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Meeting abstract

Does NO participate in cytokinin signaling?

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Background

Nitric oxide (NO) is a unique ubiquitous molecule in animals and plants [1]. It has been demonstrated that NO mediates ABA-induced stomatal closure and interferes with ethylene during the maturation and senescence of plant tissues. Recently published data [2,3] suggested that NO is also involved in cytokinin signaling. However the above-mentioned data did not provide defenitive proof of a role of NO in cytokinin signaling. In order to obtain more conclusive evidence we have performed a study using P*arr5*::*GUS* transgenic *Arabidopsis* plants which express the *GUS* reporter gene under the transcriptional control of the cytokinin-responsive *ARR5* promoter. Additional experiments were also performed using *Amaranthus* seedlings.

Materials and methods

The *Arabidopsis* and *Amaranthus* assay systems were described in detail earlier [4,5]. The cytokinin effects were measured quantitatively in both systems. For RNA blot analysis, 3-day-old Arabidopsis seedlings were incubated with BA (with oor without tested compounds) for 35 min. Total RNA was isolated and separated in a 1.5% agarose-formaldehyde gel, transferred to nylon membrane (Amersham) and hybrydized with radioactive-labeled DNA probe. For radioautography blots were exposed with BioMax MS films (Kodak) for 1–3 days. Quantification of signal was done on an integrative densitometer CD-50 (Desaga). As a control for loading, the blot was rehybridized with an *actin 2* gene probe.

Results

In our experiments, a strong competitive inhibitor of all three isoforms of animal NO synthase (NOS), L-NMMA, inhibited the accumulation of GUS activity in transgenic *Arabidopsis* harboring the *GUS* gene driven by a cytokinin-sensitive *ARR5* promoter. In accordance with earlier publication [], this inhibitor also suppressed cytokinin-dependent betacyanin accumulation in *Amaranthus*. In the *Arabidopsis* assay system, the effectiveness of L-NMMA was higher than in *Amaranthus* one: 1–2 mM L-NMMA inhibited cytokinin-induced effect in *Arabidopsis* at about 80–90%. In *Amaranthus*, similar inhibition was observed with 5 mM L-NMMA.

In addition to L-NMMA we have used its D-isomer (D-NMMA) which does not affect animal NOS. Experiments with L- and D-isomers have shown that both isomers were able to inhibit cytokinin action with similar effectiveness. Thus no functional difference between the active (in animals) L-form and its inactive D-analog was revealed in the *Arabidopsis* assay system.

To obtain more direct evidence, we treated *Arabidopsis* seedlings with NO. *Arabidopsis* seedlings were incubated in the presence of the NO donors NOR3 or SNAP. In our experiments neither SNAP nor NOR3, both tested in a wide concentration range, caused a cytokinin-like effect, namely the enhancement of GUS activity. This result argues against a role for NO as a direct messenger of the cytokinin signal. However, it does not exclude the possibility that NO has a role in a some parallel transduction pathway, which could be indispensable to effective cytokinin signaling. This possibility was tested experimentally by adding NO donors to *Arabidopsis* plants which were treated with BA and L-NMMA. However experiments showed that NO did not alleviate the L-NMMA inhibition of cytokinin-induced GUS activity. This result also does not support the participation of NO in cytokinin signal

transduction to primary response genes, at least in *Arabidopsis*.

Next we explored whether L-NMMA inhibits cytokinin signaling at an early stage (before gene activation) or acts posttranscriptionally. To this end we analysed the steady state levels of *GUS* transcripts 35 min after cytokinin treatment of *Arabidopsis* seedlings. Results demonstrated that with no cytokinin treatment *GUS* gene expression was very low, the radioactive signal being hardly detectable. 35 min after cytokinin treatment a more than 30-fold increase of the *GUS* transcript was detected. 5 mM L-NMMA, which strongly inhibits the cytokinin-induced accumulation of GUS activity, had no influence on the cytokinin-induced accumulation of *GUS* transcripts. This result shows that L-NMMA acts posttranscriptionally.

Conclusion

Together the obtained results suggest that NOS inhibitor acted after the cytokinin signal transduction stage and NO has no direct role in eliciting the primary cytokinin response in plants, at least on cytokinin primary response genes in *Arabidopsis*.

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