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Short communication

Effect of neocuproine and cuprizone on the development of amine oxidase and growth in pea seedlings

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Dedicated to Professor Bastiaan J. D. Meeuse in honor of his sixtieth birthday

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Effects of neocuproine (2,9-dimethyl-1,10-phenanthroline) and cuprizone (biscyclohexanone oxalyldihydrazone), as copper-chelating agents on the development of amine oxidase and the growth of pea plants were studied. Amine oxidase development in the apical part was inhibited by administration of neocuproine, and this effect was markedly reversed by the addition of copper disodium ethylenediaminetetraacetate(EDTA-Na_2-Cu). In this case, the amine oxidase activity was stimulated. Pea plant growth was inhibited by this chelator but not in the presence of EDTA-Na_2-Cu. On the other hand, the development of enzyme activity and growth were much less sensitive to cuprizone.

We have reported that the development of amine oxidase in germinating pea cotyledons is probably due to a de novo synthesis of proteins (*13*, *15*). The localization of amine oxidase activity in pea seedlings was examined carefully and high activity was found in the apical part and cotyledons (*2*, *5*, *8*, *17*). Hill and Mann (*2*) reported that although the total enzyme activity in whole plants decreases with age, high activity remains in the apical parts (shoot tip) of mature pea plants. Evidence that copper is an essential component of pea amine oxidase has been obtained (*1*, *9*), but the catalytic role of copper in the enzyme is still not understand (*11*). On the other hand, the role of amine oxidase in pea plant growth is interesing since tryptamine did stimulate the elongation of stem sections (*3*, *10*, *16*). In the present study, we have examined the effect of two copper chelators, neocuproine and cuprizone, on the development of amine oxidase in the apical part and on pea seedling growth. Neocuproine was used as a highly specific chelator for cuprous copper (*12*) while cuprizone, a chelator for cupric copper, is used as a specific inhibitor for copper-containing amine oxidases (cf. *4*).

Seeds of pea (*Pisum sativum* cv. Alaska) were germinated in moist vermiculite at 25°C for three days in the dark. Next, the seedlings were transferred to plastic trays containing four liters of 1/2 Hoagland's solution and/or various test solutions.

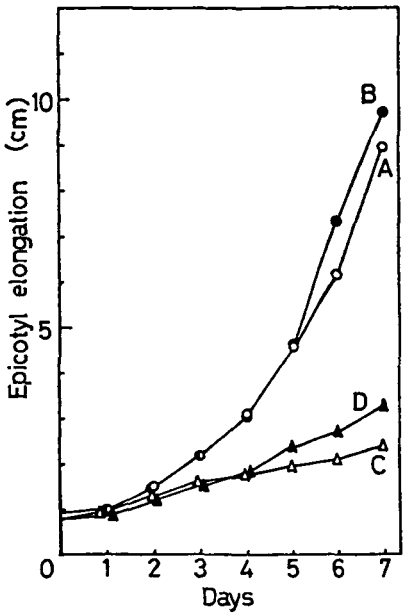

Fig. 1. *Growth patterns of pea seedlings in various culture solutions.* Culture conditions are given in the text. A: Control. B: Cuprizone (5×10^{-5} M). C: Neocuproine (5×10^{-5} M). D: Neocuproine plus EDTA-Na_2-Cu (2.5×10^{-5} M).

They were grown hydroponically under continuous light (about 2000 lux at plant level) at 25°C for seven days. Ten to twenty seedlings were used for each treatment. They were washed thoroughly with deionized water and elongation of the epicotyl and main root was measured. Enzyme extracts from apical parts were prepared by methods reported previously (*13*). Amine oxidase activity of the extract was determined using a Clark oxygen electrode and the consumption of soluble oxygen was measured with a TOA polyrecorder (Model-10A). The activities were expressed as μl O_2/min/mg protein. The reaction mixture was composed of 1.0 ml of phosphate buffer (0.1 M, pH=7.0), 250 μg of beef crystalline catalase, 0.5 ml of putrescine·2HCl (50 mM) and 0.1 ml of the extract solution. The total volume was adjusted to 2.3 ml with deionized water. The protein content of the extract was determined by the method of Lowry et al. (*7*) with bovine serum albumin as the standard.

As shown in Fig. 1 and 2 and Table 1, when neocuproine was added to the culture solution at 5×10^{-5} M concentration, elongation of the epicotyl and lateral roots was much more strongly inhibited than that of the main root. Neocuproine had no effect on the formation of lateral root primordia but did inhibit the development of amine oxidase activity in the apical part. On the contrary, in vitro enzyme activity was inhibited little (ca. 10% at 0.4 mM). The enzyme activity in the extract of pea seedlings which had been treated with neocuproine did not increased with addition of $CuCl_2$ solution (at 10^{-3} to 10^{-5} M), suggesting that no copper-free apoenzyme had been synthesized. In our experiments, the activity of amine oxidase in the apical parts was markedly stimulated to 2.3-fold of control levels when EDTA-Na_2-Cu (at 2.5×10^{-5} M) was added to the culture solution simultaneously with neocuproine. At the same time, the leaves of the apical part unfolded as shown in Fig. 2. However, such reversal of inhibition was not obtained

Table 1 *Effect of neocuproine and cuprizone on amine oxidase development and growth of pea seedlings*[a]

Treatment	Growth (cm±SD)		Specific enzyme activity (μl O_2/min/mg protein)
	Epicotyl	Main root	
H_2O (control)	8.86±1.86	10.40±1.45	0.27
Neocuproine (5×10^{-5} M)	1.97±0.62	5.43±1.35	0.13
Cuprizone (5×10^{-5} M)	9.63±1.40	10.45±1.19	0.20
Neocuproine plus EDTA-Na_2-Cu (2.5×10^{-5} M)	2.76±1.10	4.75±1.33	0.62
Neocuproine plus EDTA-Na_2-Zn[b] (2.5×10^{-5} M)	1.92±0.49	4.81±1.40	0.14
EDTA-Na_2-Cu	8.25±1.92	9.45±1.50	0.27
EDTA-Na_2-Zn	7.87±1.63	9.01±1.07	0.25

[a] Seven-day-old seedlings in culture solutions were used.
[b] Zinc disodium ethylenediaminetetraacetate.

using zinc disodium ethylenediaminetetraacetate under the same conditions. Thus, neocuproine-induced inhibition of enzyme activity, but not that of elongation of epicotyls and main roots, was reversed by EDTA-Na_2-Cu (Table 1). This differential effect is not understood yet. Fig. 1 and 2 show that cuprizone did not inhibit the growth of epicotyl and lateral roots, and had little effect on the elongation of the main root (Table 1). In contrast to the neocuproine effect, the development of amine oxidase activity was inhibited slightly; however, strong inhibition (ca. 90% at 0.4 mM) of in vitro enzyme activity was observed. Our results indicate that neocuproine may interfere with the enzyme synthesis at the stage of copper incorporation into the enzyme protein, while cuprizone does not disturb the synthesis of pea amine oxidase with its copper-chelating properties (*6*). On the other hand, corn seedlings do not contain the amine oxidase necessary for the conversion of tryptamine to indoleacetaldehyde, whereas they have polyamine oxidase which probably is not a copper protein (*14*). In contrast to pea seedlings, the growth of corn plant was inhibited little by the presence of neocuproine (unpublished findings), therefore further studies are needed to elucidate its mode of action on the growth and IAA synthesis in pea and corn seedlings.

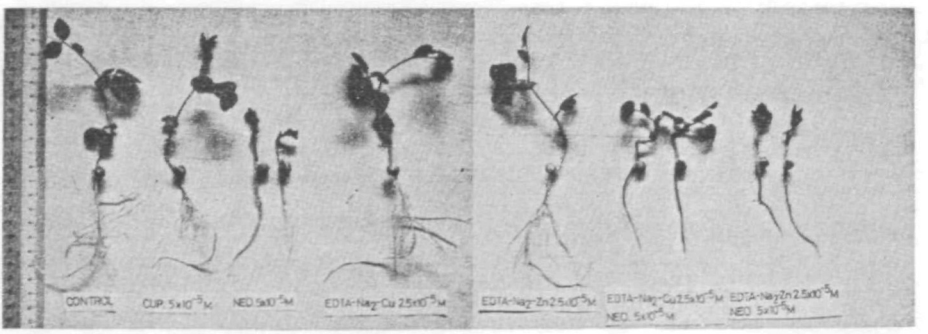

Fig. 2. *Pea seedlings grown in various culture solutions for 7 days.* Conditions were similar to those of Fig. 1.

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