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COPPER-BINDING PROTEIN IN MIMULUS GUTTATUS

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ABSTRACT

A Cu-binding protein has been purified from the roots of Mimulus guttatus using gel permeation chromatography on Sephadex G-75 and anion exchange chromatography on DEAE Biogel A. The protein has similar properties to putative metallothioneins (MTS) purified from other angiosperms. Putative MT was estimated by measuring the relative percentage incorporation of (^{35}S) into fractions containing the protein after HPLC on SW 3000-gel. In the roots of both Cu-tolerant and non tolerant plants synthesis of putative MT is induced by increased Cu concentration in the nutrient solution. The relative percentage incorporation of (^{35}S) into putative MT is significantly higher in extracts from the roots of Cu-tolerant than non tolerant M. guttatus after growth in 1 uM Cu suggesting involvement in the mechanism of tolerance.

INTRODUCTION

Copper tolerance in Mimulus guttatus is primarily controlled by a single major gene (ref 11, 12). However, the mechanism of Cu tolerance in this plant is unknown. In animals and in microorganisms MTS are thought to be involved in Cu homeostasis (ref 5, 8, 13). Proteins similar to MTS may also occur in angiosperms (ref 2, 3, 4, 6, 9, 15). The structure of these proteins remains to be elucidated although the amino acid compositions of some partially purified metal complexes are similar, in some respects, to MT (ref 16, 18, 21). Aspects of the production of these complexes correlate with Cu tolerance in some plants (ref 10, 17). We describe here the purification, partial characterization and estimation of a Cu-induced, MT-like, Cu-complex from the roots of Cu-tolerant and non tolerant M. guttatus.

MATERIALS AND METHODS

Roots of M. guttatus (ref 1), for analysis by gel permeation chromatography, (Fig 1), were grown in 1/10th Long Ashton Solution for 19 days in 16uM Cu and homogenised at 4° in 10mM Tris/HCl-5mM NaCl-5mM 2-mercaptoethanol, pH 8.0. After centrifugation and concentration by dialysis samples were applied to a column (600 x 24mm) of Sephadex G-75 in elution buffer, 10mM Tris/HCl-3.5mM NaN_3 pH7.4. Roots analysed by HPLC (Fig 2) were grown for 24 days in culture solution minus copper, for the last two days of growth they were exposed to 11u Ci (^{35}S) per plant. Then half of these plants were treated with 16µM Cu for 24h, the remainder were untreated. Roots were homogenised at 4° in 50mM Tris/HCl-5mM 2-mercaptoethanol, pH 7.9. After centrifugation and concentration extracts were applied to a SW 3000 column in elution buffer, 50mM Tris/HCl-0.1% NaN_3 pH 7.4. Roots used to obtain data shown in Table 2 were grown for 5 days without Cu, half of these plants were then grown in 1µM Cu for 17 days and 11µ Ci of (^{35}S) per plant, the remainder were grown in the absence of copper.

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RESULTS AND DISCUSSION

Sephadex G-75 gel permeation chromatography resolved the copper present in an extract from the roots of Cu-tolerant plants grown in 16 uM Cu for 19 days into three peaks (fig 1). Peak 1 corresponded to Cu bound to excluded proteins, pe. 2 had an elution volume similar to rat Cd-Th (data not shown) and a high ratio of absorbance at 254 nm:280 nm relative to other compounds eluting from the column (inset, figure 1), peak 3 corresponded to the total volume of the column and contained unbound Cu. The concentration of Cu in peak 2 was not reduced after heating an extract at 60^{o} for 3 min. under N_2, demonstrating heat stability, a feature of MT (ref 22). The MT-like Cu-complex, when further purified by anion exchange chromatography, contained more glx and gly than mammalian MT. However, insertion of the two values for these amino acids from equine MT-1 (ref 7) demonstrated that the relative percentages of the other amino acids were similar to mammalian MTS with 34% cys, 13.3% ser, 13.3% lys no phe, tyr or his. Mammalian MTS are characterized by high contents of cys (25 to 35%), ser (7 to 17.5%) and lys (10 to 15%), the absence of tyr and the absence or presence of only trace amounts of phe and his (ref 22). Thus the isolate from M. guttatus would be classified as MT if it did not contain such large amounts of glx and gly. This material is referred to as putative MT. The relatively large amounts of glx and gly suggest that putative MT is either contaminated with a compound rich in these amino acids, or differs from mammalian MT. Previous studies have reported high glx and gly contents in putative MT from angiosperms (ref 10, 16, 18, 19, 21), but it is possible that these previous isolates were also contaminated. HPLC analysis of pooled fractions from peak 2 (fig 1) showed a single major peak of Cu-binding activity with a retention time of 21 min, a retention time similar to that of Cd-Th under these conditions (ref 20). Ninety seven percent of the Cu applied to the column was recovered. A S-rich compound, with a retention time on the same column of 20 to 21 min, was present in extracts from Cu-tolerant plants grown in the presence of 16 μM Cu for 24 h but was not prominent in the absence of copper (fig 2). Ninety four percent of the (^{35}S) applied to the column was recovered. The recovery of putative MT during extraction was estimated to be 71%. To normalise for differences in the absolute incorporation of (^{35}S) into total cytosol proteins, the data shown in figure 2 has been expressed (table 1) as percentage incorporation of (^{35}S) into putative MT relative to (^{35}S) incorporation into high MW compounds (ref 14). These data show induction of putative MT in the roots of Cu-tolerant plants by increased Cu after growth in copper. The relative percentage incorporation of (^{35}S) into putative MT was significantly higher in the roots of non tolerant plants after growth in 1 μM Cu than after growth in the absence of Cu, demonstrating induction in the roots of non tolerant plants by Cu (table 2). This suggests that Cu tolerance in M. guttatus is not due to the absence or lack of expression of the genes that code for the synthesis of putative-MT in non tolerant plants. The relative percentage incorporation of (^{35}S) into putative MT was significantly higher in the roots of Cu-tolerant plants than non tolerant ones after growth in 1 μM Cu. The mechanism of Cu tolerance in M. guttatus involves, at least in part, increased production of putative MT. The primary Cu tolerance gene in M. guttatus could be either amplified putative MT genes or a regulator of the activity of genes involved in the synthesis of this compound.

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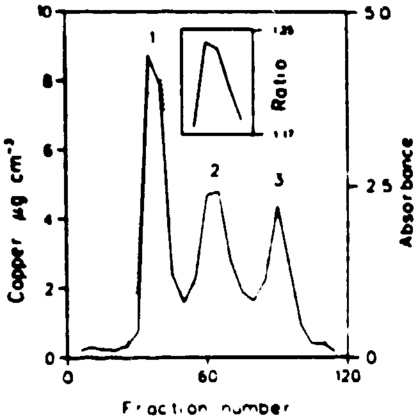

Figure 1. Gel permeation chromatogram of an extract of roots of Cu-tolerant plants grown in 16µM Cu for 19 days. Cu (——) A254 (- - -).

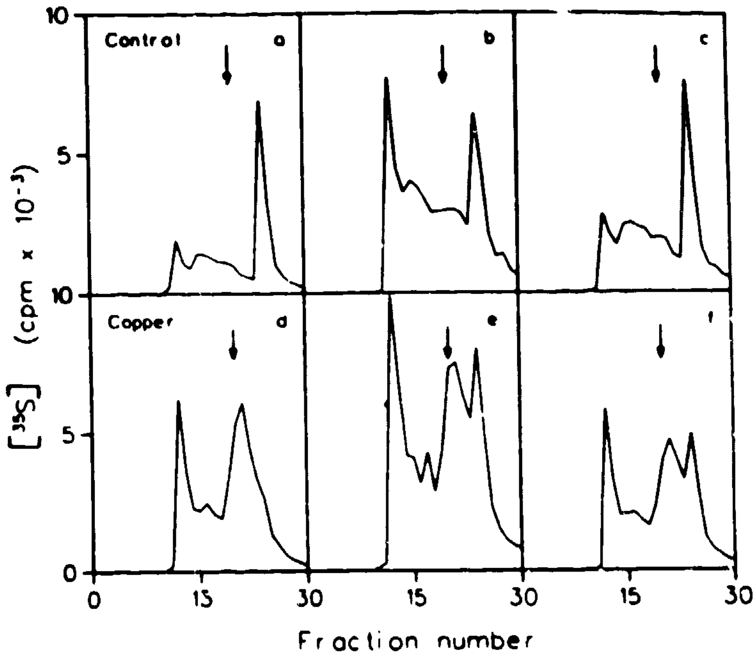

Figure 2. Replicate HPLC analyses of extracts of roots of Cu-tolerant plants grown in (^{35}S) and subsequently in the absence (a, b, c) or presence (d, e, f) of 16 µM Cu for 24 h. Arrows indicate the retention time for putative MT.

Table 1. Mean (after arcsin transformation) relative percentage incorporation of (^{35}S) into putative MT in the roots of Cu-tolerant plants after growth in the presence or absence of 16 µM Cu for 24h. Values correspond to the data in fig 2.

Treatment	Mean Relative Percentage of (^{35}S) in Putative MT	Mean+ SD of mean	Mean- SD of mean
Control	5.5	9.1	2.8
16 uM Cu	35.2	40.1	30.5

Table 2. Mean (after arcsin transformation) relative percentage incorporation of (^{35}S) into putative MT in the roots of Cu-tolerant plants after growth in 1 µM Cu for 1[illegible] days and non tolerant plants after growth in the presence or absence of 1 µM Cu also for 1[illegible] days.

Treatment	Mean Relative Percentage of (^{35}S) in Putative MT	Mean+ SD of mean	Mean- SD of mean
Non-tolerant control	[illegible]	1.2	0.8
Non-tolerant + 1 uM Cu	3.[illegible]	[illegible].3	3.1
Tolerant + 1 uM Cu	9.[illegible]	11.8	6.6

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