An Immunological Approach to ABA Receptor and its Gene

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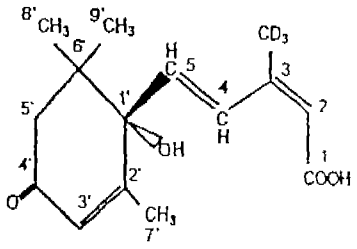
I. Abstract

Two types of immunological probes, anti-ABBP Abs, have been developed. The purified ABBP from ABA-C_1-BSA-sepharose 4B column was identified by PAGE and appeared in one band of about 56KD, as well as showed a specific binding ability and a high affinity for ABA (Kd2.0×10^{-9} mol/L). Unexpectedly, the existence of rRNA with a length of around 300 nucleotides could be found, when the ABBP was digested with protcinase K and identified by elctrophorsis on an agarose gel (1%). As a result, about 120 cDNA clones coding maize 17s RNA and only one cDNA clone coding ABBP (24cDNA) were obtained from 200,000 seperated phage plaques by the anti-ABBP pAbs. 24cDNA had 1075bp and contained an open reading frame coding 254 amino acids. The anti-idiotypic Ab raised against an ABA MAb showed the ability of either mimicking ABA or competing with ABA. The localization of ABBPs in plant cell was investigated.

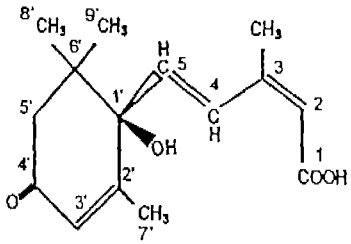
II. Instruction

Abscisic acid (ABA) is an acidic sesquitepene and unlike most other

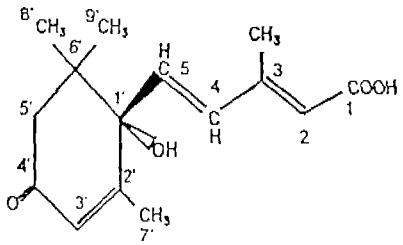
hormones has an asymmetric carbon. The naturally occurring isomer is dextrorotatory ABA. The biologically active form of (+)ABA has the cis/trans configuration, the biosynthetic trans/trans form is converted from cis/trans form by UV irradiation and becomes virtually inactive (Fig.1).



2-cis-4-trans-(+)-ABA



2-cis-4-trans-(-)-ABA



2-trans-4-trans-(+)-ABA

Figure 1. Configurations of abscisic acid. The (+)-ABA can be isomerized to its (-)-enantiomer and has cis/trans configurations. Of these configurations only (+)-ABA has biological activity.

During the last decade, our understanding about (-)ABA biosynthetic pathway in higher plants, ABA responsiveness of over 150 genes from various species as well as the relationship between ABA and second messengers such as Ca^{+2} and calmodulin, IPs has progressed greatly. On the other hand, 2-cis-(+)ABA has already been mass-produced with a strain of *botrytis cinerea* in Japan. The dream of ABA application in Agriculture and horticulture will come true.

Both of theoritical and practical advances need a corresponding progress concerning ABA receptor research. Unfortunately, however, little is known about this field. Allen & Teewavas (1994) indicated that receptor to red light, auxin and calcium have been well characterized, and those for blue light, ethylene and incompatibility are not far behind. By comparision, the site of perception of GA and ABA remains in a more troublesome area.

In order to facilitate the ABA receptor research, following molecular probes have been applied.

1. Radiolabeled probes

3-ABA is now commercially available at high specific activities (87ci/mmol) and has been used in RLBA(Radioligand Binding Assay) which is very effective for studying animal hormones. Due to a high level of non-specific binding, however, the ABA RLBA has been less successful.

2. Photoaffinity probes

ABA contains an α,β-unsaturated ketone group which can be photoactivated by UV irradiation, probably to a highly reactive triplet excited state. By this way, a hydrogen atom from a donor molecule, such as an amino acid and residue of a receptor protein can be abstracted. As a result, ABA may be covalently attached to its receptor. Using this probe, Hornberg and Weiler (1984) got some experimental results. Since then, however, further progress has been negligible.

3. Immunological probes

As compared with both probes mentioned above, the immunological probes are more promising in ABA receptor research. Sine 1988, we have been develo ping, viatwo different ways, anti-ABA Binding Protein Antibodies and anti-idio typic Antibodies mimicking (+)ABA (Fig 2)

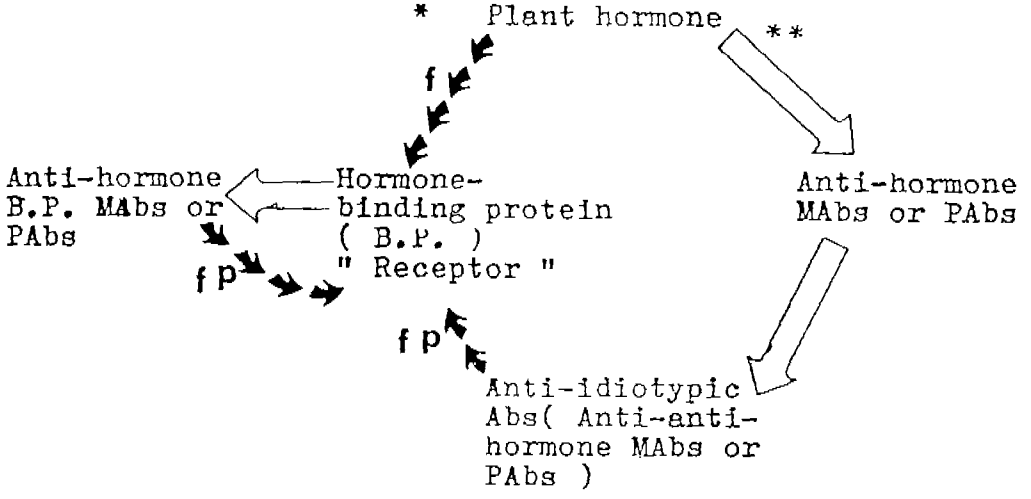


Figure 2. Two different ways to make immunological probes for "Receptor". Immunization is represented by the straight arrow. The curved arrow represents fishing outs(f) and/or probing(p) the "Receptor".
* A certain derivative of plant hormone immobilized on support matrix, ** The same conjugated with BSA,HSA or KLH.

III. TEXT

1. Developing the antibodies against ABA binding proteins(ABBPs).

Using sepharose 4B linked with ABA-C_1-BSA or ABA-C_4-BSA, two groups of ABBPs from coleoptiles and root tips of maize were extracted. As

compared with $C_{4'}$, -column, the C_1-column could offer much more ABBP. The eluting effects of 7mol/L urea and 3mol/L KSCN were compared based on antigen-immobilized ABA ELISA and PAGE. Only the latter could provide ABBP in high purity, while the former might break the linkage between 'ABA-BSA' complex and sepharose 4B and then contaminated the ABBP. The purified ABBP from C_1-column was identified by PAGE and appeared in one band of about 56KD. The ABBP also showed a specific binding ability and a high affinity for ABA($K_\alpha = 2.0 \times 10^{-9}$mol/L). Interestingly and unexpectedly, if the purified ABBP was digested with proteinase K and then identified by electrophoresis on an agarose gel (1%), the existence of rRNA with length of about 300 nucleotides would be found. As a result, the antibodies against such an immunogen would recognize both of rRNA and ABBPs.

Two kinds of antibodies, anti-C_1-ABBP PAbs and anti-$C_{4'}$-ABBP PAbs have been developed.(Xia and ZHou, 1996).

2. Developing anti-idiotypic Ab(Anti-Id) as the internal image of (+) ABA.

A monocional antibody (IgG_1), With high specificity to abscisic acid methyl ester (ABAme) was firstly prepared. This antibody was derived from an imm unogen in which carrier proteins (BSA) were linked to ABA at c_1 (-COOH). It shows very little cross-reactivity with ABA(1%)or ABA glucosyl ester(3.5 %). Other analogues such as α-cis-xanthoxin, violaxanthin and both the 2,tra ns-isomer and the (R)-enatiomer of ABA, were not found to exhibit any cross-reactivity. Based on this antibody, an ELISA which displays a linear range from 4.75×10^{-14} to 1.52×10^{-12} mol of ABAme has been set up. This ELISA was use d to monitor the ABA contamination in ABBP eluate.(ZHou,ZHeng et al.1996).

The MAb(Ab_1) was also used as an immunogen to immunize rabbits . The crude antisera of Anti-Ids were purified with an affinity chromatograph in which normal Balb/c mice IgG were linked to sepharose4B. The Anti-Ids(Ab_2) were eluted from the column with PBS.Several experiments have been carried out to determine the ability of Ab_2 mimicking ABA molecule. An overview fo immobilized antibody ELISA for this purpose or for ABA quantification was shown in Fig.3 Immobilized antigen (ABA-OVA) ELISA also showed that Anti-Id(Ab_2) could block the binding of ABA MAb to ABA-OVA. The retention time of ABA binding protein in Ab_2-column was the same as that in ABA-OVA column except that the amount of ABBPs was different. It implied that Anti-Id_s (Ab_2) could mimic ABA on fishing out ABBPs.

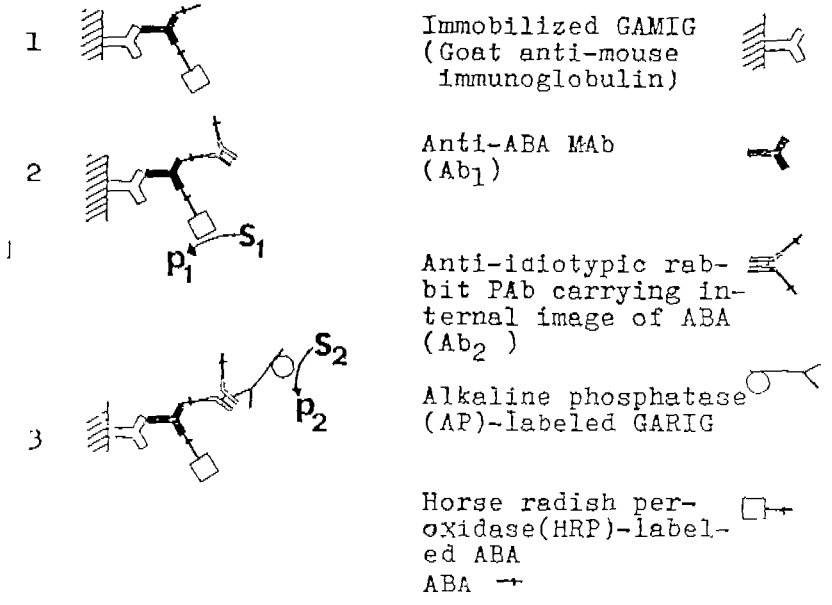


Figure 3. Overview of ELISAs for ABA measurement (1) or the identification of anti-idiotypic antibodies(2 and 3)

addition, it was found that anti-Id raised against ABA MAb could also recognize rabbit anti-ABA Ab_s and inhibit the ABA-HRPbinding to pAb.

3. A Search for ABA Receptor.

3.1. Localization of ABBPs in maize root tips using immumo gold-silver staining method.

Based on the staining pattern observed along the root tip, an apicobasal gradient of ABBP has been revealed with either anti-c_1-ABBP PAbs or anti-Id. The labels are mainly concentrated in root cap and apical meristem. In the elongation zone and root hair zone, only cells in pericycle and epidermis are obviously labeled. The silver labels are distributed evenly in the whole cell of apical meristem, while in the cells of root cap and elongation zone, only nuclei and plasma membranes are strongly labeled. The implication, therefore, is that ABBPs are present at the plasma membrane and dependant on the nucleic existence(SANG, ZHOU et al 1994).

3.2. To identify putative ABA receptor in the plant mitochondrion.

Some biophysical parameters, such as the fluidity and the phase transition temperature of mitochondria membrane, the activity of Na^+-K^+ ATPase and succinate dehydrogenase could be significantly affected by (+) ABA(CHEN, ZHOU et al. 1994). Isocitric acid dehydrogenase(ICDH), one of the important enzymes in Krebs cycle also could be increased by treating the isolated mitochondria from maize coleoptiles with (+) ABA.

After being isolated from the coleoptiles of maize grown in dark at 26℃

for 5 days, the mitochondria were treated with (+) ABA (range:0-200umol/L), then a rise in ICDH activity could be obviously detected, when the mitochondria were incubated with anti-C_1-ABBP PAbs (normal rabbit sera as a control) for 15min before adding ABA, the increasing degrees of ICDH activity were decreased by 95%, 50% and 35% for 10,100 and 200mol/L ABA respectively (Fig 4). The results indicated that the effect of ABA on ICDH activity is mediated by ABA binding sites located in mitochondria(Xia, liuetal unpublished).

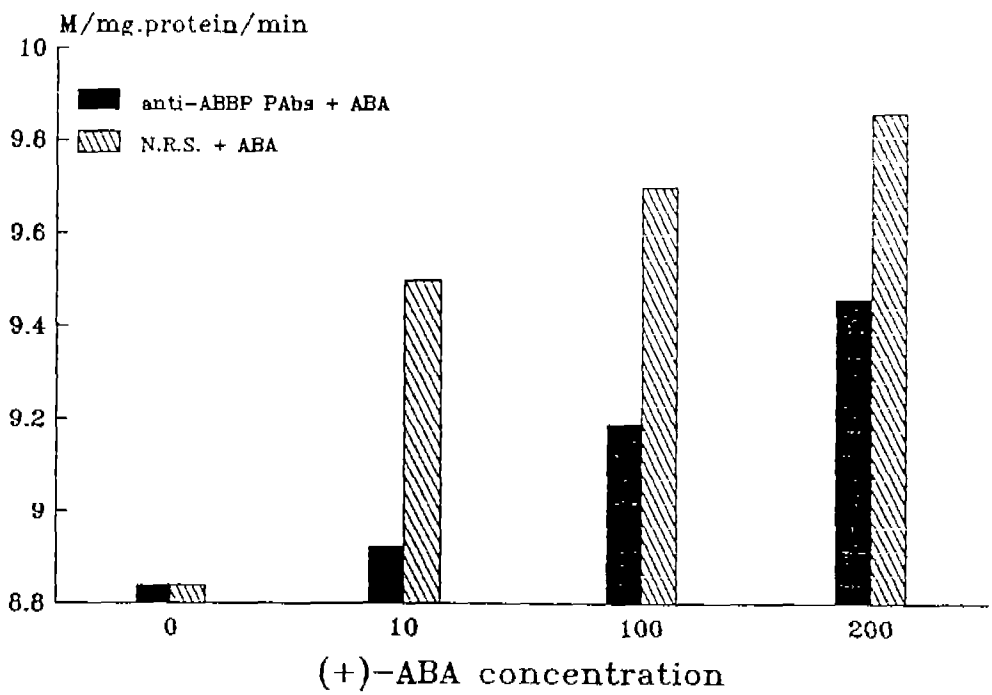


Figure 4. ABA EFFECT ON ICDH ACTIVITY COULD BE INHIBITED BY INCUBATING ANTI-AB BP PAbs WITH ISOLATED MITOCHONDRIA 15 MINUTES BEFORE TREATING THE MITOCHON-DRIA WITH (+)ABA

4. A search on coloning the gene of ABA receptor

Using the antibodies against ABBPs which contains rRNA. Several screening were carried out from the cDNA expression library of maize. From 200,000 separated phage plaques, about 120 cDNA clones coding maize 17S RNA and only one cDNA clone coding ABBP called 24 cDNA were obtained. This result demonstrated that the antibodies with high specificity onlt recognized recombinant lambda expressing maize ABBP and 17S RNA. On the other hand, it was showed that the sparsity of cDNA of ABBP was in accordance with the fact that the content of ABBP in higher plants was also extremely low.

24 cDNA had the length of 1075bp and contained an open reading frame coding 254 amino acids. The sequence was searched for the homology to those innrdatebase, indicating that it was a new gene not reported or registered so far. The comparative data about homology indicated that 24 cDNA showed relatively high homology to nucleic acid-binding proteins in the nuclei of mammalia. Its homology region was located at 346-510 of 5′ flanking with 60-65% identities.

The characteristics of deduced peptide were as follows: (1) Ser- and Leu-rich; (2) many phosphorated sites; (3) strong hydrophobic; (4) three PEST region unstable; (5) a motif similar to helix-lop-helix.

Ⅳ. Conclusion

Two types of immunological probes, anti-ABBP Abs and anti-idiotypic Abs, have been developed and applied to identify the ABA receptor and its gene. The former probe only recognized the determinents(epitopes)of ABBP,

whereas the ligand reception site in ABBP could be recognized by a kind of latter probe. However, the anti-idiotypic Abs may also recognize ABA-uptake or metabolising proteins. Using both probes, one can confirm a fact or draw a conclusion with redoubled confidence.

At least three kinds of auxin receptors have been localized. Are there also several ABA receptors? Recently, Anderson et al (1994) and Gilroy and Jones(1994) presented evidences that ABA receptors are located on the external face of the plasma membrane. On the other hand, Allan and Trewavas(1994) synthesized and microinjected caged ABA into individual guard cells and observed that internal releaze of ABA in open guard cells can induce stomatal closuse. A cytoplasmic receptor of ABA action is the favored conclusion. The latter viewpoint is supported by our experimental results. Why the purified ABBP always conjugates or links with rRNA? It is assumed that a proportion of ABBP is cytoplasmically located and appears to be secreted from rough endoplasmic reticulum. In addition, the ABA binding sites located in plant mitochondria would also be noteworthy.

We have already used highly purified anti-ABBP Abs as a probe to select an ABBPcDNA clone from the cDNA expression library of Maize. These result would be confirmed by screenings cDNA library using an oligonucleotide probe constructed on the basis of the NH_2-terminal sequence of a purified ABBP.

V. Reference

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