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Dedicated to Professor Włodzimierz Ostrowski
Review

Two plant signalling peptides: systemin and ENOD 40

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Recently several new evidences have appeared on biological role of native short peptides. This is an overview on two of them occurring in plants: systemin and ENOD 40.

During the course of evolution, plants and microorganisms have evolved a number of different relationships from beneficial such as legume — *Rhizobium* symbiosis or root — mycorrhizal association, to pathogenic ones in which microbes parasite on the host plant causing its diseases. At the early stages of these interactions, specific signals are released that elicit a discrete response of the respective partner and determine the outcome, i.e. symbiosis, pathogenesis or systemic acquired resistance.

The signalling molecules synthesized by the plant regulate the expression of defined genes which initiate the defence cascade or organogenesis. In recent years major advances have been made in understanding of the events occurring during the induction and expression of plant defence responses. In response to wounding, the plant releases localized and systemic signals that are coupled to induction of a set of defence genes. The timing and extent of the plant response to intracellular signalling determine the final outcome of a plant-pathogen interaction. The signalling molecules should meet the following criteria: i) synthesis by the plant, ii) ability to recognize the attack by pathogen or pest, iii) movement throughout the plant, iv) induction of the defence-related proteins and phytochemicals which will finally enhance the resistance to pathogens.

The signalling molecules include oligouronide fragments of plant cell walls, abscisic acid, jasmonic acid, methyl jasmonate, salicylic acid, ethylene, as well as short polypeptides [1–5]. Oligouronides presumably belong to the early defence signals, released by polygalacturonases and pectin lyases from attacking pathogens [6]. In contrast to other low molecular mass compounds they are not mobile in plants and therefore it is unlikely that they represent long range systemic signals.

Recent evidence suggests that plants may widely use signalling peptides in embryonic development as well as throughout the life cycle. A wound hormone, a 18-amino acid polypeptide called systemin, activates defence genes [2]. Another peptide named

Abbreviations: ORF, open reading frame; SBPSO, systemin binding protein; TNFR, tumor necrosis factor receptor; TNF-λ, tumor necrosis factor λ

ENOD 40 is postulated now to be a regulator of the root nodule morphogenesis as a result of a legume-*Rhizobium* symbiosis [7]. Also, the function of differentiation signals is attributed to the extracellular domain of the cr4 protein, containing a cysteine-rich region similar to the ligand binding domain in the factor receptor of mammalian tumor necrosis (TNFR) and to seven copies of a previously unknown 39-amino acid repeat [5, 8]. In bacteria, another pentapeptide coded by 23S rRNA fragment renders cells resistant to the ribosome-inhibiting antibiotic erythromycin [9].

Biologically active peptides are synthesized as inactive precursors e.g. prosystemin [10] or, as in the case of ENOD 40, the biologically active primary translation product [7]. In this review we will concentrate on the properties and postulated mode of action of these two peptides.

SYSTEMIN

Systemin (**^{1}Ala-Val-Gln-Ser-Lys-Pro-Pro-Ser-Lys-Arg-Asp-Pro-Pro-Lys-Met-Gln-Thr-Asp^{18}**) is synthesized in tomato plants as a 200 amino acids precursor protein, prosystemin, whose synthesis is essential for accumulation of the wound-induced proteinase inhibitors [10, 11]. It has been shown that systemin is translocated throughout tomato plants. The movement of labelled systemin is similar to that of sucrose which supports its role as a systemic wound signal in tomato plants [12]. Apart from serine proteinase inhibitors, other proteins, such as polyphenol oxidase, sulfhydryl proteinase inhibitor, cathepsin D inhibitor, carboxypeptidase, leucine aminopeptidase, aspartic proteinase and threonine deaminase are also systemically regulated [13]. When placed directly on leaf wounds systemin is readily transported in the phloem of young tomato plants. A signal transduction pathway for defence gene activation was proposed in which oligouronides and systemin activate the lipid-derived pathway (the octadecanoid pathway) where linolenic acid is released from membranes, resulting in the synthesis of jasmonic acid and activation of defensive genes [14, 15]. Because of the similarity of the structure of jasmonic acid and its precursor phytodienoic acid to some prostaglandins, derived from arachidonic acid released from membranes, it has been postulated that systemin activates the release of linolenic acid, synthesis of jasmonic acid and activation of proteinase inhibitor genes [1].

Interestingly, a plant signal transduction system for defense against herbivores is very similar to the signalling systems of animals against bacterial infections. In response to the infection, macrophages release the tumor necrosis factor-λ (TNF-λ), which activates phospholipase A2. This enzyme in turn releases arachidonic acid, easily converted to prostaglandins which subsequently trigger fever to fight against infection. Since in plants arachidonic acid is absent, free linolenic acid is converted to phytodienoic acid and finally to jasmonic acid. The similarity of overall polypeptide and lipid based strategies of the plant and animal systems, together with similarity of the structures of prostaglandins, phytodienoic and jasmonic acids, suggest that both systems evolved from the same ancestral organisms [1].

It has been found that systemin is processed *in vitro* at the putative furin cleavage site (Arg10-Asp11) of the plasma membrane-associated protease, which is the systemin-binding protein (SBP50). Although the function of SBP 50 is not clear, its role in degradation of systemin has been suggested [16]. Systemin at levels of femtomoles per young tomato plant is involved in the induction of proteinase inhibitors, which is one of the most powerful gene-activating signals known. It was shown that systemin leads to alkalization associated with an increase of K^+ concentration in the extracellular medium of *Lycopersicon peruvianum* cells [17]. The peptide triggered also a transient depolarization of the tomato mesophyll cell membrane and transient acidification followed by an increase of extracellular pH of the tomato mesophyll tissue [18].

Recently, the tertiary structure of systemin has been solved using two-dimensional NMR spectroscopy. The identified *cis* isomer, shows a Z-like-β-sheet structure [19–24]. It is well known that the β-sheet domain is

present in various DNA-binding proteins [25]. One can therefore suggest that systemin having such a motif should be able to bind to DNA. This indeed appeared to be the case since random DNA immobilized on cellulose retards systemin [20]. These observations suggested also direct interactions of DNA with systemin.

As it was mentioned above, systemin activates synthesis of proteinase inhibitors I and II in tomato [12, 26, 27]. This can be explained by direct interaction of systemin with promoter regions of the respective genes. DNA forms a complex with peptide and was footprinted with deoxynuclease I. The data showed strong protection of nucleotides in the major groove of DNA against DNase I [28]. Thus it can be postulated, that the peptide having a Z-like-β-sheet structure could easily be located in the major groove of DNA (Fig. 1). Interestingly, several defined interactions could lead to this hypothetical model: amino group of arginine 10 interacts with oxygen 4 (O4) of thymine residue (T6), lysine 5 binds to phosphate oxygen of adenine 5, the oxygen backbone proline 7 is in close contact with N7 of adenine 3. Moreover, serine 8 and glutamine 16 interact with phosphate oxygen of adenine 3 and thymine 6, respectively. Furthermore, lysine 14 interacts with phosphate oxygen. The proposed model is not only in agreement with the experimental data but also conforms with earlier observation that residues near the C-terminus of systemin are necessary for its function [29].

ENOD 40 OLIGOPEPTIDE

The morphogenesis of legume root nodule — a *sui generis* organ in which atmospheric nitrogen is bound and reduced to ammonia — is activated by lipochito-oligosaccharide signal molecules (*Nod factor*) synthesised by rhizobia (nitrogen fixing symbiotic bacteria) [30–35]. This process is initiated by local dedifferentiation of root cortical cells. It has been postulated that *Nod factor* affects the local changes of the phytohormones — auxin/cytokinin ratio, which subsequently trigger cell division during morphogenesis [36].

As demonstrated for several legumes, the essential plant response to *Nod factor* induction is the expression of nodule-specific genes. One of them is *enod 40* gene [34]. The expression of this gene occurs at the early stage of symbiotic interaction in the root pericycle and induces corticall cell divisions in the initial phase of root nodule morphogenesis [35, 37]. In soybean, the ENOD 40 mRNA is detectable one day after inoculation with rhizobia at the time which corresponds to the development of nodule primordium. It seems that *enod 40* gene expression might be one of the key elements involved in nodule initiation, serving to trigger hormonal imbalances in the inner cortical cells of the legume root.

Sequence analysis of *enod 40* genes from different legumes and tobacco revealed two highly conserved regions. The region at the 5′ end contains a small open reading frame (ORF) starting the first ATG available encoding a peptide of 13 (alfalfa, pea, vetch) with 12 (soybean, lupin) or 10 (tobacco) amino acids [38–45]. The second conserved region lacking ORF is located in the central part of the gene. Since this region has a tendency to form particularly stable secondary structures characteristic of biologically active RNA species, it was postulated that the *enod 40* transcript might act as "riboregulator" controlling cell division and differentiation [40].

However, the recent finding of van de Sande *et al.* [7] revealed that although the riboregulator hypothesis cannot be excluded, *enod 40* encodes in fact a small oligopeptide of 10–13 amino-acid residues which confers resistance of tobacco protoplasts to elevated auxin concentrations. The same effect was demonstrated upon the addition of synthetic oligopeptide. A homologue of legume *enod 40* gene was recently identified in tobacco and it was found that the ENOD 40 peptide derived from legumes was also functional in tobacco protoplasts [7]. This finding suggests that ENOD 40 oligopeptide may play a more universal regulatory role in plants than that confined to legumes as it was originally thought.

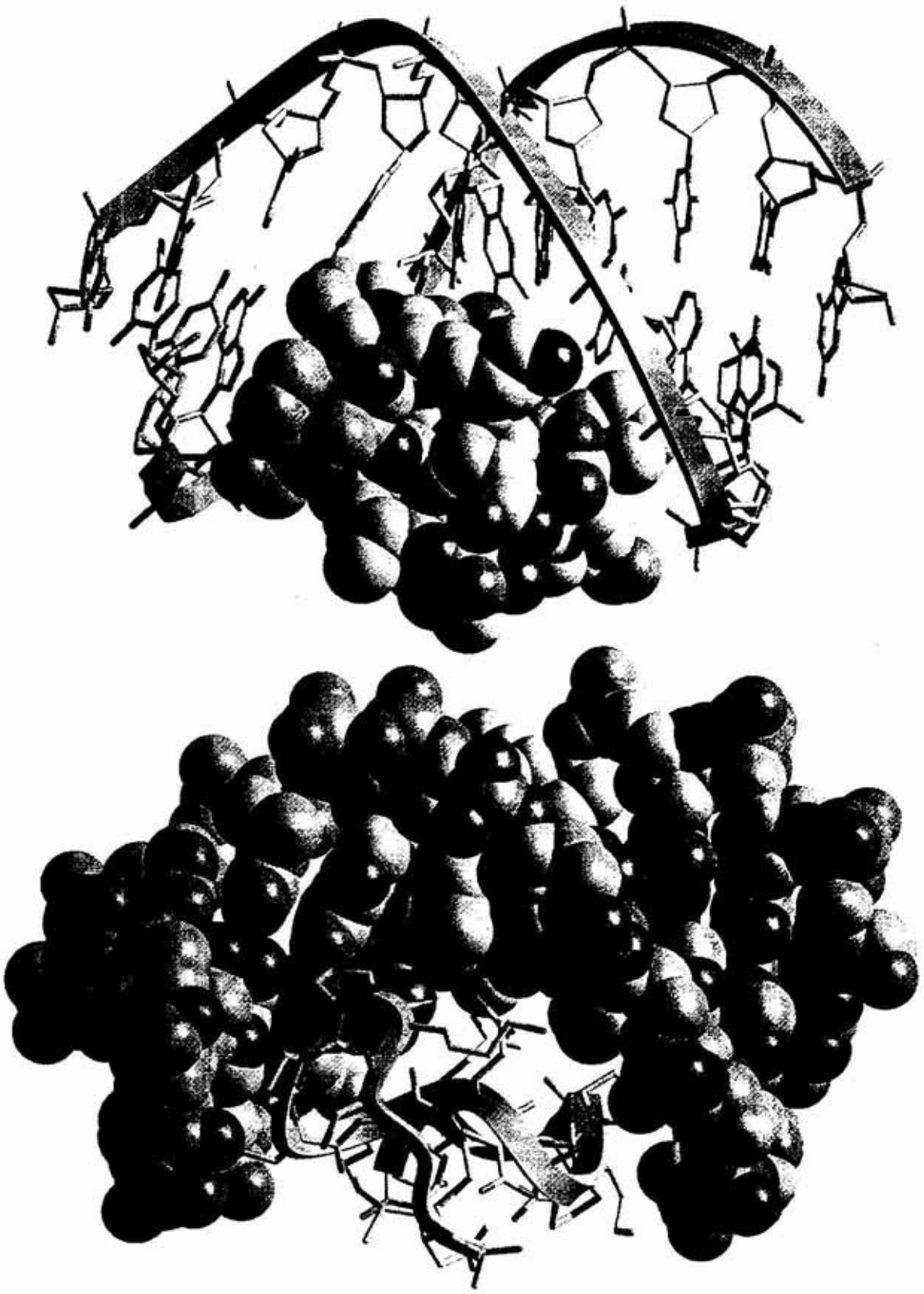

Figure 1. Hypothetical model of systemin-DNA interaction.

It was obtained by docking of the systemin NMR structure to the promoter region of the tomato proteinase inhibitor I gene (–96 to –65). One turn of DNA double helix is shown (stick model with ribbon on the left and space filling on the other). Systemin (space filling model on the left and stick model on the right) is located in the major groove of B DNA.

MsENOD40	atg	aag	ctt	ctt	tgt	tgg	caa	aaa	tca	atc	cat	ggt	tct
	Met	Lys	Leu	**Leu**	Cys	**Trp**	Gln	Lys	Ser	**Ile**	**His**	**Gly**	**Ser**
MtENOD40	atg	aag	ctt	ctt	tgt	tgg	gaa	aaa	tca	atc	cat	ggt	tct
	Met	Lys	Leu	**Leu**	Cys	**Trp**	Glu	Lys	Ser	**Ile**	**His**	**Gly**	**Ser**
PsENOD40	atg	aag	ttt	ctt	tgt	tgg	caa	aaa	tca	atc	cat	ggt	tct
	Met	Lys	Phe	**Leu**	Cys	**Trp**	Gln	Lys	Ser	**Ile**	**His**	**Gly**	**Ser**
VsENOD40	atg	aag	ctt	ctt	tgt	tgg	caa	aaa	tca	atc	cat	ggt	tct
	Met	Lys	Leu	**Leu**	Cys	**Trp**	Gln	Lys	Ser	**Ile**	**His**	**Gly**	**Ser**
GmENOD40-1	atg	gag	...	ctt	tgt	tgg	caa	aca	tcc	atc	cat	ggt	tct
	Met	Glu	...	**Leu**	Cys	**Trp**	Gln	Thr	Ser	**Ile**	**His**	**Gly**	**Ser**
GmENOD40-2	atg	gag	...	ctt	tgt	tgg	ctc	aca	acc	atc	cat	ggt	tct
	Met	Glu	...	**Leu**	Cys	**Trp**	Leu	Thr	Thr	**Ile**	**His**	**Gly**	**Ser**
LlENOD40B	atg	gaa	...	ctc	tct	tgg	caa	aaa	tcc	atc	cat	ggt	tct
	Met	Glu	...	**Leu**	Ser	**Trp**	Gln	Lys	Ser	**Ile**	**His**	**Gly**	**Ser**
NtENOD40	atg	cag	...	...	...	tgg	gat	gaa	gca	atc	cat	ggg	tct
	Met	Gln	...	...	...	**Trp**	Asp	Glu	Ala	**Ile**	**His**	**Gly**	**Ser**

Figure 2. The primary structure of ENOD 40 peptides isolated from different plants.

Ms ENOD 40, *Medicago sativa* [40]; Mt ENOD 40, *Medicago truncatula* [40]; PsENOD 40, *Pisum sativum* [44]; VsENOD 40, *Vicia sativa* [42]; Gm ENOD 40-1 and GmENOD 40-2, *Glycine max* [38, 43], respectively; LlENOD 40B, *Lupinus luteus* [45]; NtENOD 40, *Nicotiana tabacum* [38].

The above data suggest that the ENOD 40 peptide represents a novel plant growth regulator active in plant organogenesis [46]. This hypothesis is supported by the finding that *enod 40* gene is expressed not only during root nodule morphogenesis but also in lateral root development [40].

Since neither the ENOD 40 oligopeptide nor systemin appears to have an N-terminal transit sequence it seems unlikely that they are synthesized in the Golgi system. While systemin, like animal polypeptide hormones, appears to be processed from a larger precursor, the ENOD 40 oligopeptides are synthesized *de novo* as short translation products. This property seems to be a quite distinct feature of ENOD 40 oligopeptides.

The important question which still remains to be answered is how universal to the plant kingdom is the regulatory role of signal polypeptides. Systemin regulates the production of two other defence-signalling molecules: phytodienoic acid and jasmonic acid. The ENOD 40 oligopeptide affects the action of a potent developmental phytohormone, auxin.

The identification of signal peptides active in plant morphogenesis was a landmark discovery in plant biology opening new challenges to recognize detailed mechanisms of their functioning.

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