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Review

Plant signalling peptides: some recent developments

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Abstract: The subject of this review is plant signalling peptides, peptides of a new generation which regulate growth, differentiation, and other plant physiological functions. These peptides include systemin, the phytosulfokines (PSKs), ENOD40, CLAVATA3, Locus-S, POLARIS, IDA, and ROT4. On the basis of literature data and our own results we discuss their structure, biological properties, and structure/biological function relationship, especially for the more studied systemin and PSK-α. Copyright © 2007 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: plant peptide hormone; phytosulfokines; systemin; CLAVATA; Locus-S; POLARIS; IDA; ROT4

INTRODUCTION

It was noticed in as early as the 19th century that plants contain chemical transmitters that regulate their physiological processes. In the 1930s auxine, a growth hormone of uncomplicated structure, was isolated from plants [1,2]. In subsequent years, other plant hormones of diverse structure, such as giberelin [3], cytokines [4], abscisic acid [5], jasmonianes [6], ethylene [7], and brasinosteroids [8], were isolated. These substances are responsible for growth, development, proliferation and division of cells, tropism, maturation, ageing, and enzyme synthesis in plants. It is surprising that till the 1990s no peptide signalling substances had been described. Such compounds had been isolated earlier from lower and higher plants but these were mostly plant metabolites, antibiotics, or toxins.

Only in the last decade of the last century were endogenous signal and growth plant peptides identified. The discovery of these hormones gave rise to new studies in the field of plant phytochemistry and physiology.

We now know several groups of peptide hormones, such as systemin [9], phytosulfokines (PSKs) [10–13], ENOD40 peptides [14–17], CLAVATA3 [18], S-locus [19–22] POLARIS [23–25], inflorescence deficient in abscission (IDA) [26,27] and ROT4 [28,29] which are the subject of the present review. Studies concerning systemin and PSK-α predominate in the literature, and will therefore be discussed here in the greatest detail.

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Systemin

Systemin, an octadecapeptide of the sequence Ala-Val-Gln-Ser-Lys-Pro-Pro-Ser-Lys-Arg-Asp-Pro-Pro-Lys-Met-Gln-Thr-Asp, was isolated for the first time in 1991 by Ryan *et al.* [9] from infected leaves of the tomato *Lycopersicon esculentum*. The injury may be a result of insects feeding on the plant or other mechanical damage. Systemin activates the synthesis of proteinase inhibitors not only in the damaged tissues but also in the distant undamaged parts of the plant [30]. In subsequent studies the peptide was also isolated from other plant species of the *Solanaceae* family, including potato (*Solanum tuberosum*), bell pepper (*Capsicum annuum*), and black nightshade (*Solanum nigrum*) [31].

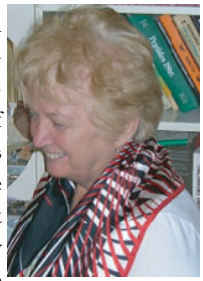
Systemin is present in the whole plant except roots [32]. The search for systemin in other plant species, such as lupin [33] or poplar leaves [34] has not given the expected results. The sequences of systemin isolated from various plant species of the *Solanaceae* family differ slightly from each other (Table 1) [31].

It has been shown that systemin is released from its precursor, prosystemin [30,36], and is the 179–196 octadecapeptide fragment of prosystemin located near its *C*-end. The peptide is flanked by a Leu residue at the *N*-end and a tetrapeptide fragment H-Asn-Asn-Lys-Leu-OH at the *C*-end (Figure 1).

Owing to the fact that prosystemin is a precursor of systemin it should be given some attention. It consists of 200 amino acid residues, of which 43% are polar [30]. It is stored in low quantities in leaves and its level increases in response to injury [36].

Stress evoked by plant damage activates the biosynthesis of prosystemin along with a group of proteolytic enzymes whose task is to release systemin [30]. One should mention that, e.g., in transgenic tomatoes there is the excessive expression of prosystemin (as if the plants were constantly damaged), which shows that

BIOGRAPHY



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Bahyrycz Agata She is a member of Chemistry of Natural Products Team, Faculty of Chemistry of Wrocław University. She obtained her M. Sc. in chemistry at Wrocław University in 2000 and begun graduate studies at the Faculty of Chemistry of Wrocław University. She obtained Ph.D. degree in organic chemistry in 2005, and started to work at University as an assistant. The main object of her studies are synthesis and structure/function relationship investigations of plant peptide hormone- phytosulfokine-α. She is the co-author of 7 scientific papers published in international journals and several conference presentations. As a research associate at University, she has lectures, seminars and laboratories in organic chemistry for students of chemistry and biology.

prosystemin overexpression is a sufficient factor in starting the octadecanoic pathway [31]. On the basis of their studies, Ryan [37] has proposed a mechanism of systemin action in plants. It is a complex process of enzyme-regulated transformations initiating a complicated signal cascade [35]. It results in releasing jasmonic acid, which activates the defensive gene (Figure 2).

This signal system shows an analogy with the eicosane signal system in animals [38]. A similarity between defensive signalling in plants and animals suggests that both signal paths could have developed from common ancestral beginnings [38].

Meindl *et al.* [39] and Scheer and Ryan [40,41], studying systemin binding to the receptor sites on the plant membranes, have found that it binds to a protein of 160 kDa which they called systemin receptor-160. They performed their studies on cell cultures from a potato (*Lycopersicon peruvianum*) with radioisotopically labelled systemin analogue [Tyr(^{125}J)^{2}, Ala^{15}]-systemin as a ligand [40].

Structurally, systemin is highly polar, containing in its peptide chain both basic and acidic amino acid residues. A striking feature is the presence of four Pro residues (positions 6, 7, 12, and 13), which form an important structural element and conformational influence. There is a Gln residue at position 3 in systemin isolated from the tomato leaves, whereas systemin from potatoes and Turkish pepper contains a His residue at this position. Systemin obtained from black nightshade, *S. nigrum*, contains Arg and Pro residues at position 3 and 11, respectively (Table 1).

During studies on the structure–function relationship of systemin, a series of its analogues have been synthesized. Modifications of systemin consisted in the synthesis of:

1. analogues devoid of the *N*-terminal Ala and *C*-terminal Asp residues;
2. the *C*-terminal fragment H-Met-Gln-Thr-Lys-OH;
3. analogues with the peptide chain substituted by Ala at all positions.

In biological studies on systemin and its analogues their properties as activators of proteinase inhibitor biosynthesis have been evaluated [42].

Table 1 The amino acid sequence of systemin in different plant species [35]

Plant species	Amino acid sequence
Lycopersicon esculentum	AVQSKPPSKRDPPKMQTD
Solanum tubcrosum-2	AVHSTPPSKRDPPKMQTD
Solanum tubcrosum-2	AAHSTPPSKRDPPKMQTD
Solanum nigrum	AVRSTPPPKRDPPKMQTD
Capsium annuum	AVHSTPPSKRPPPKMQTD

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It has been found that substitution of Pro-13 or Thr-17 by Ala drastically decreases and abolishes biological activity [42]. Further studies have shown that these analogues exhibit antagonist activity.

Analogues of systemin devoid of the *N*- and *C*-terminal residues are not able to induce inhibitor biosynthesis. Weak systemin activity is preserved in the systemin *C*-terminal tetrapeptide fragment. The results obtained show that Pro-13, Thr-17, and the *N*- and *C*-terminal residues are indeed important for the systemin–receptor interaction [42].

A striking feature of systemin is the symmetrical appearance of two pairs of Pro residues at positions 6 and 7, and 12 and 13. This general symmetry is complemented by the presence of three Lys and Arg residues, and between the Pro pairs is the tetrapeptide Ser-Lys-Arg-Asp. The presence of Gln residues at positions 3 and 16 is also interesting (Figure 1).

In 2001, octapeptides were isolated from tobacco leaves, which were named TobHypSysI and TobHypSysII (Table 2) [43]. They show functional similarity to the previously described systemin. Like systemin, the octapeptides are released by the plant cells in response to tissue damage by pathogens, and provoke the proteinase inhibitor synthesis. In the course of further studies, peptides showing systemin activity have been isolated from tomato leaves and characterized [44]. They are composed of 18 (TomHypSysI), 20 (TomHypSysII), and 15 (TomHypSysIII) amino acid residues (Table 2). Each of these peptides contains an amino acid sequence rich in Hyp residues surrounded by polar residues. These compounds are glycopeptides, though the carbohydrate attachment sites have not yet been established [43,44]. The carbohydrate component is essential for biological activity.

Conformational studies on systemin. On the basis of their CD investigations, Toumadje and Johnson [45] have postulated that the structure of the systemin central part is analogous to that of polyproline II. Ślósarek *et al.* [46] have used two-dimensional NMR to find the presence of the *cis* isomer in systemin, which showed that the peptide adopts a Z-like β-sheet conformation (Figure 3).

It follows from the results presented that systemin has so far been identified in plants of the *Solanaceae* family. The biological activity of systemin is dependent on its structure and modifications of the systemin amino acid sequence result in compounds of the decreased biological activity.

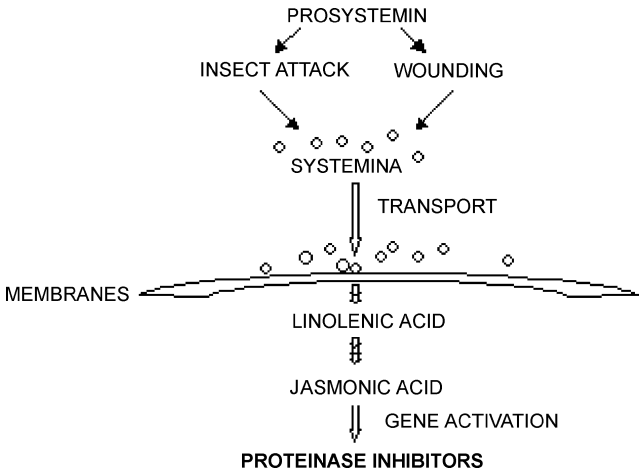

Figure 2 A proposed model for the activation of proteinase inhibitor genes by systemin [32].

Table 2 The amino acid sequences of systemin: TobHypSysI, TobHypSysII, TomHypSysI, TomHypSysII, and TomHypSysIII are shown compared with the sequence of tomato systemin [37]

Peptide	Amino acid sequence	Pentose units
TobHypSysI	RGANLPOOSOASSOOSKE	9
TobHypSysII	NRKPLSOOSOKPADQRP	6
TomHypSysI	RTOYKTOOOOTSSSOTHQ	8–17
TomHypSysII	GRHDYVASOOOOKPQDEQRQ	12–16
TomHypSysIII	GRHDSVLPOOSOKTD	10
Systemina	AVQSKPPSKRDPPKMQTD	0

Phytosulfokines

Discovery and biological properties of PSKs. PSKs, plant signal peptides of the sequences H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH (PSK-α) and H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-OH (PSK-β), were isolated in 1996 by Matsubayashi *et al.* from the mesophyll cell cultures of asparagus – *Asparagus officinalis* [10], rice – *Oryza sativa* [11], zinnia – *Zinnia elegans* [12], and carrot – *Daucus carota* [13]. These peptides possess mitogenic properties and are involved in plant cell proliferation [10] and in re-differentiation of cells (e.g.

MGTPSYDIKNKGDDMQEEPKVKLHHEKGGDEKEKIIEKETPSQDENNKDTIS
SYVLRDDTQEIPKMEHEEGGYVKEKIVEKETESQYIIKIEGDDDAQEKLKV
EYEEEEYEKEKIVEKETPSQDINNKGDDAQEKPDVEHEEGDDKETPSQDIIKM
EGEGALEITKVVCEKIIVREDL**AVQSKPPSKRDPPKMQTD**NNKL

Figure 1 The amino acid sequence of prosystemin. The systemin sequence is indicated within the *C*-terminus [24].

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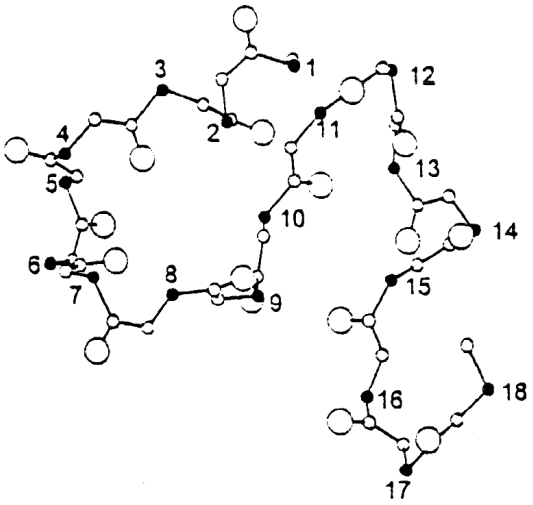

Figure 3 Stereoview structure of systemin [46].

transformation of the leaf mesophyll cells from zinnia to the tracheary element) [12].

Both PSK-α and PSK-β stimulate the proliferation of plant cells at a concentration 10^{-9} M; PSK-β acts more weakly and retains 8% of the PSK-α activity [47].

The presence of PSKs was confirmed for the first time in the conditioned medium (CM) prepared from rapidly growing mesophyll cells in culture of *A. officinalis* [10].

The mitogenic activity of single cells in the culture is strongly associated with the so-called initial cell density [10] (the minimum density of cells necessary for initiating the division and differentiation of cells in the culture). It can be initiated in a culture of the low population density by introducing it to the CM.

PSKs, besides activation of cell proliferation and differentiation processes, fulfill other functions in plants. They have stimulatory effects on chlorophyll synthesis in etiolated cotyledons of cucumber [48] as well as on the growth and chlorophyll content of *Arabidopsis* seedlings under high night-time temperature conditions [49]. Moreover, PSKs have stimulatory effects on adventitious root formation by hypocotyls of cucumber [50] and adventitious bud formation in *Antirrhnum majus* [51], influence the activation of alkaloid synthesis in *Atropa belladonna* [52], and reinforce the frequency of somatic embryogenesis in carrot cultures [13,53].

The species diversity of the plants in which PSKs are present and the variety of functions these compounds play suggest they are universal plant peptide hormones.

The PSK precursor. After the discovery of the PSKs, an attempt to search for their precursor was undertaken [47]. It has been found that the precursor of PSK-α is preprophytosulfokine (PP-PSK) consisting of 89 amino acid residues (Figure 4) and the pentapeptide discussed is released from it by proteolysis. PP-PSK has been isolated from the rice line Oc cells which are characterized by a high content of PSK-α in comparison with other plant species [54].

Figure 4 Precursor protein structure of PSK-α. (A) Structure of preprophytosufokine, with important domains: the potential *N*-terminal signal peptide and the active oligopeptide, PSK-α, are highlighted (B) Amino acid sequence around PSK-α.

Two important peptide chain segments can be distinguished in the structure of PP-PSK, which are important for biological activity. The first one is an *N*-terminal signal fragment of 22 amino acid residues including 10 Leu residues, and the second is the 80–84 fragment of PSK-α flanked at both termini by Asn residues [54]. There are 22 hydrophobic amino acid residues in the PP-PSK chain. Among the hydrophilic residues, acidic amino acids (Asp and Glu) and their derivatives (Asn and Gln) are often observed.

The mechanism of releasing PSK-α from its precursor is not fully understood yet, and little is known about the sites at which the hormone is cleaved by endoproteolytic enzymes.

It is interesting that PP-PSK does not show a homology with any plant protein found so far. There are, however, slight analogies with animal preprohormones, e.g. the epidermal growth factor (EGF) of rat [55].

These similarities are such that, as with animal preprohormones, PP-PSK contains a 22-amino-acid *N*-terminal signal peptide, the release of which gives a 67-amino-acid prohormone is formed [54]. When animal prohormones and the PSK prohormone have been compared, it was observed that the PSK-α sequence, as opposed to prohormones of the animal origin, is observed only once in the *C*-terminal precursor section [54].

The PSK receptor. Further studies on PSKs have sought a receptor site in plants. They have been carried out using PSK-α analogue labelled with ^{125}I and having a 4-azidosalicoyl group on the side chain of Lysine-5. The results of these studies indicate that there are two receptor proteins, of 120 and 160 kDa, respectively, in the rice plasmatic membrane [56].

Valuable information on the PSK receptor have been obtained by the studies of Matsubayashi and Sakagami [57], in which [^{3}H]PSK-α was used. They showed the influence of pH and selected metal ions on the specificity of receptor binding. The results of these studies suggest that the ligand–receptor binding is controlled by the ionic interactions with the side chains of basic amino acid residues located close to the receptor active site, which may have a significant influence on the receptor conformation.

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Structure–physiological function of PSK. The structure of PSK-α is characterized by the presence of Tyr(SO_3H) residues, at position 3 of the peptide chain.

To study the structure–biological function of PSK-α, a series of its peptide analogues have been synthesized (Table 3). The biological effects of these peptides have been evaluated by tests in which the mitotic activity of PSK-α and its analogues towards *A. officinalis* cells [47] and the binding of radioisotopically labelled peptide [^{3}H]PSK-α to carrot membrane protein [58] were studied. The structural modifications in the studies of Matsubayashi *et al.* [47] consisted in:

1. Substitution of the PSK-α sulphate residues by the esterified Tyr residues;
2. Simultaneous removal of the *C*- and *N*-terminal residues of the PSK chain;
3. Removal of the *N*-terminal residue of PSK;
4. Exchange of Ile^2 by Val or Thr^4 by Ser;
5. Elongation of the peptide chain by a triglycine fragment from the *N*- or *C*-end (Table 3).

Further studies on the PSK structure–biological activity relationship have been undertaken [59,60] involving:

(1) substitution of the Tyr(SO_3H) residues at position 1 or 3 or simultaneous exchange of both Tyr(SO_3H) residues at positions 1 and 3 of the peptide chain;
(2) change of amino acid configuration from L to D at position 1 or 3 or at both positions at the same time;
(3) substitution of Thr^4 by Ser (Tables 4–6).

Among a series of analogues, the greatest affinity for the receptor (30%) was shown by [Phe(4-Cl)^1]-, [Phe(4-I)^1]-, and [Phe(4-Cl)^3]-PSK-α. [Phe(4-NO_2)^1]-, [Phg(4-NO_2)^1]- [Phe(4-F)^3]- and [Tyr(PO_3H_2)^3]-PSK-α preserved 10% of the native peptide activity (Tables 4 and 5). A low affinity for the receptor (3%) was observed for the analogue modified at position 1 of the peptide chain by Phe(4-Br) and D-Phe(4-$NHSO_2CH_3$). [D-Phg(4-NO_2)]- and [Phg(4-OSO_3H)]-PSK-α also showed a low

Table 3 Mitogenic activities of PSK analogues. Mesophyll cells of asparagus were incubated in the presence of each PSK analog. Mitogenic activities were determined on the 6th day of culture and ED_{50} value was defined as the concentration of compound required for 50% cell division [47]

Peptide	ED_{50} (nM)	Relative activity
H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH	4	100
H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-OH	50	8
H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-OH	20	20
H-Tyr(SO_3H)-Ile-OH	>1000	<0.1
H-Ile-Tyr(SO_3H)-Thr-Gln-OH	>1000	<0.1
H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-Gly-Gly-Gly-OH	100	4
H-Gly-Gly-Gly-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH	500	0.8
H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-Gly-Gly-Gly-Cys-OH	40	10
H-Tyr(SO_3H)-Ile-Tyr(OH)-Thr-Gln-OH	100	4
H-Tyr(OH)-Ile-Tyr(SO_3H)-Thr-Gln-OH	700	0.6
H-Tyr(OH)-Ile-Tyr(OH)-Thr-Gln-OH	>1000	<0.1
H-Tyr(SO_3H)-Val-Tyr(SO_3H)-Thr-Gln-OH	100	4
H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Ser-Gln-OH	200	2

Table 4 Biological activity of PSK-α analogues modified in position 1 of the peptide chain [59,60]

Peptide	Biological activity (%)
H-D-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH (I)	<0.1
H-Phe(4-SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH (II)	<0.1
H-D-Phe(4-SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH (III)	<0.1
H-Phe(4-$NHSO_2CH_3$)-Ile-Tyr(SO_3H)-Thr-Gln-OH (IV)	<0.1
H-D-Phe(4-$NHSO_2CH_3$)-Ile-Tyr(SO_3H)-Thr-Gln-OH (V)	3
H-Phe(4-NO_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH(VI)	10
H-D-Phe(4-NO_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH (VII)	<0.1
H-Phe(4-Cl)-Ile-Tyr(SO_3H)-Thr-Gln-OH (VIII)	30
H-Phe(4-Br)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XIX)	3
H-Phe(4-F)-Ile-Tyr(SO_3H)-Thr-Gln-OH (X)	<0.1
H-Phe(4-I)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XI)	30
H-Tyr(PO_3H_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XII)	<0.1
H-D-Tyr(PO_3H_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XIII)	<0.1
H-Phg(4-SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XIV)	<0.1
H-D-Phg(4-SO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XV)	<0.1
H-Phg(4-$NHSO_2CH_3$)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XVI)	<0.1
H-Phe(4-NO_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XVII)	10
H-D-Phg(4-NO_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XVIII)	1
H-Phg(4-OSO_3H)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XIX)	1
H-Phg(4-OPO_3H_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XX)	<0.1
H-Hpa(4-NO_2)-Ile-Tyr(SO_3H)-Thr-Gln-OH (XXI)	<0.1

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Table 5 Biological activity of PSK-α analogues modified in position 3 or 4 of the peptide chain [59,60]

Peptide	Biological activity (%)
H-Tyr(SO_3H)-Ile-Phe(4-NO_2)-Thr-Gln-OH (XXII)	3
H-Tyr(SO_3H)-Ile-Phe(4-Cl)-Thr-Gln-OH (XXIII)	30
H-Tyr(SO_3H)-Ile-Phe(4-F)-Thr-Gln-OH (XXIV)	10
H-Tyr(SO_3H)-Ile-Tyr(PO_3H_2)-Thr-Gln-OH (XXV)	10
H-Tyr(SO_3H)-Ile-D-Tyr(PO_3H_2)-Thr-Gln-OH (XXVI)	<0.1
H-Tyr(SO_3H)-Ile-Phg(4-NO_2)-Thr-Gln-OH (XXVII)	<0.1
H-Tyr(SO_3H)-Ile-Hpa(4-NO_2)-Thr-Gln-OH (XXVIII)	<0.1
H-Tyr(4-SO_3H)-Ile-Tyr(4-SO_3H)-Val-Gln-OH (XIX)	<0.1
H-Tyr(SO_3H)-Ile-Tyr(SO_3H)-Ile-Gln-OH (XXX)	<0.1

receptor activity (1%). Other analogues were practically inactive.

On the basis of those results, it is difficult to discuss importance of the individual amino acid residues for the plant biological activity. Nevertheless, it should be noted that the presence of sulfate esters at position 4 of the Tyr aromatic ring at positions 1 and 3 of the PSK-α peptide chain is essential for preservation of biological activity. It follows also from these studies that the configuration of the Tyr residues at positions 1 and 3 is fundamental for the PSK-α activity because the substitution of L-Tyr by D-Tyr results in the loss of biological activity.

OTHER PLANT SIGNAL PEPTIDES

ENOD40

There are nodules in the roots of legume plants in which the atmospheric nitrogen is bound and reduced to ammonia. These nodules are activated by the signal lipo-chito oligosaccharide molecule (*nod factor*) synthesized by *Rhizobia* bacteria [61]. This process is initiated by local root cells. Studies on several legume plant species have demonstrated that an important role in the response to the *nod factor* is played by nodulin genes. One of them is *ENOD40* [14–17,] which is detected in soya one day after its inoculation with the *Rhizobia* bacteria. It induces proliferation and division of cells in the internal part of the root bark at the early stage of nodule organogenesis, and hence its name (*Early NOD*) [61,62]. The structure of the gene has been described in detail in many papers [14–17].

Table 6 Biological activity of PSK-α analogues modified in position 1 and 3 of the peptide chain [59,60]

Peptide	Biological activity (%)
H-Phe(4-SO_3H)-Ile-Phe(4-SO_3H)-Thr-Gln-OH (XXXI)	<0.1
H-Phe(4-NO_2)-Ile-Phe(4-NO_2)-Thr-Gln-OH (XXXII)	<0.1
H-Phe(4-Cl)-Ile-Phe(4-Cl)-Thr-Gln-OH(XXXIII)	<0.1
H-Phe(4-F)-Ile-Phe(4-F)-Thr-Gln-OH (XXXIV)	<0.1
H-Tyr(PO_3H_2)-Ile-Tyr(PO_3H_2)-Thr-Gln-OH (XXXV)	<0.1
H-D-Tyr(PO_3H_2)-Ile-D-Tyr(PO_3H_2)-Thr-Gln-OH (XXXVI)	<0.1
H-Phe(4-Cl)-Ile-Tyr(3-SO_3H)-Thr-Gln-OH (XXXVII)	<0.1
H-Phe(4-Br)-Ile-Tyr(3-SO_3H)-Thr-Gln-OH (XXXVIII)	<0.1
H-Phe(4-F)-Ile-Tyr(3-SO_3H)-Thr-Gln-OH (XXXIX)	<0.1
H-Phe(4-I)-Ile-Tyr(3-SO_3H)-Thr-Gln-OH (XL)	<0.1
H-Tyr(3-SO_3H)-Ile-Phe(4-Cl)-Thr-Gln-OH (XLI)	<0.1
H-Tyr(3-SO_3H)-Ile-Phe(4-F)-Thr-Gln-OH (XLII)	<0.1
H-Phg(4-NO_2)-Ile-Phg(4-NO_2)-Thr-Gln-OH (XLIII)	<0.1
H-Hpa(4-NO_2)-Ile-Hpa(4-NO_2)-Thr-Gln-OH (XLIV)	<0.1

On the basis of sequential analysis of genes derived from several legume plant species, the sequences of peptides rated among the ENOD40 group (Table 7) have been deduced. A constant appearance of the Trp residue and the repeated *C*-terminal peptide fragment IHGS (Table 7) in the chains of these peptides is observed [63]. The presence of these peptides in plants plays an important role, as it seems, in the binding of nitrogen in the plant nodule. The *ENOD40* gene expression may be the factor initiating a hormonal action of nodule in the legume plants root cells [62]. Very few studies have been carried out to establish the role and mechanism of action of these peptides.

CLAVATA3 (CLV3)

CLAVATA3 (CLV3) is a signal polypeptide found in *Arabidopsis thaliana* [18]. The structure of the peptide, like that of ENOD40, has been deduced from gene *CLV3* sequential analysis [18]. It consists of 96 amino acid residues, and the octadecapeptide signal sequence located at the *N*-end, which is a hydrophobic fragment rich in Leu residues (Figure 5). The peptide is responsible for the proliferation of cells in the

MDSKSFVLLLLLFCFLFLHDASDLTQAHAHVQGLSNRKMMMMKMESEWVGANGE

AEKAKTKGLGLHEELRTVPSGPDPLHHHVNPPRQPRNNFQLP

Figure 5 The CLV3-predicted amino acid sequence. The signal peptide is indicated within the *N*-terminus [18].

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plant shoot apical meristem. Probably it counteracts excessive cell proliferation [64]. The shoot apical meristem controls a steady increase of the shoot and root length, differentiation of cells, and their building into the leaves and flower buds. As has been shown, in the Arabidopsis CLAVATA genes (*CLV1, CLV2*, and *CLV3*) are responsible for keeping the equilibrium between proliferation and differentiation of cells. The role of individual gene has been the subject of broader research including also studies on transgenic plants [64–66]. For clarity we omit the description of individual gene roles and mechanisms of action, referring the reader to the literature [67]. Until now, CLAVATA 3 have been the subject of studies by plant physiologists only, and there are no investigations concerning its structure–biological function relationships.

S-locus. There are three genes in locus S: *SLG, SRK*, and *SCR* [68]. They code the proteins that are involved in the mechanism of self-incompatibility. This complex process prevents self-fertilization of plants by their own pollen. In the pollination process, the pollen grains land on a stigma and undergo the steps of recognition, adherence, and hydration, followed by pollen germination and pollen tube growth through the pistil. If the pollen is recognized by polymorphic protein receptors (S-locus) as their own, then the pollination is stopped. This process prevents self-pollination, and in the long run counteracts plant degeneration. The self-incompatibility (self-infertility) is the most effective mechanism protecting against self-pollination, which consists in the incompatible pollen grain not sprouting at all or, in case the sprouting is started, short stili not penetrating the stigma. The self-incompatibility in *Brassicaceae* is controlled by a single multiallelic locus S. When the stigma is contacted by a pollen grain with the alleles identical to those of the pistil stigma, that pollen is recognized as 'self' and rejected while 'non-self' pollen can develop normally which leads to the fertilization of a cell [69]. This phenomenon has been best studied in plants of the *Brasicaeceae* family.

Gene *SRK* codes the protein functioning as a female self-incompatibility determinant [69]. The role of the SLG protein has not been studied so far. On the basis of accumulated proofs, a hypothesis has been put forward that the SLG proteins may (i) be a necessary component of the receptor complex [70], (ii) participate in the posttranscriptional maturation of SRK [71], (iii) take part in adhesion of the pollen to stigma [72], or (iv) function as carriers of the pollen ligand [73].

The best known is gene *SCR* (S-*locus cysteine – rich protein*), also known as *SP11* [74,75], which was isolated in 1999 from *Brassica compestis* (rapa) plants [75]. *SCR* codes the protein which acts as a male self-incompatibility determinant [75]. It is a signal polypeptide containing 74–83 amino acid residues and characterized by the presence of eight Cys residues (so-called *Cys-rich peptide*). The sequence of that polypeptide has been deduced from the sequence of gene S_8 which codes SCR/SP11 [74]. SCR is a new member of the pollen coat protein (PCP) protein family [76] (Figure 6). The structure–biological function relationship of Locus S peptide has not been studied so far.

The peptide is the subject of other intensive biological studies.

Table 7 The amino acid sequence of ENOD40 in different plant species [35]

Plant species	Sequence of ENOD40
Medicago sativa	MKLLCWQKS**IHGS**
Medicago trunculata	MKLLCWEKS**IHGS**
Pisum sativum	MKFLCWQKS**IHGS**
Vicia sativa	MKLLCWQKS**IHGS**
Glicine max-1	ME - LCWQTS**IHGS**
Glicine max-2	ME - LCWLTT**IHGS**
Lupinus luteus	ME - LSWQKS**IHGS**
Nicotiana tobacum	MQ --- WDEA**IHGS**

POLARIS

POLARIS (PLS) is a 36-amino-acid peptide (4.6 kDa) [23]. Its sequence has been deduced from the genome of *Arabidopsis* (Figure 7). It does not possess a signal sequence, which suggests that it acts in the cytoplasm, though there is no direct evidence for its presence in the intracellular region. *PLS* is expressed in the embryonic root and in the seedling's primary and lateral root tips [24]. Among *pls* mutant plants, a reduced primary root length due to reduced longitudinal cell expansion and increased radial expansion has been observed. Moreover, vascularization of the rosette leaves is also reduced with the fewer higher-order veins arising from the major strands. It has followed from successive studies that the *pls* mutants show hyper-responsiveness to exogenous cytokinin and reduced responsiveness to auxin mutants, two hormones responsible for proper root growth and the development of leaves' vascular system [25]. Basing on these observations, a hypothesis has been put forward that *PLS* is responsible for the maintenance of equilibrium between auxins and cytokins. Nevertheless, the precise mechanism of the peptide action has not been recognized yet.

IDA

Abscission is a physiological process of cell separation, which allows plants to discard unwanted organs, like old leaves or flowers. It starts with the formation of an abscission zone, which separates the plant body from the organs to be shed.

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SP11-9

*MK-SAIYALLCFIFIVSSHVQEV-E--A*NLRKT-**C**VHRLNSGGS**C**GKSGQHD**C**EAFYTNKTN
QKAFY**C**NCT-SPFRTRY**C**D**C**AIK**C**KVR

SP11-8

*MK-SAVYALLCFIFIVSGHIQEL-E--A*NLMKR-**C**TRGFRKLGK**C**TTLEEEK **C**KTLYP-R----
-GQ**C**T**C**SDSKMNTHS**C**D**C**-KS**C**

SP11-12

*MK-SAIYALLCFIFIVSSHVQELTEVGA*D--KQQ**C**KKNFP--GH**C**ETS--ER**C**ENTYK-RLN
KKVFD**C**H**C**Q--PFGRRL**C**T**C**-K-**C**

SP11-52

*MK-SVLYALLCFIFIVSSHVQDV-E--A*NLMNR-**C**TRELPFPGK**C**GSSEDGG**C**IKLYSSEKKL
HPSR**C**E**C**-EPRYKARF**C**R**C**-KI**C**

PCP-A1

*MKNTVKLSLIGFVMLTVLLLGETVI--A*QKRKP-CYSQEP-DKTCEVN---RCKANCVKKHK
KILAFTSCIKENNGNMYCRCQYPCPP

Figure 6 Alignment of predicted amino acid sequences of four allelic variants SP11 and PCP-A1. The amino acids of putative signal peptide for each protein are shown in italics; conserved cysteine residues in all four allelic variants of SP11 are shown in bold [76].

MKPRLCFNFRRRSISPCYISISYLLVAKLFKLFKIH

Figure 7 The amino acid sequence of POLARIS [77].

A few days after anthesis the *Arabidopsis* plants discard intact turgid flower petals, sepals and stamens.

Among *Arabidopsis* plants mutants have been identified that do not shed the flowers even after the shedding of mature seeds. These plants have been described as IDA [26]. It follows from the studies that they release the *IDA* gene in the abscission zone, which codes a 77-amino-acid polypeptide with the *N*-terminal signal sequence (Figure 8). It has been established by sequential analysis that there is a conservative region close to the *C*-terminal peptide section, rich in basic amino acids, which resembles the PSK and CLV3 precursor structure.

During studies carried out so far, the role and mechanism of action of IDA have not been established yet; nevertheless, it has been found that it probably plays a role of ligand for HAESA, kinase of the LRR-RLK-type engaged in the process of floral organs abscission [27].

ROTUNDIFOLIA4, DEVIL1(DVL1)

Leaf shape is determined by polar cell expansion and polar cell proliferation along the leaf axes. As shown by studies on *Arabidopsis* genes *ROTUNDIFOLIA4* (*ROT4*) and *DEVIL1* (*DVL1*) [28,29] are among genes engaged in the cell proliferation process. It follows from studies that plants with the gene *ROT4* overexpression possess round leaves, shortened floral organs, and short inflorescence stems. A similar phenotype is observed among *dvl1-1D* mutants. They are characterized by shortened petioles, shortened siliques, and moderately horned fruit tips. On the basis of these observations, it has been evaluated that the *ROT4* and *DVL* genes serve a similar function [28].

ROT4 and *DVL1* are members of the family described as *RTFL* (ROT FOUR-LIKE) (Figure 9). So far, the peptides coded by these two genes have not been isolated. Nevertheless, genome sequential analysis has established that they code peptides consisting of 53 and 51 amino acid residues, respectively.

All peptides coded by the *RTFL* genes are characterized by the presence of a conservative 29-amino-acid segment (RTF). These peptides are engaged in the cell

MAPCRTMMVLLCFVLFLAASSSCVAAARIGATMEMKKNIKRLTFKNSHIFGYLPKGV
PIPPSAPSKRHNSFVNSLPH

Figure 8 Alignment of predicted amino acid sequences of IDA [77].

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Rot4
MAP - - - - - EENGTCEPCK-TFGQKCSHVVKKQRAKFYLLRRCIAMLVCWHDQNHDRKDS
DVL1
MEMKRVMMSSAERSKEKKRSISRRLGKYMKEQKGRIYIIRRCMVMLLCSHD - - - - - - - -
DVL2
MES - - - IMS – LKR - KEKK-SQSRRLGKYLKEQKGRIYIIRRCMVMLLCSHD - - - - - - - -
DVL3
MKG - - - - - T - - - - - - KKKT-PCNKKLGGKYLKEQKGRIYIIRRCMVMLLCSHD - - - - - - - -
DVL4
MK - - - - - - - - MGG- SKRR-VSSKGLGAVLKEQKGRIYIIRRCMVMLLCSHD - - - - - - - -
DVL5
MKT - - - TGSSVGG-TKRK-MWSRGVGGVVREQKAKLIIRRCMVMLLCSHD - - - - - - - -

Figure 9 Deduced amino acid sequences of ROT4/DVL [77].

proliferation process at the different plant development stages.

SUMMARY

The discovery of plant peptide hormones has given rise to new directions of research in phytochemistry and plant physiology. The incentive to undertake those studies has not only been the cognitive aspect but also a practical one, especially to agriculture. The results of studies on the plant signal peptides presented here are mainly the results of studies performed by biologists. The material contained in the present article is not chemically uniform. These articles may be rated among pioneer studies aimed at the molecular explanation for obscure plant physiological mechanisms, which are controlled by plant endogenous peptide substances. The aim of this review is the arousal of interest in a wider group of scientists who could take up the task of explanation of the role of individual factors presented here in the plant physiological functions.

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