Molecules in focus
Natriuretic peptides—a class of heterologous molecules in plants

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Abstract

Immunological and physiological evidence suggests the presence of biologically active natriuretic peptide hormones (NPs) in plants. Evidence includes specific binding of rat atrial NP, [rANP (99–126)] to plant membranes and the promotion of cyclic guanosine-3′,5′-monophosphate (cGMP) mediated stomatal responses. Furthermore, anti-ANP affinity purifies biologically active plant immunoreactants (irPNPs) and a biologically active Arabidopsis thaliana irPNP (AtPNP-A) has been identified. AdPNP-A belongs to a novel class of molecules that share some similarity with the cell wall loosening expansins but do not contain the carbohydrate-binding wall anchor, thus suggesting that irPNPs and ANP are heterologues. We hypothesise that irPNP-like molecules have evolved from primitive glucanase-like molecules that have been recruited to become systemically mobile modulators of homeostasis acting via the plasma membrane. Such a function is compatible with localisation in the conductive tissue and the physiological and cellular modes of action of irPNPs reported to-date.

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Keywords: Plant hormones; Natriuretic peptides; Homeostasis

1. Introduction

The molecular structures and physiological functions of natriuretic peptides (NPs) in animals are the subject of a large and rapidly growing literature. Atrial NPs (ANPs), also referred to as atrial natriuretic factors (ANFs) or atriopeptins, were first discovered in extracts of rat atria (deBold, Borenstein, Veress, & Sonnenberg, 1981). ANP synthesis and processing are complex and the term ANP is subsequently used for the biologically active C-terminus (ANP, 99–126). NPs constitute a family of peptide hormones that are implicated in the regulation of salt and water homeostasis and their effects are mediated by the guanylate cyclase coupled NP receptors (NPR-A and NPR-B) or the clearance receptor (NPR-C) (Takei, 2001).

The first indications for NPs in plants came from radioimmunoassays on Florida beauty (Dracena godseffiana) (Vesely & Giordano, 1991) where antibodies against the N-terminus (ANP, 1–98), the mid-portion (ANP, 31–67) and the C-terminus (ANP, 99–126) recognised peptides in leaves and stems. Subsequently, it was demonstrated that synthetic cANP can induce stomatal opening in Tradescantia sp. in a concentration dependent manner (Gehring, Md Khalid, Toop, & Donald, 1996). It is noteworthy that Naa is required in the medium for activity in animal systems,

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which is not the case in plants (Gehring et al., 1996). This suggests that in plant NPs operate on processes other than Na\(^+\) transport, such as K\(^+\) transport or the synthesis of compatible solutes.

Evidence for receptor ligand interactions comes from binding studies where isolated leaf membranes were exposed to rat 3]-\[125\text{I}\]iodotyrosol 28 ANP (Gehring et al., 1996; Suwastika, Toop, Irving, & Gehring, 2000). Approximately 50% of the labelled ligand could be displaced by 0.1 \(\mu\)M rANP, indicating that plant membranes contain a low-affinity NP binding site for the heterologous rANP.

Isolation and affinity purification of immuno-reactive plant NPs (irPNPs) from ivy (Hedera hedges) and potato (Solanum tuberosum) with rabbit anti-[125\text{I}]-ANP (99–126) (human) antiserum yielded several different molecules, indicating that plants either contain more than one type of NP or that different precursors also contain the epitope(s) (Pharmawati, Billington, & Gehring, 1998a). IrPNPs promote stomatal opening (Pharmawati et al., 1998a), affect ion transport in plants (Pharmawati, Shabala, Newman, & Gehring, 1999), displace ANP-binding (in situ and in vitro) (Suwastika et al., 2000) and cause rapid, reversible cGMP increases (Pharmawati, Gehring, & Irving, 1998b; Pharmawati, Maryani, Nikolakopoulos, Gehring, & Irving, 2001). Responses to irPNP and ANP showed a considerable, and perhaps, astonishing degree of similarity.

2. Structure

We have since identified and characterised an irPNP from Arabidopsis thaliana (AdPNP-A) and several closely related sequences in different plant species (Ludidi, Heazlewood, Seoighe, Irving, & Gehring, 2002). AdPNP-A is a small protein of 126 amino acids in length (MW: 14016 kD; pl: 9.22) that is encoded by a gene with a single intron of 100 bp (accession no.: AAD08935). The protein contains a 24 amino acid signal peptide (MW: 2249) (Fig. 1a). AdPNP-A, its related sequence ApNP-B, and orthologues in other higher plant species, share domains with the cell wall loosening expansins (Fig. 1b) and can be classified as \(\gamma\)-expansins on the basis of this homology. Interestingly, the C-terminal domain of \(\alpha\) and \(\beta\) expansins (Cosgrove, 2000) is encoded by an entire exon that is absent in irPNP-like molecules (Ludidi et al., 2002). Expansins are distantly related to glucanases and cellulases; in the latter, the C-termini have been proven to be cell wall binding (Linder & Teeri, 1997) and the same function has been suggested for the \(\alpha\) and \(\beta\) expansin C-terminus (Cosgrove, 2000). Since expansins, which are the closest relatives of irPNPs, and the more distantly related glucanases and cellulases, all contain the C-terminus, it is reasonable to argue that irPNP-like molecules have lost this domain. This is in keeping with the fact that the domain is delineated by an intron-exon border. Loss of the wall binding domain could result in increased mobility of irPNP-like molecules. It also follows from the phylogenetic data, that similarities between AdPNP-A and ANP (Fig. 1c) may be the result of convergent evolution.

3. Synthesis and degradation

Loci of synthesis and degradation as well as induction patterns of irPNPs are currently under investigation. RT-PCR has revealed AdPNP-A transcripts in leaf tissue from un-stressed Arabidopsis plants (Ludidi et al., 2002), affecting transport in plants (Pharmawati, Shabala, Newman, & Gehring, 1999), displace ANP-binding (in situ and in vitro) (Suwastika et al., 2000) and cause rapid, reversible cGMP increases (Pharmawati, Gehring, & Irving, 1998b; Pharmawati, Maryani, Nikolakopoulos, Gehring, & Irving, 2001). Responses to irPNP and ANP showed a considerable, and perhaps, astonishing degree of similarity.

Loco of synthesis and degradation as well as induction patterns of irPNPs are currently under investigation. RT-PCR has revealed AdPNP-A transcripts in leaf tissue from unstressed Arabidopsis plants (Ludidi et al., 2002), indicating that the protein is not only found, but synthesised, in leaves. It is also possible that stress induced up-regulation of AdPNP-A transcription occurs either in leaves or elsewhere. Data on irPNP-B-like molecules comes from citrus (CjBAp12; accession no.: AAD03398). While the biological role of the molecule is as yet undetermined, CjBAp12 transcription occurs in the root only and is up-regulated in response to blight infection (Cecardi, Barthe, & Derrick, 1998). The protein is also found in shoots (Cecardi et al., 1998), thus strongly suggesting that the molecules are transported, possibly in the xylem, and have a systemic mode of action. Our in situ localisation data indicates that irPNPs are systematically mobile proteins since they are found in conductive tissue; a highly unlikely place for synthesis, but not transport. We have also detected biologically active immunoreactants in xylem sap and this is indicative of synthesis in the root (G. Bradley and C. Gehring, unpublished observation). Further information on temporal and spatial induction patterns will be forthcoming when transgenic plants containing GUS or GFP promoter fusions are available.
4. Biological function

There is evidence that irPNPs have a role in plant homeostasis. The difficult task ahead is to elucidate their role and mode of action. An overview of processes directly or indirectly affected by irPNPs is shown in Fig. 2. Net water uptake into plant cells is osmotic in nature and occurs in response to highly regulated ion movements. Under unfavourable osmotic conditions, e.g., high salinity, plants can synthesise intracellular compatible solutes such as proline or mannitol, thus increasing intracellular osmotic pressure and drawing in water. Enhanced water uptake does occur in response to NPs in plants (Gehring, 1999; Maryani, Bradley, Cahill, & Gehring, 2001) and may be the driving force of the observed stomatal opening which is conditional on water uptake. Since irPNP does not appear to change water permeability (J. P. Lassalles, personal communication) and net ion flux appears with a delay (>20 min) (Pharmawati et al., 1999), it is interesting to speculate that irPNPs stimulate rapid synthesis (or re-compartmentalisation) of compatible solutes. Such a mode of action is in accordance with previously observed NP-dependent increases in water movement out of the conductive tissue (xylem) (Gehring, 1999), increases in cell volumes (Maryani et al., 2001), the delayed net K⁺ and Na⁺ uptake (Pharmawati et al., 1999) and the
promotion of stomatal opening (Gehring et al., 1996; Pharmawati et al., 1998a; Gehring, 1999).

5. Possible biotechnological applications

Preliminary data suggest that trPNP increases in shoots in response to NaCl stress. An trPNP response to environmental disturbance is not unexpected and in agreement with the blight induced protein (Ceccardi et al., 1998). The pathogen causes severe disruption and plugging of xylem vessels leading to wilting and limb death. Accordingly, CjBAp12, found predominately in the xylem, may have a role in countering the severe homeostatic disturbance caused by the pathogen. We are currently making A. thaliana transgenics with modified AtPNP-A expression to test changes in plant response to drought and salinity stress.

References


