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Effect of Jasmonic, Salicylic, and Abscisic Acids on [^{14}C]Leucine Incorporation into Proteins of Pea Leaves

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Abstract—All investigated exogenous phytohormones (jasmonic, salicylic, and abscisic acids) induced the appearance of ^{14}C-label in a polypeptide with molecular mass 29 kD that was not found in the control; these acids also increased [^{14}C]leucine incorporation into a 25-kD polypeptide and decreased such incorporation into a 45-kD polypeptide. This can be considered as a nonspecific response of the plants to the action of these hormones. Salicylic and abscisic (but not jasmonic) acids induced the synthesis of a 19-kD polypeptide, and jasmonate induced the synthesis of a 96-kD polypeptide.

Key words: protein synthesis, jasmonate, salicylate, abscisic acid, signal systems in plant cells, stress

Investigation of the molecular mechanisms of the response of plants to biogenic and abiogenic stressors has demonstrated that the response includes a significant increase in stress phytohormones (jasmonic acid (JA), salicylic acid (SA), and abscisic acid (ABA)) production due to activation or induction of the synthesis of the enzymes catalyzing their formation. It is known that the formation of JA and SA is enhanced on activation by stressors of lipoxygenase [1] and superoxide synthase [2] signal systems, respectively. Intensive ABA synthesis in plant cells begins with the stressor-induced expression of the gene for zeaxanthin oxidase [3, 4], one of the key enzymes for formation of this phytohormone from carotenoids.

The synthesis of protective enzymes can be induced by stressor phytohormones—JA [5, 6], SA [7-9], and ABA [10]. Studies of the effect of two or more exogenous stress phytohormones on protein synthesis are very rarely performed on a single object. However, this is important at least because of the fact that under the effect of stressors, a set of stress phytohormones is usually synthesized, and it is of doubtless interest to determine the contribution of each of them to the formation of the stress reaction of a particular plant. So, the goal of our study was to determine the effect of each of the three most important stress phytohormones—jasmonate, salicylate, and abscisic acid—on protein synthesis in pea leaves.

MATERIALS AND METHODS

The leaves of 8-day-old sprouts of pea strain Danko were used. The plants were grown in cells with tap water at 23°C and lit with luminescent lamps; the light intensity was 30 kerg/sec per cm^2 with 16 h/day illumination. Cut leaves were placed on solutions of the exogenous acids, which were changed daily, where they were exposed for 6 days. The concentrations of abscisic, jasmonic, and salicylic acids were $1 \cdot 10^{-5}$ M. Leaves exposed on to pure water were used as the control. After 6 days of contact with the studied exogenous organic acid, the leaves were placed on a solution of L-[2-^{14}C]leucine with radioactivity 100 μCi/ml for 2 h. Samples with mass of 1 g were fixed with liquid nitrogen. The absorption of [^{14}C]leucine by the leaves was determined radiometrically using a Delta-300 radiometer (Tracor Analytic, USA). Soluble proteins were isolated in 20 mM Tricine-NaOH buffer (pH 8.3) at 4°C.

Basic proteins were separated by SDS-PAGE in a 12% gel (2% SDS) according to Laemmli [11]. Equal amounts of protein were placed on each lane. Standard proteins with molecular masses of 67 (BSA), 45 (ovalbu-

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min), 25 (chymotrypsinogen), 17.8 (myoglobin), and 12.3 kD (cytochrome *c*) were used as protein markers.

Protein concentration was determined according to Bradford [12]. Radioactivity of polypeptides was assayed by autoradiography; for this, the gels were dried and then used to expose RM-1 X-ray film. Four biological replicates of all experiments were performed. The reagents used in this work were from Sigma (USA).

RESULTS AND DISCUSSION

The overall [^{14}C]leucine content in proteins changed in response to the exogenous stress phytohormones JA, SA, and ABA (Fig. 1). Jasmonic and salicylic acids enhanced protein synthesis, whereas abscisic acid decreased label incorporation into proteins.

Judging by the autoradiographs of gels with applied extracts with equal protein content, stress phytohormones first changed the spectrum of labeled polypeptides and second, ambiguously changed the incorporation level of the labeled amino acid into the polypeptides (Fig. 2). The appearance of radioactivity in a polypeptide with molecular mass 29 kD in all experimental samples in contrast to its absence in the control attracted our attention. SA and ABA induced the appearance of radioactivity in a 19-kD polypeptide, and JA induced a 96-kD polypeptide. However, JA blocked the incorporation of the labeled amino acid into a 104-kD polypeptide.

The values of radioactivity corrected for the total radioactivity of the protein extracts are presented in Fig. 3. In the control, [^{14}C]leucine most intensively incorporated into polypeptides with molecular masses 90, 57, 45, 38, and 18 kD.

Exogenous phytohormones changed the radioactivity of many polypeptides. Jasmonate enhanced label incorporation into polypeptides with molecular masses 90, 71, 57, 38, 35, 25, and 18 kD and decreased that into a 45-kD polypeptide. Salicylate enhanced the label incorporation into polypeptides with molecular masses 90, 64, 57, 38, 32, 25, and 18 kD and decreases that into the 45-kD polypeptide. Abscisic acid increased the radioactivity of some polypeptides (64, 42, and 25 kD) as compared with the control (Fig. 3) in spite of the fact that the total radioactivity of proteins decreased (Fig. 1). The label content decreased in most polypeptides of this series (104, 90, 80, 71, 57, 50, 45, 43, 38, 35, 32, 24, 23, 22, 18, 15, and 12 kD).

The appearance of the ^{14}C-label in the 29-kD polypeptide, increase in the label incorporation into the 25-kD polypeptide, and decrease in that for the 45-kD polypeptide can probably be considered as a nonspecific response of plants to the action of all the stress phytohormones (ABA, JA, and SA) investigated by us. However, specificity of the reaction of plants to the action of exogenous ABA, JA, and SA was also found. For example, only

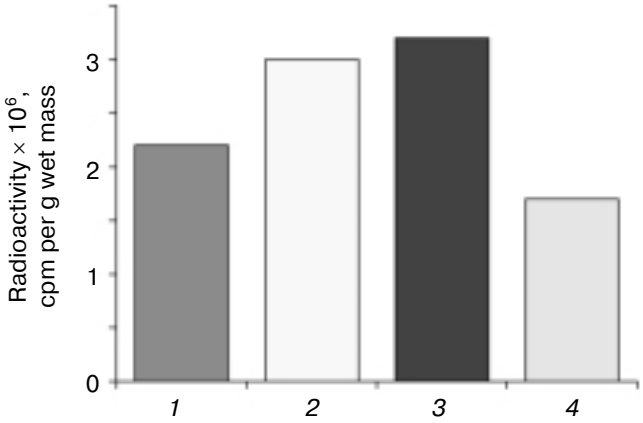

Fig. 1. Effect of jasmonate (*2*), salicylate (*3*), and abscisic (*4*) acid on [^{14}C]leucine content in soluble proteins; (*1*, control). The concentration of the phytohormones was $1 \cdot 10^{-5}$ M.

by the action of JA the appearance of the label in the 96-kD polypeptide, disappearance of the label in a 104-kD protein, and increase in formation of polypeptides with molecular masses 35 and 71 kD were observed.

The action of phytohormones on plants is known to involve various cell signaling systems that receive,

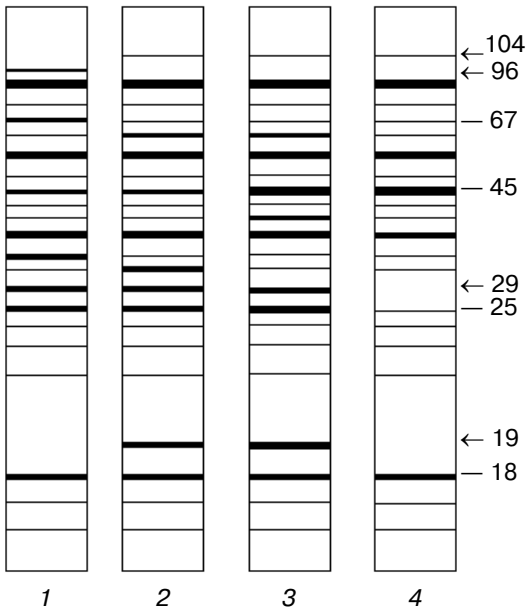

Fig. 2. Effect of jasmonate (*1*), salicylate (*2*), and abscisic acid (*3*) on the radioactivity of polypeptides (*4*, control). The concentration of the phytohormones was $1 \cdot 10^{-5}$ M. Molecular masses of proteins in kD are given on the right; positions of protein markers are indicated by the horizontal lines.

enhance, and transmit external signals (in this case, stress phytohormones) to the genetic apparatus. Jasmonate (and methyljasmonate) activate the lipoxygenase [13, 14] and superoxide synthase [15] signaling systems.

Salicylic acid activates lipoxygenase [16], MAP-kinase [17-19], superoxide synthase [20], and NO-synthase [21, 22] signaling systems.

Abscisic acid was found to activate lipoxygenase [23], calcium [24, 25] (including that with participation of cADP-ribose [26, 27]), MAP-kinase [28], superoxide synthase [29], and phosphatidic acid [30] signaling systems.

Comparing the literature data and our results, attention should be given to the fact that two very important signaling systems, lipoxygenase and NADPH-oxidase, are "switched on" by each of the investigated stress phytohormones (Fig. 4). This seems to cause a nonspecific response (Fig. 3) from a part of the cell genome (formation of a new 29-kD polypeptide, increase in the synthesis of the 25-kD polypeptide, and inhibition of the synthesis of the 45-kD polypeptide). The specificity of gene expression and thus caused [^{14}C]leucine incorporation into polypeptides can be explained by specific induction of the cell signaling systems by various phytohormones as mentioned above.

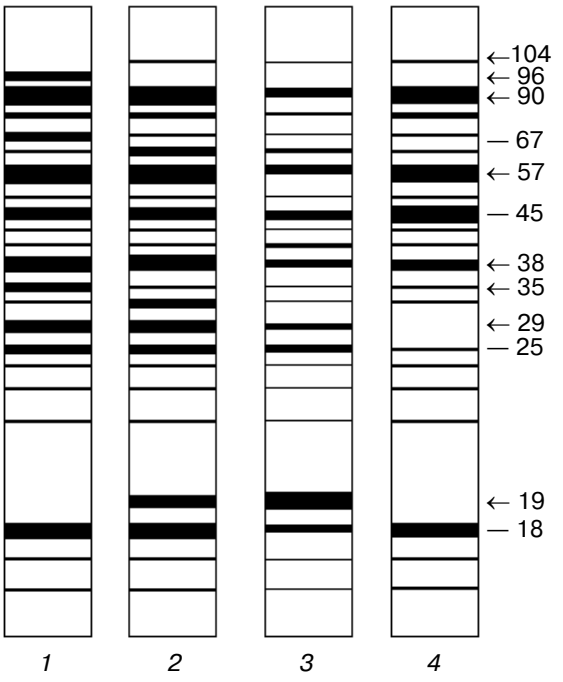

Fig. 3. Effect of jasmonate (*1*), salicylate (*2*), and abscisic acid (*3*) on the radioactivity of polypeptides corrected for the total radioactivity of protein extracts (*4*, control). The concentration of the phytohormones was $1 \cdot 10^{-5}$ M. Molecular masses of proteins in kD are given on the right; positions of protein markers are indicated by the horizontal lines.

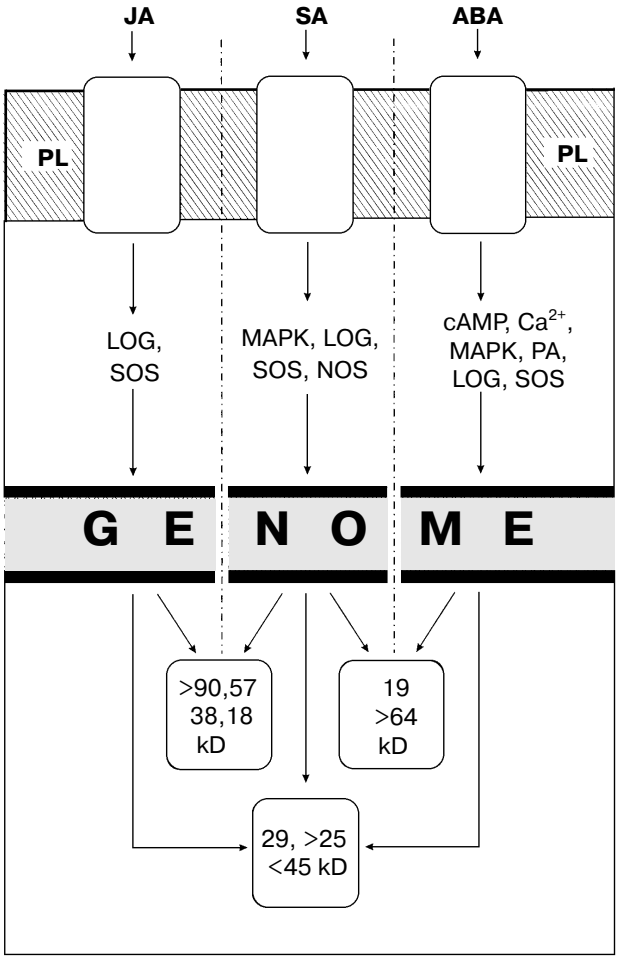

Fig. 4. Possible participation of signal systems in the nonspecific plant response to the action of phytohormones. LOG, lipoxygenase; SOS, superoxide synthase; MAPK, MAP-kinase; PA, phosphatidic acid; cAMP, cycloadenylate; NOS, NO-synthase; Ca^{2+}, calcium signal systems; PL, plasmalemma.

Decrease in [^{14}C]leucine incorporation into the proteins under the influence of ABA (Fig. 1) might be explained by the fact that this phytohormone "switches on" more signal systems simultaneously than JA and SA; this can cause over-excitation of the signaling network and the inhibition of cell response as a whole.

It is also necessary to consider that stress phytohormones can effect the formation of each other, e.g., JA causes a significant decrease in ABA content in cells [31], and SA inhibits the synthesis of JA [32]. SA was also found to suppress JA- [33] and ABA-induced [34] synthesis of some proteins.

There are reasons to assume the existence of a coordinated signaling network in plant cells consisting of interrelated signaling systems, activation of some signaling system (caused, e.g., by the action of a certain stress phytohormone) being able to influence the functioning of other signaling systems.

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