, March 29, 2001, and in revised form, June 27, 2001
Published, JBC Papers in Press, July 3, 2001, DOI 10.1074/jbc.M102783200

Karine David‡§, Eugénie Carnero-Diaz¶, Nathalie Leblanc‡**, Michèle Monestiez¶, Jeanne Grosclaude‡‡, and Catherine Perrot-Rechenmann‡§§

From the ‡Institut des Sciences du Végétal, CNRS, 91198 Gif sur Yvette, Cedex, the ¶Laboratoire d'Électrophysiologie des Membranes, Université de Paris 7, 75000 Paris, and ‡‡Virologie et Immunologie Moléculaires, INRA, 78352 Jouy en Josas, France

The auxin-binding protein 1 (ABP1) has been proposed to be involved in the perception of the phytohormone at the plasma membrane. Site-directed mutagenesis was performed on highly conserved residues at the C terminus of ABP1 to investigate their relative importance in protein folding and activation of a functional response at the plasma membrane. Detailed analysis of the dynamic interaction of the wild-type ABP1 and mutated proteins with three distinct monoclonal antibodies recognizing conformation-dependent epitopes was performed by surface plasmon resonance. The influence of auxin on these interactions was also investigated. The Cys¹⁷⁷ as well as Asp¹⁷⁵ and Glu¹⁷⁶ were identified as critical residues for ABP1 folding and action at the plasma membrane. On the contrary, the C-terminal KDEL sequence was demonstrated not to be essential for auxin binding, interaction with the plasma membrane, or activation of the transduction cascade although it does appear to be involved in the stability of ABP1. Taken together, the results confirmed that ABP1 conformational change is the critical step for initiating the signal from the plasma membrane.

Plant growth and developmental processes are controlled by the action of plant hormones among which auxin is implicated in embryogenesis, lateral root formation, vascular development, regulation of tropistic responses, maintenance of apical dominance, and senescence. At the cellular level, auxin is involved in the regulation of cell elongation, cell division, and cell differentiation. The isolation and characterization of auxin-responsive mutants have led to the identification of several genes involved in auxin transport (1–4) and auxin signaling (5–7). For example, the molecular analysis of the *TIR1* and *AXR1* genes has demonstrated the fundamental role of ubiquitin and ubiquitin-related pathways in auxin action. Recent molecular studies of auxin-regulated genes including the iden-

tification of transcription factors and the characterization of related *Arabidopsis* mutants have greatly contributed to our understanding of auxin responses (8–11). Nevertheless, details of the molecular mechanisms underlying auxin responses remain fragmentary.

A large number of auxin-binding proteins have been identified from soluble and membrane fractions, but their function in auxin signaling, transport, or metabolism has rarely been established (12, 13). The auxin-binding protein 1 (ABP1),¹ initially isolated from maize coleoptiles (14, 15), was rapidly designated as a putative auxin receptor (13, 16). For years, the involvement of this protein in auxin action remained uncertain (17–19). Transgenic tobacco plants conditionally overexpressing the *Arabidopsis* ABP1 were shown to exhibit an increased capacity for auxin-mediated cell expansion (20). The involvement of ABP1 in the early activation of the auxin-dependent electrical response at the plasma membrane has been demonstrated by using a collection of monoclonal antibodies to the tobacco protein (21). Despite the presence of a KDEL sequence at the C terminus of ABP1 acting as a retention signal in the endoplasmic reticulum (22, 23), it has been shown that a fraction of ABP1 reaches the plasma membrane (21, 24). The C-terminal domain of ABP1 has also been proposed to be implicated in the interaction with plasma membrane components. A synthetic peptide comprising the last 12 residues of the C terminus of maize ABP1 was shown to modulate the K⁺ currents of *Vicia faba* guard cells mimicking the effect of supra-optimal auxin concentrations (25, 26). The C-terminal maize peptide was also shown to provoke a cytosolic pH alkalinization of *Paphiopedilum tonsum* L. guard cells and consequently stomatal closure (27) and to increase GTPγS binding on microsomal fractions (28). The same maize peptide as well as a 15-amino acid-long synthetic peptide corresponding to the C terminus of the tobacco protein and the Nt-abp1 protein itself have also been shown to induce the hyperpolarization of tobacco mesophyll protoplasts in the absence of auxin (29, 30). The possible role of the KDEL sequence in the action of the C-terminal domain is unclear. The KDEL-truncated peptide was shown to promote GTPγS binding (28) but was ineffective in altering cytosolic pH and stomatal closure suggesting that the C terminus is important in ABP1 function (27).

To analyze further the relative importance of specific residues within the C-terminal domain of ABP1 in protein folding as well as in the activation of the functional electrical response

* This work was supported in part by funds from Direction de la Recherche et des Études Doctorales, Ministère de l'Éducation Nationale et de la Culture Grant EA 291. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

§ Supported by a grant from the Ministère de l'Enseignement Supérieur, de la Recherche et de la Technologie, France. Present address: School of Biological Sciences, University of Auckland, Auckland, New Zealand.

¶ Present address: Université de Paris-Jussieu 75251 Paris Cedex 05, France.

** Present address: Université Blaise Pascal, 63177 Aubière Cedex, France.

§§ To whom correspondence should be addressed. Tel.: 33 1 69 82 35 88; Fax: 33 1 69 82 35 84; E-mail: catherine.rechenmann@isv.cnrs-gif.fr.

¹ The abbreviation used are: ABP1, auxin binding protein 1; mAb, monoclonal antibody; NAA, naphthalene acetic acid; Nt-abp1, *Nicotiana tabacum* ABP1; SPR, surface plasmon resonance; WT, wild type; GTPγS, guanosine 5'-3-O-(thio)triphosphate; mAb, monoclonal antibody; RAM, rabbit anti-mouse.

at the plasma membrane, site-directed mutagenesis was performed on highly conserved residues among ABP1 proteins. In addition to studies on the effect of each mutation in the functional assay, a detailed analysis of the dynamic interaction of the wild-type and mutated proteins with a set of mAbs recognizing distinct conformation-dependent epitopes have been performed by SPR. The influence of auxin on these interactions was also investigated. We report on the residues critical for ABP1 action at the plasma membrane. The functional assay and dynamic interaction analysis provide new insights on the structure-function relationship of ABP1.

EXPERIMENTAL PROCEDURES

Material—Tobacco plants (*Nicotiana tabacum* cv. xanthi) were grown from seeds in a greenhouse (22 °C, 9-h photoperiod). Mesophyll protoplasts were isolated from young tobacco leaves using the procedure described by Caboche (31).

Site-directed Mutagenesis—Mutated Nt-abp1 proteins were generated using polymerase chain reaction-based site-directed mutagenesis on the plasmid containing the cDNA sequence of *Nt-abp1* (32). All amplifications were performed using a unique 5' primer corresponding to the N-terminal end of Nt-abp1 and including a *Bam*HI site 5'-ATACGATCCATGGCTCGCCATGTTCTCGTAGTGGTAG in combination with distinct reverse primers for mutagenesis at the C terminus. A single mutation was introduced to convert Asp¹⁷⁴ into Asn using the primer 5'-ATCTTTCCACGAAGTTGTCTGATAACATTCCTCATTACC. For ABP1-NQ, the double mutation Asp¹⁷⁴ and Glu¹⁷⁵ to Asn and Gln, respectively, was performed using the primer 5'-ATCTTTCCACGAAGTTGTCTGATAACATTCCTCATTACC. For ABP1-S, the Cys¹⁵⁷ was converted into Ser using the primer 5'-ATCTTTCCACGAAGTTGTCTGATAAGATTTC. The same primer was used to obtain the triple mutation of Asp¹⁷⁴, Glu¹⁷⁵, and Cys¹⁵⁷ using the *ABP1-NQ* sequence as a template. Another triple mutation corresponding to the conversion of Asp¹⁷⁴, Glu¹⁷⁵, and Cys¹⁵⁷ into Ala was achieved using the primer 5'-ATCTTTCCACGAAGTTGTCTGATAAGCTTCCGCAGCCCA.

Amplified fragments were subjected to a second round of polymerase chain reaction amplification using the reverse primer 5'-CGCGGATCCTTAAAGCTCATCTTTCCACGAAGTTGT adding the last two codons, the stop codon and a *Bam*HI site at the 3' end. ABP-ΔK was generated by deletion of the last 12 nucleotides encoding the C-terminal motif KDEL (Lys¹⁸⁴-Asp¹⁸⁵-Glu¹⁸⁶-Leu¹⁸⁷) using the primer 5'-CGCGGATCCTTACCACGAAGTTGTCTGATAACATTC. Each polymerase chain reaction amplification was performed using *Taq* polymerase (Promega) under the following conditions: 94 °C for 3 min, then 25 cycles (94 °C for 30 s, 65 °C for 1 min, 72 °C for 1 min) followed by 72 °C for 5 min. The amplification products were cloned into pBlueScript vector. Clones of each mutated Nt-abp1 were double strand-sequenced using the ABI prism Dye Terminator kit (PerkinElmer Life Sciences) and an ABI 373A automated DNA sequencer (Applied Biosystems, Inc.). Mutated sequences were cloned in the pQE30 expression vector (Qiagen). Recombinant proteins were produced in *Escherichia coli* cells (M15) and purified as described previously (32).

Monoclonal Antibodies—Isolation and initial characterization of monoclonal antibodies (mAbs) to wild-type recombinant Nt-abp1 have been reported previously (21). Pairwise epitope mapping was conducted by SPR using both competitive binding and sandwich procedures as described (21). Three mAbs were selected for the present work on the basis of their characteristics of interaction with ABP1: all recognize highly discontinuous epitopes, mAb12 and mAb50 are auxin antagonists on the early auxin electrical response of tobacco protoplasts and recognize non-overlapping epitopes, whereas mAb28 is hormonometric and overlaps with mAb12 but not with mAb50. Western blot analyses were performed as described previously (21).

SPR Analysis—The Biacore analytical system based on surface plasmon resonance was used to characterize extensively the interaction of mAbs raised to Nt-abp1 with wild-type and mutated proteins (33). mAb reactivity with each purified proteins was analyzed as follows. Rabbit anti-mouse (RAM) Fc affinity-purified immunoglobulins were covalently immobilized by amino linkage onto chip CM-dextran and then hybridoma supernatants of each mAb were injected for 4 min (flow 10 μl/min) to reach a level of ~1000 resonance units. A 4-min injection of each protein followed, either in HBS buffer (25 mM Hepes, pH 7.4, 150 mM NaCl, and 0.05% Nonidet P-20) or in HBS with 5 μM NAA, and dissociation was observed for 4 min after the end of injection. Regeneration of the RAM was achieved through injection of two 3-min pulses of 10 mM HCl. For kinetics analysis of protein capture by mAbs, serial

TABLE I

Site-directed mutagenesis at the C-terminal domain of *Nt-abp1*

The consensus sequence results from the alignment of the tobacco (32), maize (16), arabidopsis (42), strawberry (43), and hot pepper (44) sequences. Unique or multiple mutations were introduced by PCR as indicated. Residue numbers are indicated on the Nt-abp1 sequence from Met¹.

Name	C-terminal sequence	Mutations
Nt-abp1	YWDEECYQTTSWKDEL	
Consensus	.WDE.C.....KDEL	
ABP-S	YWDEE S YQTTSWKDEL	C178S
ABP-N	YW N EECYQTTSWKDEL	D174N
ABP-NQ	YW NQ EECYQTTSWKDEL	D174N; E175Q
ABP-NQES	YW NQES YQTTSWKDEL	D174N; E175Q; C178S
ABP-AAEA	YW AAEA YQTTSWKDEL	D174A; E175A; C178A
ABP-ΔKDEL	YWDEECYQTT SW	Deletion of KDEL185-188

½ dilutions of protein from 500 to 62.5 nM concentrations were injected. The association and dissociation constants (k_{on} and k_{off} , respectively) were calculated using BIA224 software.

Measurement of the Electrical Response of Tobacco Protoplasts—For each experiment, about 10⁴ protoplasts in suspension were incubated with different concentrations of mutated ABP1 for 5 min at room temperature. The transmembrane E_m was then measured by the microelectrode technique as described previously (34, 35). Membrane potential variations (ΔE_m) reported for each experimental condition correspond to the average of 15–20 individual measurements. Dose-response curves were established by plotting ΔE_m values as a function of protein concentrations. For each protoplast preparation, the electrical response was investigated in response to the optimal NAA concentration of 3×10^{-6} M, and in response to 10^{-9} M Nt-abp1 was shown to induce the maximal hyperpolarization (30).

RESULTS

Site-directed Mutagenesis at the C Terminus of *Nt-abp1*—The comparison of ABP1 C-terminal domains isolated from different species has defined a consensus sequence composed of strictly conserved residues (Table I). This consensus corresponds to 8 of the last 15 residues at the C terminus of ABP1, including Trp¹⁷³, Asp¹⁷⁴, Glu¹⁷⁵, the third cysteine (Cys¹⁷⁷), and the C-terminal tetrapeptide KDEL (Lys¹⁸⁴-Asp¹⁸⁵-Glu¹⁸⁶-Leu¹⁸⁷). Given the intrinsic activity of the ABP1 C-terminal domain in the hyperpolarization of tobacco mesophyll protoplasts (30), we used site-directed mutagenesis of highly conserved residues to analyze their relative importance in the activation of the response. Single and multiple mutations were introduced on conserved residues as indicated in Table I. The Asp and Glu residues were converted into their corresponding amine, whereas the cysteine was converted into serine to remove the -SH group. A more drastic change was introduced by mutation of these 3 residues into Ala. To address in more detail the possible role of the KDEL sequence, a truncated protein was also constructed by deletion of the last 4 amino acids. After mutagenesis and systematic sequencing, the different proteins were produced in *E. coli* and purified as described previously (32) for further characterization. The immunoblot presented in Fig. 1 shows that mAb12, a monoclonal antibody to Nt-abp1 (21), recognized each protein. Deletion of the KDEL sequence corresponds to a decrease of 557 Da, which can be seen on the Western blot. In the case of ABP1-NQES, the protein has migrated slightly less than the wild-type protein. This reflects the charge modification introduced by mutation of the two acidic residues (Asp and Glu) into their corresponding amines (Asn and Gln) hence altering their interaction with SDS.

Dynamic Interaction of WT and Mutated *Nt-abp1* with mAbs—We have previously characterized a set of monoclonal

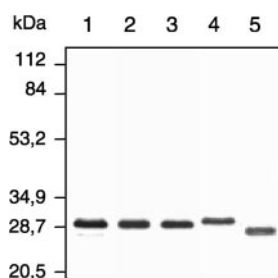


FIG. 1. Western blot showing mutated forms of Nt-abp1. Wild-type Nt-abp1 (lane 1) and recombinant mutated proteins (ABP1-N, ABP1-S, ABP1-NQES, and ABP1-ΔKDEL, lanes 2–5) were expressed in *E. coli*, separated by SDS-polyacrylamide gel electrophoresis, and revealed on Western blot using mAb12 (21). Each lane represents 200 ng of purified protein.

antibodies to Nt-abp1 and their interaction with the wild-type protein. Most of these mAbs were demonstrated to recognize conformational epitopes (21). Surface plasmon resonance was used to follow the dynamic interaction of each mutated protein with three distinct mAbs to Nt-abp1. Fig. 2 illustrates a selection of sensorgrams showing interaction of wild-type Nt-abp1, ABP1-ΔKDEL, and ABP1-NQES with mAbs 12, 28, and 50, respectively. Each sensorgram corresponds to the capture of the injected ABP1 (WT or mutated) at 0.25 mM on the immobilized mAb indicated. ABP1-NQES showed much lower association with each mAb than the WT and ABP1-ΔKDEL proteins. However, the association observed for ABP1-NQES, albeit low, was relatively stable. Analysis of the sensorgrams suggests that the deletion of the C-terminal KDEL tetrapeptide did not strongly affect the binding of the protein to the different mAbs but that the interaction was less stable, in particular with mAb50. To study more precisely these interactions, kinetic constants were calculated on the basis of a series of sensorgrams obtained with a whole range of concentrations of injected protein. Values of k_{on} , k_{off} , and deduced K_d are plotted in Table II. Analysis of association and dissociation kinetics confirmed the high affinity of the mAbs for the wild-type Nt-abp1 with a K_d value in the nanomolar range. Although drastic changes of K_d values were observed for the two mutated proteins, association and dissociation constants are more informative. For example, the increased K_d values calculated for the interaction of ABP1-NQES with mAb28 and mAb50 resulted mainly from a severe decrease in the association constant by a factor of 32 and 90, respectively. On the contrary, the increased K_d value for the interaction of ABP1-ΔKDEL with mAb50 resulted mainly from an increased dissociation by a factor of 10, whereas the association is decreased by only 4.8. Interactions of the mutated proteins with mAb12 were less affected by the modifications introduced within the sequences than for the other antibodies. Interestingly, the reduction of the association constant of ABP1-NQES with mAb12 is counteracted by an increase in the stability of the interaction.

Functional Assay of Mutated Forms of ABP1—To determine if the different mutations generated in ABP1 were able to modify the ability of the protein to activate the electrical response of tobacco protoplasts, we examined the effect of each mutated protein in this assay. The effects of single and multiple mutations are illustrated in Figs. 3 and 4. Addition of ABP1-N to tobacco mesophyll protoplasts was able to induce a membrane hyperpolarization but with a reduced efficiency compared with the WT protein (Fig. 3). The mutated protein containing a Ser¹⁷⁷ instead of Cys was unable to induce an electrical response (Fig. 3), nor were ABP1-NQES (Fig. 4) and ABP1-AAEA (not shown). An absence of signal was also observed using the ABP1-NQ protein carrying the double muta-

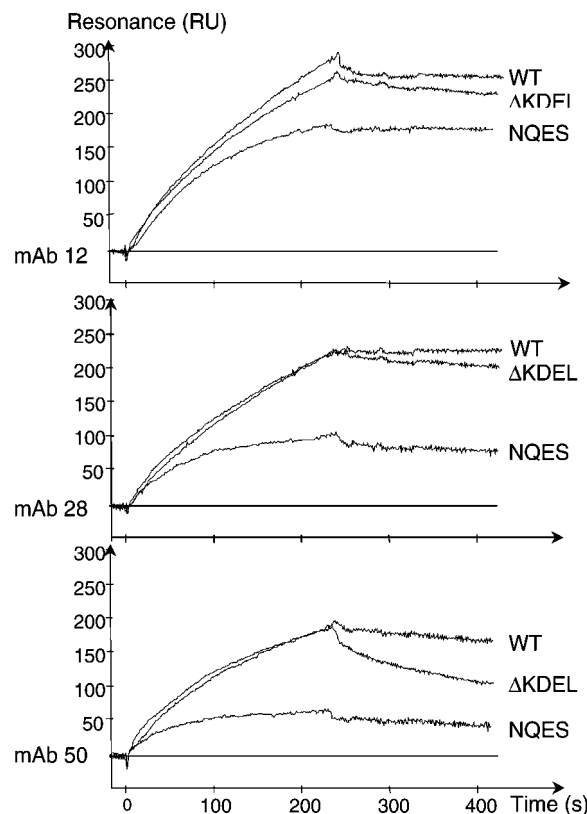


FIG. 2. Interaction of the wild-type (WT) and mutated ABP1 with mAb12, mAb28, and mAb50. Resonance signals (RU) were plotted as a function of time. Each overlay plot of three sensorgrams illustrates the binding of injected ABP1 (WT, -NQES, or -ΔKDEL) to a mAb (mAb12, mAb28, or mAb50) immobilized on the biosensor. The injection of each protein was followed for 250 s. The base line corresponds to the signal of continuous flow of buffer over the matrix after immobilization of RAM and one of the mAb to Nt-abp1.

TABLE II
Calculated kinetic constants characterizing the interaction of WT and mutated ABP1 with mAbs 12, 28, and 50

Kinetic constants	Nt-abp1 WT	ABP1-ΔKDEL	ABP1-NQES
mAb 12 k_{on} *	4.36 ± 0.35	2.17 ± 0.25	0.75 ± 0.06
k_{off} †	0.47 ± 0.01	3.80 ± 0.22	0.10 ± 0.01
K_d ‡	1.07	17.5	1.3
mAb 28 k_{on} *	1.95 ± 0.21	0.67 ± 0.08	0.06 ± 0.007
k_{off} †	0.37 ± 0.03	4.5 ± 0.35	2.8 ± 0.17
K_d ‡	1.92	67	500
mAb 50 k_{on} *	2.70 ± 0.38	0.56 ± 0.04	0.03 ± 0.15
k_{off} †	1.80 ± 0.14	18 ± 1.2	1.02 ± 0.11
K_d ‡	6.6	320	290

* k_{on} values are expressed in $\times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$.

† k_{off} values are expressed in $\times 10^{-4} \text{ s}^{-1}$.

‡ Calculated K_d values expressed in nM.

tion changing the two acidic residues into amines (not shown). These mutations correspond to an important change in the charge of the C-terminal domain of the protein resulting from the removal of the two negative charges of the aspartic and glutamic acids. The membrane hyperpolarization of tobacco mesophyll protoplasts induced by ABP1-ΔKDEL is similar to

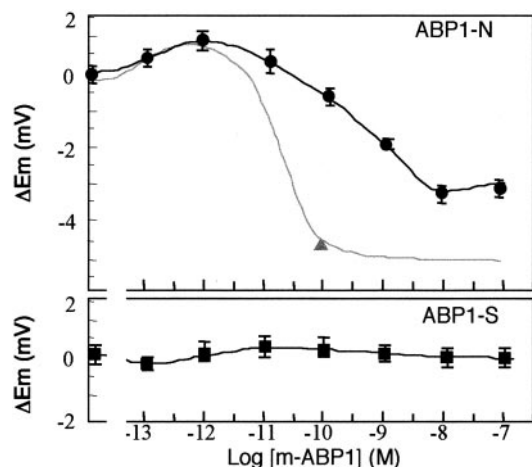


FIG. 3. Effects of single mutations of Nt-abp1 on the transmembrane potential of tobacco protoplasts. Mutated proteins ABP1-N (●), ABP1-S (■), or WT Nt-abp1 (△) were added to the protoplast medium just prior to measurement. The dose-response curves were established by plotting ΔE_m values as a function of protein concentration. Data are given for one representative experiment among three independent experiments. The mean E_m variation induced in these experiments by the optimal auxin concentration ($3 \mu\text{M}$, 1-NAA) was of -3.4 mV. Maximal S.E. is indicated for each point.

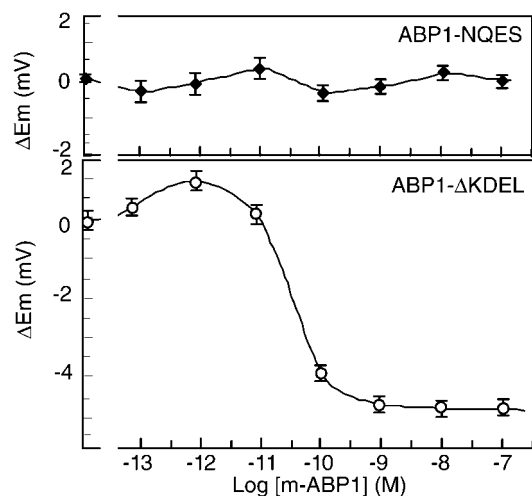


FIG. 4. Effects of multiple mutations of Nt-abp1 on the transmembrane potential of tobacco protoplasts. Mutated proteins ABP1-NQES (◆) or ABP1- Δ KDEL (○) were added to the protoplast medium. The dose-response curves were established as for Fig. 3. Data are given for one representative experiment among three or four independent experiments. The mean E_m variation induced by the optimal auxin concentration ($3 \mu\text{M}$, 1-NAA) was of -3.8 mV. Maximal S.E. is indicated for each point.

the one induced by the WT protein. The maximal hyperpolarization of -5 mV was obtained for concentrations of 10^{-10} M and over (Fig. 4). The deletion of the KDEL sequence has no effect on the capacity of the protein to activate the electrical response when acting at the plasma membrane.

Influence of Auxin on the Dynamic Interaction of ABP1 with mAbs—The Nt-abp1-induced electrical membrane response was demonstrated previously (30) to be related to the biological activity of auxin at the plasma membrane, both sharing the same pathway. We have already reported evidence (21) suggesting that the activation of the auxin signal transduction cascade results from conformational change of Nt-abp1 in response to specific stimuli. To understand better the relationship between the conformation of the protein and its ability to induce a response, we have studied the interaction of the mutated forms of ABP1 with the series of mAbs in the presence of

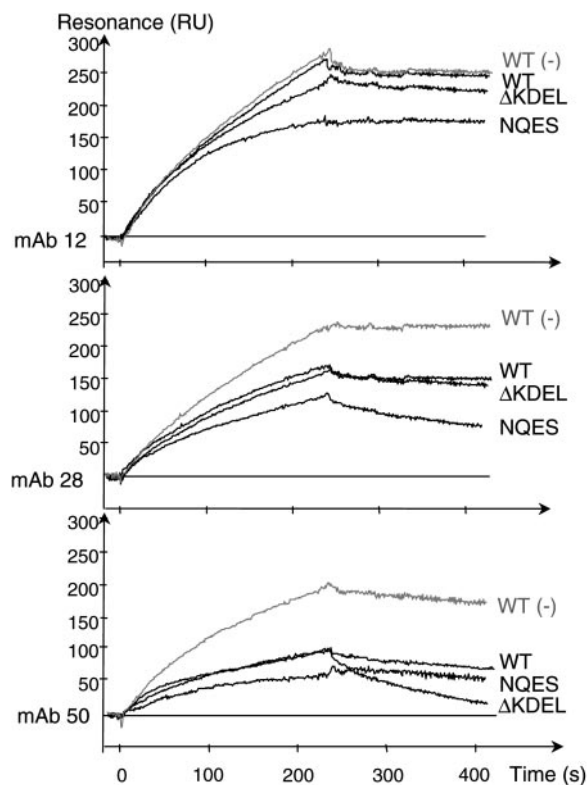


FIG. 5. Influence of auxin on the interaction of the WT Nt-abp1 and mutated proteins with mAb12, mAb28, and mAb50. Resonance signals (RU) were plotted as a function of time. Each overlay plot of three sensorgrams illustrates the binding of injected ABP1 (WT, -NQES, or Δ KDEL) in the presence of $5 \mu\text{M}$ NAA to a mAb immobilized on the biosensor (mAb12, mAb28, or mAb50). The base line corresponds to the signal of continuous flow of buffer over the matrix after immobilization of RAM and specific anti-Nt-abp1 antibody. Gray curves correspond to the interaction of the WT protein with each antibody in the absence of auxin.

auxin. Sensorgrams and related kinetic parameters describing the interaction of wild-type ABP1, ABP1-NQES, and ABP1- Δ KDEL with mAbs 12, 28, and 50 in the presence of $5 \mu\text{M}$ NAA are presented in Fig. 5 and Table III, respectively. The curve corresponding to the interaction of the WT protein in the absence of auxin with each mAb has been plotted as a reference on each group of sensorgrams (gray curve). The interaction of Nt-abp1 with mAb12 was not modified by the addition of auxin. On the contrary, the capture of Nt-abp1 in the presence of auxin by mAb28 and mAb50 was strongly reduced as confirmed by the association constants that were decreased by a factor of 5 and 118, respectively. However, the dissociation phase was much less affected. A slight increase of 2-fold was measured for the k_{off} value for the interaction of Nt-abp1 with mAb28, whereas for mAb50 the dissociation constants with and without auxin were identical. These two mAbs recognize highly conformation-dependent epitopes that appear to be less accessible when the protein has bound auxin.

The interaction of mAbs with mutated ABP1 in the presence of auxin was significantly altered compared with the wild-type protein. Deletion of the KDEL tetrapeptide affected the stability of the interaction with all mAbs. The most severe dissociation was observed with mAb50 with an increase in k_{off} up to $72.5 \times 10^{-4} \text{ s}^{-1}$ compared with the wild-type protein ($1.82 \times 10^{-4} \text{ s}^{-1}$) and the protein in the absence of auxin $k_{\text{off}} 18 \times 10^{-4} \text{ s}^{-1}$ (Table II). The mutation carried by ABP1- Δ KDEL destabilizes the interaction with the antibodies, and this effect is amplified in the presence of auxin. However, the auxin effect observed on the association of the wild-type protein with the

TABLE III
Calculated kinetic constants characterizing the interaction of WT and mutated ABP1 with mAbs 12, 28, and 50 in the presence of auxin

Kinetic constants in the presence of NAA	Nt-abp1 WT	ABP1-ΔKDEL	ABP1-NQES
k_{on}^*	4.05 ± 0.38	2.02 ± 0.25	0.78 ± 0.55
mAb 12 k_{off}^\dagger	0.48 ± 0.03	3.45 ± 0.19	0.08 ± 0.01
K_d^\ddagger	0.83	17.1	1.02
k_{on}^*	0.39 ± 0.40	0.13 ± 0.017	0.07 ± 0.08
mAb 28 k_{off}^\dagger	0.75 ± 0.06	3.75 ± 0.24	10.5 ± 1.50
K_d^\ddagger	19.3	279	1540
k_{on}^*	0.023 ± 0.003	0.16 ± 0.18	0.009 ± 0.0002
mAb 50 k_{off}^\dagger	1.82 ± 0.22	72.5 ± 0.55	0.65 ± 0.04
K_d^\ddagger	790	439	720

* k_{on} values are expressed in $\times 10^4 \text{ M}^{-1} \cdot \text{s}^{-1}$.
 † k_{off} values are expressed in $\times 10^{-4} \text{ s}^{-1}$.
 ‡ Values are calculated K_d expressed in nM.

different mAbs is maintained with ABP1-ΔKDEL. Indeed, the interaction of ABP1-ΔKDEL with mAb12 is not changed in the presence of auxin, whereas a marked reduction in its capture with the two other mAbs was observed. This suggests that the deletion of the KDEL sequence does not inhibit the binding of auxin to the protein.

As shown in Fig. 2, the triple mutation introduced on ABP1-NQES strongly decreases the association of the protein with mAbs. Auxin has no marked effect on the interaction of this ABP1-NQES with the different mAbs (Fig. 5). However, calculation of kinetic constants revealed an attenuation of the strength of its interaction with mAb28 and a slight modification of both the association and the dissociation with mAb50. The effect of auxin on the interaction of ABP1-NQES with the mAbs is severely decreased by the NQES mutation suggesting that this protein has lost the capacity to interact with auxin.

DISCUSSION

Monoclonal Antibodies to Nt-abp1 Are Markers for Protein Conformation—We have investigated whether 3 mAbs, each exhibiting distinct characteristics of interaction with Nt-abp1 (21), could detect changes induced by auxin binding. Our primary aim was thereby to evaluate their suitability as sensors of ABP1 conformation. Auxin was demonstrated by circular dichroism spectroscopy measurements (15) to induce a conformational change of ABP1. These data were also supported by enzyme-linked immunosorbent assay experiments (36) and more recently by structural analyses (37). A change in mAb and ABP1 interaction after auxin binding reflects an alteration of mAb accessibility to its epitope which results either from a steric hindrance due to the auxin molecule or from the intrinsic conformational change of ABP1 following auxin binding. The monoclonal antibody mAb12 recognizes a discontinuous epitope formed by the assembly of boxes A and C (both proposed to be involved in the auxin-binding site) and the C-terminal domain of ABP1 and is a strong auxin antagonist for the electrical response of tobacco protoplasts. The interaction of mAb12 with Nt-abp1 is not significantly affected by auxin (Fig. 5). Either the accessibility of mAb12 to the epitope is not modified in the presence of auxin, despite the involvement of domains A and C in auxin binding, or the interaction of mAb12 with each sub-domain of the epitope is strong enough to assemble them and reconstitute the optimal structure. The mAb28

recognizes a highly discontinuous epitope overlapping the one of mAb12 and is hormonomimetic toward the auxin electrical response. The interaction of mAb28 with Nt-abp1 is weakened in the presence of auxin. As mAb28 mimics the auxin effect on the electrical response, it was proposed that the binding of this mAb to the protein generated a conformational change in Nt-abp1 similar to the one induced by auxin (21). When this change has already occurred, via auxin binding, the accessibility to the mAb28 epitope is reduced as revealed by the kinetic data of the interaction (Tables II and III). In this case, the alteration could result either from direct hindrance with auxin or from the conformational change of ABP1. mAb50 also recognizes a highly discontinuous epitope, does not recognize non-folded ABP1, and does not overlap with mAb12 and mAb28 and is therefore distant from boxes A and C involved in the auxin-binding site (data not shown). These characteristics taken together with the severe reduction of mAb50 association to Nt-abp1 in the presence of auxin (Tables I and II and Fig. 5) show that the decrease in the accessibility to the epitope results from the conformational change induced either by auxin binding or by interaction with the hormonomimetic mAb28 and not from steric hindrance. Knowing that mAb50 is an IgG3, it is likely that mAb50 interacts with a cryptotope less accessible than an epitope mapped at the surface.

On the basis of kinetic data, the population of Nt-abp1 injected on the sensorchip in the presence of auxin was homogenous indicating that the whole population has been modified by auxin binding. These mAbs that recognize conformation-dependent epitopes are able to discriminate between ABP1 and ABP1 which has bound auxin, and at least one of them (mAb50) reveals genuine conformational changes. As such they appear to be highly sensitive markers of probing Nt-abp1 conformation.

Relative Importance of the C-terminal KDEL Tetrapeptide on ABP1—The KDEL tetrapeptide located at the C terminus of ABP1 is known to act as a retention signal in the lumen of the endoplasmic reticulum. However, it has been demonstrated that at least part of the protein reaches the plasma membrane and is involved in auxin signaling at the outer face of the membrane (21). The mechanism controlling the targeting of the protein to the plasma membrane is not yet known. To determine whether the KDEL sequence is necessary for the interaction of Nt-abp1 with the plasma membrane, we have constructed a truncated protein with no KDEL at the C terminus, and we have checked the ability of this mutated protein to induce a hyperpolarization of tobacco protoplasts as reported for the wild-type protein (30). This protein, termed ABP1-ΔKDEL, induced the hyperpolarization of tobacco protoplasts with the same efficiency as the wild-type protein demonstrating that the KDEL sequence is not required for the interaction with the plasma membrane and the activation of the electrical response (Fig. 4).

The dynamic interaction of ABP1-ΔKDEL with the different mAbs, studied by SPR, was altered compared with the wild type. In each case, the interaction was less stable. The strongest destabilization was observed with mAb50 which is the most responsive to conformational changes of ABP1. mAbs to Nt-abp1 detected more subtle conformational changes than the functional assay at least when combined with the Biacore analysis. It has been reported that addition of a KDEL sequence even to soluble and cytosolic proteins increased their stability (38). The decrease of the interaction stability suggests that the C-terminal KDEL sequence could play a role in the stabilization of ABP1 folding.

As for the wild-type protein, a conformational change was also observed with ABP1-ΔKDEL in the presence of auxin

suggesting that the truncated protein was still able to bind auxin and to adopt the corresponding conformation. Again the interaction was unstable indicating that the stabilization effect of the KDEL sequence occurs in both conformations (with and without auxin). These data confirm that the KDEL sequence may act to stabilize the protein but is not essential for ABP1 activity, including auxin binding and interaction with the plasma membrane.

Critical Residues at the C-terminal Domain of ABP1 for Its Action at the Plasma Membrane—In addition to the KDEL sequence, the C-terminal domain of ABP1 contains three highly conserved residues, namely Asp¹⁷⁴, Glu¹⁷⁵, and Cys¹⁷⁷. By using synthetic peptides corresponding to the last 12 or 15 C-terminal amino acids of Nt-abp1, we have suggested previously (30) that the two acidic residues could play an important role in the activation of the electrical response of tobacco protoplasts. To overcome the difficulties inherent in the use of short synthetic peptides instead of full-length proteins, we have introduced single or multiple mutations on these residues to determine their importance in the protein as a whole. Proteins carrying such mutations showed distinct effects on the electrical response of tobacco protoplasts. In particular, the electrical response was abolished in the case of ABP1-NQ in which Asp¹⁷⁵ and Glu¹⁷⁶ have been converted into their corresponding amine (not shown). The suppression of the two negative charges of the acidic residues was responsible for the functional inactivation of the protein. Conversely, the mutation of only one of these residues still produced a response, although weaker than using the wild-type protein. These results suggest that the negative charges upstream of Cys¹⁷⁷ are important either for the interaction of the protein with the plasma membrane or for the folding of the protein.

The absence of activity of the protein carrying the mutation of the Cys¹⁷⁷ into Ser is consistent with the idea that Cys¹⁷⁷ is involved in a disulfide bridge that is essential for ABP1 activity at the plasma membrane. We have shown that the C-terminal domain of the protein is associated with the boxes A and C of ABP1 (21) (boxes proposed to be responsible for auxin binding), suggesting the possible interaction of Cys⁸² from box A with Cys¹⁷⁷ and the formation of a disulfide bridge that could stabilize the protein in a functional conformation. Our data reinforce the importance of the Cys¹⁷⁷ at the C terminus of ABP1. In the future, site-directed mutagenesis on the two other cysteines will help to demonstrate which of them is involved together with Cys¹⁷⁷ in the disulfide bridge corresponding to the functional folding of Nt-abp1.

Our working hypothesis is that the two negative charges coming from Asp¹⁷⁴ and Glu¹⁷⁵ are necessary for the folding of the protein and the formation of the disulfide bridge involving Cys¹⁷⁷. In the absence of these charges, the electrostatic proximity of interacting domains would not be possible, resulting in the formation of an inactive protein. Proteins carrying the triple mutations -NQES (or -AAEA, not shown) were also unable to generate a hyperpolarization of tobacco protoplasts, confirming the absence of effect recorded with single and double mutations. The loss of functional response results from an alteration in folding which contributes to a loss of the interaction with the plasma membrane or to an inability of the protein to adopt the conformation promoting the activation of the response. An alteration in folding is confirmed by the SPR data that showed that each mAb recognized less efficiently ABP1-NQES than the wild-type protein and that the interaction is not modified by the presence of auxin. Similarly, the interaction of the mAbs with the wild-type protein treated with reducing agents is also unaffected (not shown) suggesting a loss of auxin binding. It has been reported previously (39, 40) that

auxin binding was reversibly inactivated by reducing agents revealing the presence of a reducible group essential for auxin binding activity. Taken together, these experiments all suggest that the introduced mutations lead to an irreversible loss of auxin binding which is correlated to an inadequate and inactive conformation of the protein.

Conformational Change of Nt-abp1 in Relation to the Functional Response at the Plasma Membrane—The activation of the electrical response of tobacco protoplasts by exogenous ABP1, in the absence of auxin, has been demonstrated to be related to the auxin electrical response (30). We have reported previously (21) that the activation of the auxin-dependent electrical response results also from a conformational change of the protein after either auxin binding or interaction with an homonomimetic antibody responsible for a modification of the interaction with the plasma membrane. In addition to the identification of important residues for the folding and activity of the protein, the study of mutated proteins supports the idea that the conformation adopted by the exogenous ABP1 influences the effect generated at the plasma membrane. It is possible that the added ABP1 could interact with plasma membrane components and consequently generates the response. However, it has been shown that low amounts of ABP1 present at the plasma membrane do not induce such response in the absence of auxin. An alternative explanation is that the added ABP1 also interacts with existing ABP1, the latter protein-protein interaction generating a conformational change of the protein similar to the one induced by auxin binding. Increased amount of ABP1 at the surface of tobacco protoplasts could force the formation of multimers, modifying the dynamic interaction with the plasma membrane. This could explain the shape of the curves where the hyperpolarization is maintained for increasing concentrations of functional ABP1.

In conclusion, we have identified residues critical for ABP1 folding and activity at the plasma membrane. Furthermore, we demonstrated that the C-terminal KDEL sequence is important for the stability of the protein but not necessary for auxin binding, interaction with the plasma membrane, or activation of the transduction cascade. We have confirmed that ABP1 conformational change is the critical step for propagating the signal. All the data also suggest that the activation of the auxin signal transduction cascade does not require a highly stable interaction of activated ABP1 with the plasma membrane. A transient change of ABP1 conformation and subsequent modification of the interaction with membrane components could be sufficient to initiate the biological signal as it has been reported for animal receptor systems (41, 42).

Acknowledgments—We thank Dr. James Baully for critical reading of the manuscript. We thank Suzanne Labiau and Liliane Troussard for technical assistance in hybridoma cultures and automatic DNA sequencing, respectively.

REFERENCES

- Bennett, M. J., Marchant, A., Haydn, G. G., May, S. T., Ward, S. P., Millner, P. A., Walke, A. R., Schultz, B., and Feldmann, K. A. (1996) *Science* **273**, 948–950
- Chen, R. J., Hilson, P., Sedbrook, J., Rosen, E., Caspar, T., and Masson, P. H. (1998) *Proc. Natl. Acad. Sci. U. S. A.* **95**, 15112–15117
- Luschnig, C., Gaxiola, R. A., Grisafi, P., and Fink, G. R. (1998) *Genes Dev.* **12**, 2175–2187
- Muller, A., Guan, C. H., Galweiler, L., Tanzler, P., Huijser, P., Marchant, A., Parry, G., Bennett, M., Wisman, E., and Palme, K. (1998) *EMBO J.* **17**, 6903–6911
- Deruere, J., Jackson, K., Garbers, C., Soll, D., and Delong, A. (1999) *Plant J.* **20**, 389–399
- Gray, W. M., and Estelle, I. (2000) *Trends Biochem. Sci.* **25**, 133–138
- Christensen, S. K., Dagenais, N., Chory, J., and Weigel, D. (2000) *Cell* **100**, 469–478
- Leyser, O., Pickett, F. B., Dharmasiri, S., and Estelle, M. (1996) *Plant J.* **10**, 403–413
- Sessions, A., Nemhauser, J. L., McColl, A., Roe, J. L., Feldmann, K. A., and Zambryski, P. C. (1997) *Development* **124**, 4481–4491

10. Hardtke, C. S., and Berleth, T. (1998) *EMBO J.* **17**, 1405–1411
11. Tian, Q., and Reed, J. W. (1999) *Development* **126**, 711–721
12. Jones, A. M. (1994) *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **45**, 393–420
13. Venis, M. A., and Napier, R. M. (1995) *Crit. Rev. Plant Sci.* **14**, 27–47
14. Löbler, M., and Klämbt, D. (1985) *J. Biol. Chem.* **260**, 9848–9853
15. Shimomura, S., Sotobayashi, S., Futai, M., and Fukui, T. (1986) *J. Biochem. (Tokyo)* **99**, 1513–1524
16. Hesse, T., Feldswisch, J., Balshusemann, D., Bauw, G., Puype, M., Vandekerckhove, J., Löbler, M., Klämbt, D., Schell, J., and Palme, K. (1989) *EMBO J.* **8**, 2453–2461
17. Goldsmith, M. H. M. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 11442–11445
18. Hertel, R. (1995) *J. Exp. Bot.* **46**, 461–462
19. Venis, M. A. (1995) *J. Exp. Bot.* **46**, 463–465
20. Jones, A. M., Im, K. H., Savka, M. A., Wu, M. J., Dewitt, N. G., Shillito, R., and Binns, A. N. (1998) *Science* **282**, 1114–1117
21. Leblanc, N., David, K., Grosclaude, J., Pradier, J. M., Barbier-Brygoo, H., Labiau, S., and Perrot-Rechenmann, C. (1999) *J. Biol. Chem.* **274**, 28314–28320
22. Pelham, H. (1990) *Trends Biochem. Sci.* **15**, 483–486
23. Napier, R. M., Fowke, L. C., Hawes, C., Lewis, M., and Pelham, H. R. B. (1992) *J. Cell Sci.* **102**, 261–271
24. Diekman, W., Venis, M. A., and Robinson, D. G. (1995) *Proc. Natl. Acad. Sci. U. S. A.* **92**, 3425–3429
25. Thiel, G., Blatt, M. R., Fricker, M. D., White, I. R., and Millner, P. (1993) *Proc. Natl. Acad. Sci. U. S. A.* **90**, 11493–11497
26. Blatt, M. R., and Thiel, G. (1994) *Plant J.* **5**, 55–68
27. Gehring, C. A., McConchie, R. M., Venis, M. A., and Parish, R. W. (1998) *Planta* **205**, 581–586
28. Millner, P. A., Groarke, D. A., and White, I. R. (1996) *Plant Growth Regul.* **18**, 143–147
29. Barbier-Brygoo, H., Zimmermann, S., Thomine, S., White, I. R., Millner, P., and Guern, J. (1996) *Plant Growth Regul.* **18**, 23–28
30. Leblanc, N., Perrot-Rechenmann, C., and Barbier-Brygoo, H. (1999) *FEBS Lett.* **449**, 57–60
31. Caboche, M. (1980) *Planta* **149**, 7–18
32. Leblanc, N., Roux, C., Pradier, J. M., and Perrot-Rechenmann, C. (1997) *Plant Mol. Biol.* **33**, 679–689
33. Johnsson, B., Löfas, S., and Lindquist, G. (1991) *Anal. Biochem.* **198**, 268–277
34. Ephritikhine, G., Barbier-Brygoo, H., Muller, J. F., and Guern, J. (1987) *Plant Physiol.* **83**, 801–804
35. Rona, J. P., Ptiman, G. M., Lüttge, U., and Ball, E. (1980) *J. Membr. Biol.* **57**, 25–37
36. Napier, R. M., and Venis, M. A. (1990) *Planta* **182**, 313–318
37. Warwicker, J. (2001) *Planta* **212**, 343–347
38. Schouten, A., Roosien, J., de Boer, J. M., Wilmink, A., Rosso, M. N., Bosch, D., Stiekema, W. J., Gommers, F. J., Bakker, J., and Schots, A. (1997) *FEBS Lett.* **415**, 235–241
39. Venis, M. A. (1977) *Nature* **266**, 268–269
40. Ray, P. M., Dohrman, U., and Hertel, R. (1977) *Plant Physiol.* **59**, 357–364
41. Gertler, A., Grosclaude, J., Strasburger, C., Shlomo, N., and Djiane, J. (1996) *J. Biol. Chem.* **271**, 24482–24491
42. Philo, J., Aoki, K., Arakawa, T., Owers Narhi, L., and Wen, J. (1996) *Biochemistry* **35**, 1681–1691
43. Palme, K., Hesses, T., Campos, N., Garbers, Yanofsky, M. F., and Schell, J. (1992) *Plant Cell* **4**, 193–201
44. Lazarus, C. M., and Macdonald, H. (1996) *Plant Mol. Biol.* **31**, 267–277
45. Choi, S. Y. (1996) *Plant Mol. Biol.* **32**, 995–997

Conformational Dynamics Underlie the Activity of the Auxin-binding Protein, Nt-abp1

Karine David, Eugénie Carnero-Diaz, Nathalie Leblanc, Michèle Monestiez, Jeanne Grosclaude and Catherine Perrot-Rechenmann

J. Biol. Chem. 2001, 276:34517-34523.

doi: 10.1074/jbc.M102783200 originally published online July 3, 2001

Access the most updated version of this article at doi: [10.1074/jbc.M102783200](https://doi.org/10.1074/jbc.M102783200)

Alerts:

- [When this article is cited](#)
- [When a correction for this article is posted](#)

[Click here](#) to choose from all of JBC's e-mail alerts

This article cites 45 references, 18 of which can be accessed free at <http://www.jbc.org/content/276/37/34517.full.html#ref-list-1>