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Effect of antagonists of ethylene action on binding of ethylene in cut carnations

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Abstract. Five hours after cut carnations had been treated with a pulse of 1 or 4 mM silver thiosulfate (STS), *in vivo* ethylene binding in petals was inhibited by 22 and 29%, respectively. When binding was measured 4 days after the 4-mM STS treatment, binding was inhibited by 81%. 2,5-Norbornadiene, which substantially delays carnation senescence, inhibited ethylene binding by 41% at a concentration of 1000 µl/l. The Kd for ethylene binding in carnations was estimated to be 0.1 µl/l in petals and 0.09 µl/l in leaves. The concentration of binding sites was estimated to be 6.0×10^{-9} mol/kg of petals and 2.0×10^{-9} mol/kg of leaves

Introduction

In two recent reports [8, 9] it has been shown, using an isotope competition technique, that ethylene binds specifically to plant tissues. The amount of ethylene needed to displace one-half of the bound ethylene is very similar to the concentration of ethylene required for half-maximal activity in biological systems. In addition, propylene and carbon monoxide, both active analogs of ethylene, are competitive inhibitors of binding [8].

Apart from this correlative evidence, there are presently no firm data indicating that the binding sites assayed by this technique are the physiological receptor sites for ethylene. We report here the results of experiments investigating the effect of two antagonists of ethylene activity, Ag^+ and 2,5-norbornadiene, on ethylene binding in carnation flowers. Both Ag^+ [6] and 2,5-norbornadiene [12] are known to preserve carnations, flowers which are very sensitive to ethylene [3, 4, 14].

Materials and methods

Plant material

Cut carnations (*Dianthus caryophyllus* L. cv. White Sim) were obtained from a commercial greenhouse and stored at 2 °C prior to treatment.

Flowers were treated with Ag^+ by placing the stems in solutions of the anionic silver thiosulfate complex (STS) containing 1 or 4 mM Ag^+ as indicated [7]. Some flowers were treated with 2,5-norbornadiene during the binding assay; 2,5-norbornadiene was injected into a sealed reaction vessel containing the flowers to generate a concentration in the vapor phase of 1000 or 4000 μl/l.

Measurement of ethylene binding

Binding experiments carried out in a 2.5-litre reaction vessel fitted with an injection port and a trap containing NaOH to absorb CO_2 and to remove impurities in the radioactive ethylene. [^{14}C] Ethylene (Amersham, 120 mCi/mmol) was trapped as the mercuric perchlorate complex [16]. Aliquots containing either 0.05 or 0.5 μCi of [^{14}C] ethylene were pipetted into a small Erlenmeyer flask containing a magnetic stirring bar, and were then placed in the reaction vessel. Plant parts, 28 g of intact flowers, 24 g of petals, or 20 g of leaves, were placed in the chamber, which was then sealed. To start the experiment, 1 ml of LiCl was injected into the flask; with stirring, 99% of the trapped ethylene was released into the gas phase within 10 min [8]. In some chambers, the released radioactive ethylene was diluted by the addition of sufficient unlabeled ethylene to bring the concentration in the reaction vessel to 1000 μl/l.

Intact flowers or petals [4] were exposed to the radioactive ethylene for 1 h, and leaves were exposed for 3 h. The plant tissues were then removed from the reaction vessel, ventilated for a fixed time, and placed in 1-liter jars. Release ethylene was trapped in mercuric perchlorate in a liquid scintillation vial which contained a piece of fiberglass filter to increase the efficiency of the trap. After 15–20 h the vials were removed, scintillation fluid was added, and the radioactivity was determined using a liquid scintillation spectrometer. The optimum time for ventilating the tissues during the transfer from the reaction vessel to the collection container was determined from time-diffusion curves [12]. The time that gave maximum binding with minimum residual free-space ethylene was 3 min for both petals and leaves. Experiments were performed a minimum of 3 times with 3 replicates per treatment. Results are the means ± S.E.

Results

Binding in flowers, petals, and leaves

When intact flowers were used to study ethylene binding, the non-bound component took 8 min to diffuse completely from the tissue. The bound component was lost even more slowly from the tissues. When separated petals or leaves were used for the binding assay, the non-bound component had diffused out within 3 min (Figure 1).

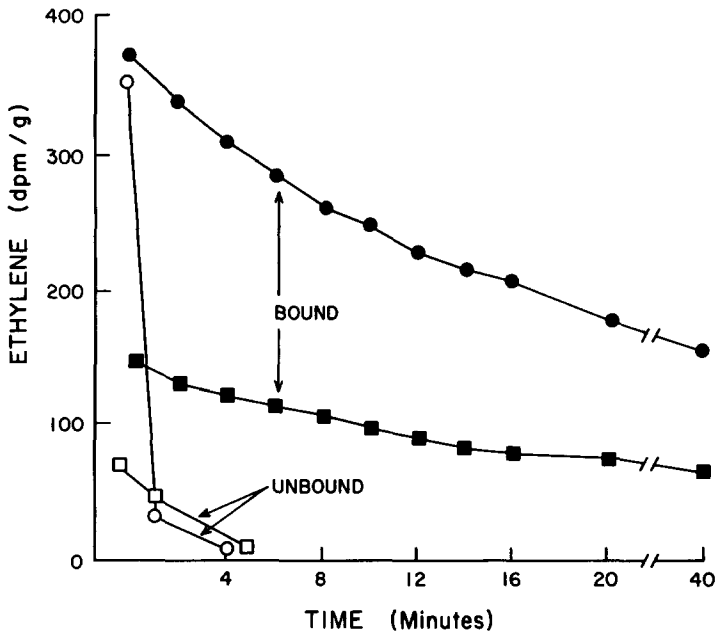

Figure 1. Diffusion of bound [^{14}C]ethylene from carnation petals (○, ●) and leaves (□, ■) as a function to time. Tissue was exposed to 0.5 μCi/l of [^{14}C]ethylene. The sum of bound and unbound is the total amount of [^{14}C]ethylene present at any given time.

Release of bound ethylene from the petals and leaves was relatively slow, with one-half being lost in approximately 12 min; however, substantial concentrations remained in the tissues after 40 min (Figure 1). On a fresh weight basis, leaf tissues bound less than half as much as petals.

Data from experiments where the tissues were treated with radioactive ethylene in the presence of different concentrations of unlabeled ethylene are plotted as a Scatchard plot in Figure 2. From the slopes and intercepts of the lines, one can calculate the concentrations of binding sites [8]. The petals and leaves contain 0.18 and 0.068 μCi of [^{14}C]ethylene/kg of tissue, respectively. The specific activity of the ethylene was 8.4×10^{-9} mol/μCi. This gives 1.5×10^{-9} mol/kg of petals and 5.7×10^{-10} mol/kg of leaves. The gas phase concentration of ethylene was 3.7×10^{-2} μl/l, and from the Scatchard plot this would occupy 25% of the binding sites in petals and 28% of the binding sites in leaves, with the total number of sites being 6.0×10^{-9} mol/kg of petals and 2.0×10^{-9} mol/kg of leaves. The Kd of binding in petals was 0.1 μl/l and 0.09 μl/l in leaves.

Effect of Ag^+ on binding. Treatment of carnation flowers with 4 mM sodium thiosulfate and 1 mM Ag^+NO_3 reduced ethylene binding, assayed 4 h later, by 22% and 26% in petals and leaves, respectively (Table 1). However, when the

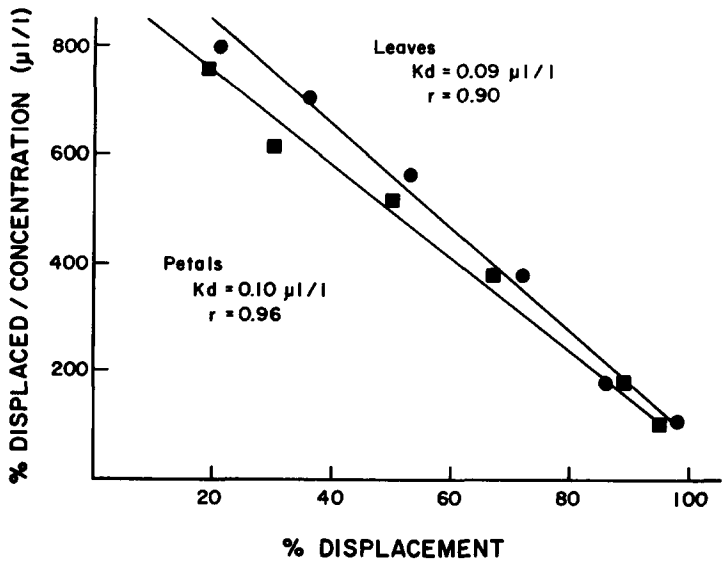

Figure 2. Scatchard plot of [^{14}C] ethylene displacement from the binding site by unlabeled ethylene. r = correlation coefficient.

carnations were treated with 4 mM STS for 4 days, ethylene binding was inhibited 81% in petals and 100% in leaves.

Table 1. Effect of Ag^+ (as STS) on ethylene binding in carnation petals and leaves

	Petals		Leaves	
Silver concentration mM	dpm/24 g fresh wt	% inhibition	dpm/20 g fresh wt	% inhibition
0	444 ± 25	0	230 ± 20	0
1 (5 h)	348 ± 24	22	170 ± 16	26
4 (5 h)	330 ± 25	29	146 ± 16	37
4 (4 d)	84 ± 11	81	0	100

[a]Exposed to 0.05 µCi of [^{14}C] ethylene in 2.9-litre volume.
[b]Took up 0.5 ml of $Ag(S_2O_3)^{-3}$/24 g flower in 40 min followed by 4 ml of water during 5 h. $S_2O_3^{-2}$ as $Na_2S_2O_3$ was in 4-fold excess, and without Ag^+NO_3 was without effect.

Effect of 2,5-norbornadiene on binding. Inclusion of 2,5-norbornadiene at 1000 and 4000 µl/l [11, 13] in the reaction vessel during the binding assay inhibited ethylene binding in petals by 41% and 66%, respectively (Table 2). Use of 4000 µl/l resulted in 86% inhibition of binding in leaves. This amount of 2,5-norbornadiene substantially delays the senescence of carnation flowers [12].

Table 2. Effect of 2,5-norbornadiene on ethylene binding in carnation petals

Concentration (μl/l)	Petals		Leaves	
	dpm/24 g fresh wt	% inhibition	dpm/20 g fresh wt	% inhibition
0	432 ± 40	0	–	
1000	256 ± 20	41		
0	460 ± 41	0	220 ± 20	0
4000	156 ± 11	66	32 ± 4	86

Discussion

The data reported here show that the attributes of an ethylene-binding site determined in carnations by a simple isotopic competitions technique [8] are consistent with the physiological effects of ethylene in these flowers. The concentration of ethylene at which half the binding sites are occupied is 0.1 μl/l, close to the concentration required for half-maximal stimulation of carnation senescence [5].

Ag^+ (applied as STS) strongly and specifically inhibits ethylene action in plants, and appears to act very early in the sequence of events leading to symptoms [1], lending credence to the suggestion that it might inactivate the physiological ethylene receptor. For this reason, Ag^+ has been used as a tool to examine the physiological nature of the binding activity measured by isotopic competition. Although Ag^+ readily inactivated binding by a Triton X-100 extract of Mung bean sprouts [10], it was without effect on ethylene binding by a particulate fraction prepared from *Phaseolus vulgaris* [2].

The data present here indicate inhibition of ethylene binding by an *in vivo* application of Ag^+ sufficient to inhibit ethylene action [6, 7]. Although inactivation of binding was relatively slow (35% after 5 h, 80% after 5 days), we know that some time is required for STS to move to the petals of carnation flowers [15], and it may be that the tightly complexed Ag^+ in STS activates the binding site slowly as the complex reaches the site and then dissociates. Inhibition of binding in leaves treated with $AgNO_3$ is rapid [R Goren, AK Matto and JD Anderson, personal communication]. The inhibition of binding activity by STS in carnation flowers was similar to that achieved by exposing them to 2,5-norbornadiene (a competitive inhibitor of ethylene binding [13]), at concentrations known to increase their longevity [12].

The data reported here, together with other correlative data on the relationship between the effect of a range of substances on ethylene binding and on its physiological effects, thus provide good evidence that the physiological receptor for ethylene is the binding site measured by isotopic dilution.

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