

Chemical Forms of Manganese and Zinc in Phloem Exudates

By

B. J. VAN GOOR and D. WIERSMA

Institute for Soil Fertility, Haren (Gr.), the Netherlands

(Received 16 May, 1975; revised 20 October, 1975)

Abstract

Investigations were performed to study the chemical form in which manganese and zinc are transported in sieve tubes. As a test plant *Ricinus communis* was used. From this plant phloem sap can be obtained in a rather pure state. The plants received labelled manganese or zinc. In the experiments on manganese translocation a radioactive phosphorus isotope was added to the nutrient solution to test if complex compounds of the cation contain also phosphorus. Components of the phloem exudate were determined by means of physicochemical separation methods.

Almost all zinc but only a part of the manganese were bound to organic compounds. The major part of the manganese was in ionic form. The molecular weight of the complexing compound(s) of manganese was estimated to be between 1000 and 5000 and of zinc between 1000 and 1500. The complexes probably contain some phosphorus. The charge of the zinc complex is negative.

Introduction

Cations are translocated by the xylem vessels from the roots of the plant to the leaves. Redistribution from leaves to other plant parts as fruits, storage-organs and growing points takes place through the sieve tubes (Van Goor 1974).

About the chemical form in which the cations are translocated in the xylem some information is available. Tiffin (1972) found negatively charged compounds of copper and zinc in the xylem exudates of plants, while iron is transported as the citrate. Höfner (1970) proposes that iron and manganese in the xylem exudate of *Helianthus annuus* is chelated by amino acids and carbohydrates with molecular weights below 1500.

Much less is known about the chemical form in which the cations are translocated in the phloem. This investigation is intended to provide some information on this question for manganese and zinc.

Materials and Methods

Plant material

The experiments were carried out with *Ricinus communis* L. var. *gibsonii*. The plants were sowed in moist peat. About 2 weeks after sowing they were transferred to 5-litre pots containing the culture solution. As a nutrient medium we used a solution according to Steiner (1961) as given in Table 1. The solutions were changed every week. The plants were cultivated in a growth chamber with a constant temperature of 25°C and a relatively high, variable humidity. The plants were artificially lighted during 14 h each day by three Philips HPI/T-400 Watt lamps on a surface of 1.5 m² and at a distance of about 60 cm from the tops of the plants.

About 8 weeks after sowing, one plant for each experiment was transferred to the isotope laboratory with temperatures of 20–25°C. The plant was lighted here with one HPI/T-lamp as closely above the plant as possible. For the experiments on manganese translocation, 100 μCi ⁵⁴MnCl₂ and 100 μCi ³²P were added to the nutrient solutions. For the experiments on zinc translocation the addition was 100 μCi or 500 μCi ⁶⁵ZnCl₂. The isotopes used were carrier-free and were mixed with the proper quantity of the non-radioactive element before addition to the water culture. About a week after the addition of the isotopes to the solutions, tapping of phloem exudate was started. Milburn's method (1971) of making an incision in the bark was used. The sap from the incision was drained off via a glass capillary. During a period of 2 to 3 weeks the incision was renewed daily. For the separations we always used exudate tapped on the preceding day and stored at 4°C during the night.

Electrophoresis of the radioactive phloem sap

Phloem exudate was carried upon the dry paper strips; 0.03 ml in the double-labelling experiments with manganese

Table 1. Nutrient solution according to Steiner (1961).

Macro-elements	g/l	Micro-elements	mg/l
KNO ₃	0.620	H ₃ BO ₃	2.02
KH ₂ PO ₄	0.127	ZnSO ₄ ·H ₂ O	0.576
K ₂ SO ₄	0.238	CuSO ₄ ·5H ₂ O	0.144
Ca(NO ₃) ₂ ·4H ₂ O	0.599	MnSO ₄ ·4H ₂ O	1.51
MgSO ₄	0.468	MnSO ₄ ·4H ₂ O	0.203 (in ⁵⁴ Mn experiment)
KOH	0.018	Na ₂ MoO ₄	0.100
		FeEDTA	35.75
		FeEDTA	17.88 (in ⁵⁴ Mn experiment)
pH = 6.2		Co(NO ₃) ₂ ·6H ₂ O	0.054

and phosphorus, and 0.08 ml in the experiments with labelled zinc. The strips were introduced into a Beckman electrophoresis apparatus (type Durrum), after which they were saturated with a buffer of pH 8.2, containing 0.05 M HCl and 0.1 M tris(hydroxymethyl)aminomethane ("tris") in water. For comparison 0.04 and 0.08 ml of a solution of ⁵⁴MnCl₂ or ⁶⁵ZnCl₂ was used. The zinc and manganese concentrations of these reference solutions were about the same as those of the phloem exudate. The electrophoresis was carried out at 300 V for 3½ h.

The pH 8.2 was chosen because this is the pH of phloem exudate. After electrophoresis the paper was cut in strips of 5 mm. For counting of ⁵⁴Mn and ⁶⁵Zn the strips were put into vials and counted directly in a Philips gamma counter PW 4520. In order to count the ³²P, the strips were ashed at 400°C. The ash was dissolved in 0.5 ml 2 N HCl and then diluted with 10 ml distilled water. The β-radiation of the ³²P was counted by means of the Cerenkov-effect. Counting took place in a Philips liquid scintillation counter PW 4510. A correction was applied for the mutual influence of the radiation of ³²P and ⁵⁴Mn on the counting results. Counting time was always 40 min.

Sephadex gel filtration

Pharmacia glass columns were filled with the dextran gels Sephadex G10, G15 or G25 medium. The fractionating range for these gels is for peptides and globular proteins respectively for G10 up to molecular weights of 700, for G15 up to 1500 and for G25 from 1000 up to 5000. Of course these data have to be applied with some caution to molecules of unknown composition. The columns were saturated with the tris-HCl buffer as described for the electrophoresis. As a sample we used 0.4 ml phloem exudate or reference solution in the same way as for electrophoresis. Elution was carried out with the tris-HCl buffer. In each elution procedure at first fractions of 3 ml and later of 5 ml were collected. The dead volume of the column was determined with blue dextran 2000. ⁵⁴Mn and ⁶⁵Zn were determined in the gamma-counter, while ³²P was counted in the liquid scintillation counter.

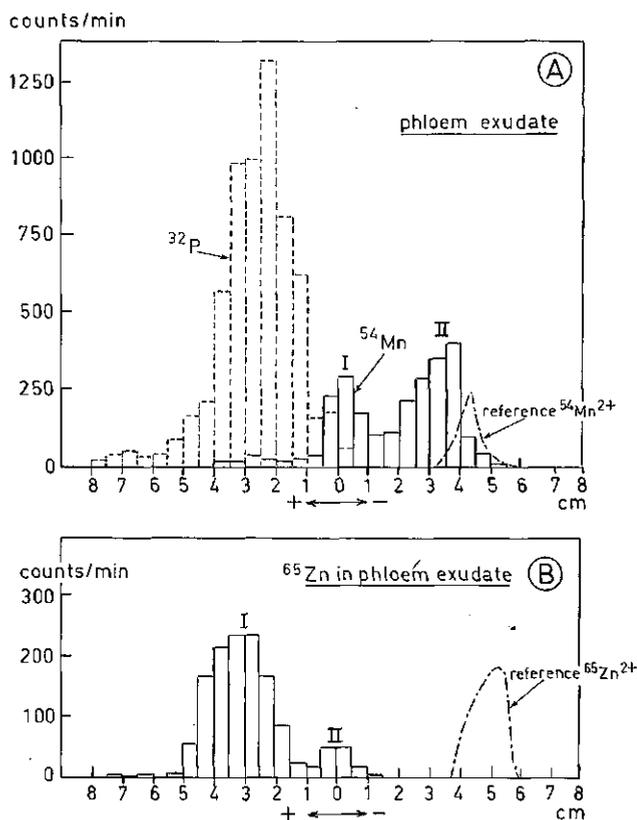


Figure 1. Paper electrophoresis of phloem sap of *Ricinus* after cultivation in a liquid medium supplied with ⁵⁴Mn and ³²P (A) and with ⁶⁵Zn (B).

Ultraviolet spectrometry

The extinction of all fractions from the column chromatography was determined in a Zeiss spectrophotometer PMQ II. As a blank we used the buffer solution. The determinations were performed at 260 and 280 nm. In the case of the elution of the zinc peaks on Sephadex G10 a more detailed spectrum was determined.

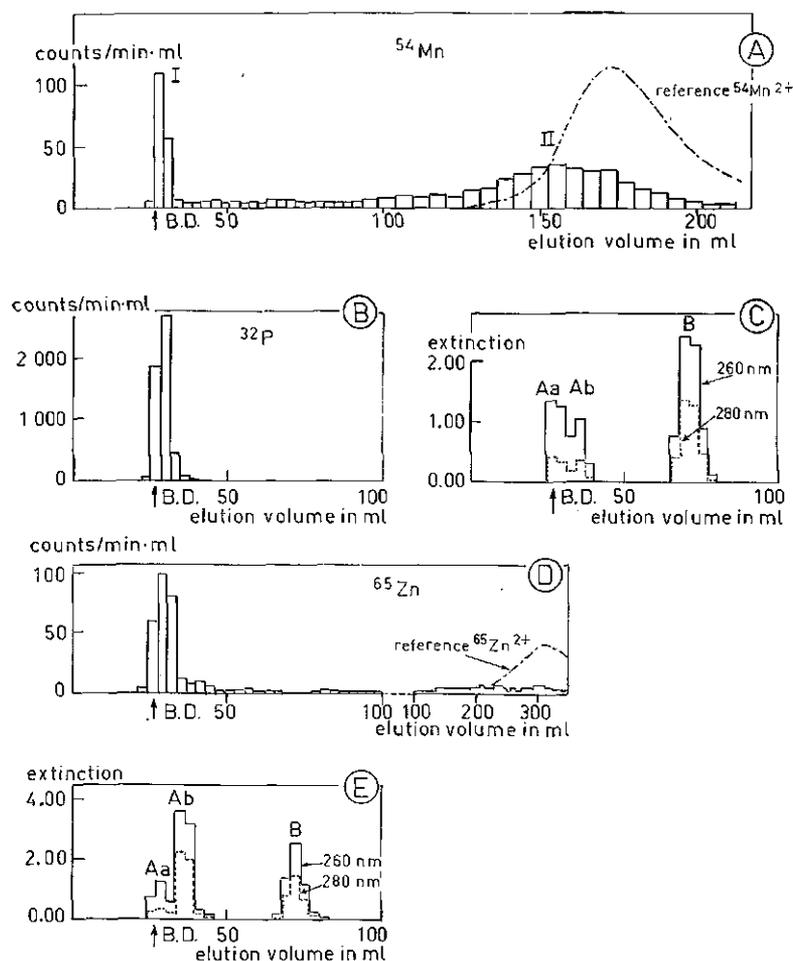


Figure 2. Gel filtration of phloem exudate of *Ricinus* through a Sephadex G10 column. Elution with tris-HCl buffer. (A) determination of ^{54}Mn ; (B) determination of ^{32}P ; (C) determination of ultraviolet absorption corresponding to (A) and (B); (D) determination of ^{65}Zn ; (E) determination of ultraviolet absorption corresponding to (D); \uparrow B.D. = blue dextran "2000". Separate UV peaks are referred to as A, B, C, sub-peaks Aa, Ab, etc

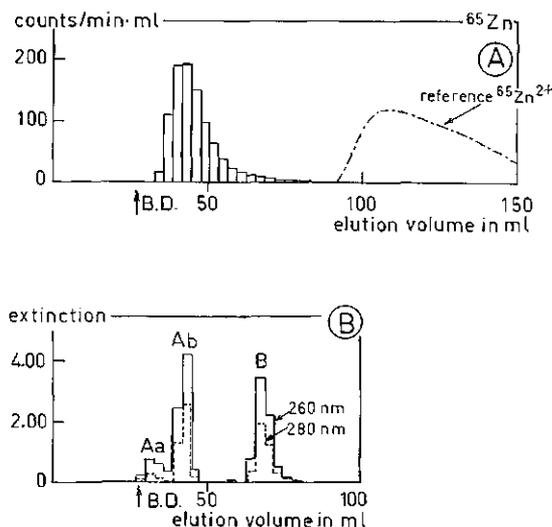


Figure 3. Same as Figure 2 but a Sephadex G15 column. (A) determination of ^{65}Zn ; (B) determination of corresponding ultraviolet absorption. \uparrow B.D. = blue dextran "2000".

Replications

All experiments were carried out in duplicate with different plants. As the results were almost the same, only one of them is given.

Results and Discussion

For the manganese the presence of two forms is probable. One is the cationic manganese, the other an organic compound. We can see the ionic manganese as the peaks II in Figures 1A, 2A and 4A, respectively, in the electrophoresis and in the gel chromatography on Sephadex G10 and G25. These peaks do always coincide with the ionic manganese used as reference. It was evident from Figures 1A and 1B that about 60-70% of the ^{54}Mn -radioactivity was ionic manganese. Some properties of the organic compound can be deduced from the results given in the graphs. The compound runs unhampered through the G10 column, while it is fractionated in the G25 column (Figures 2A and 4A). Taking the data for peptides and globular proteins into

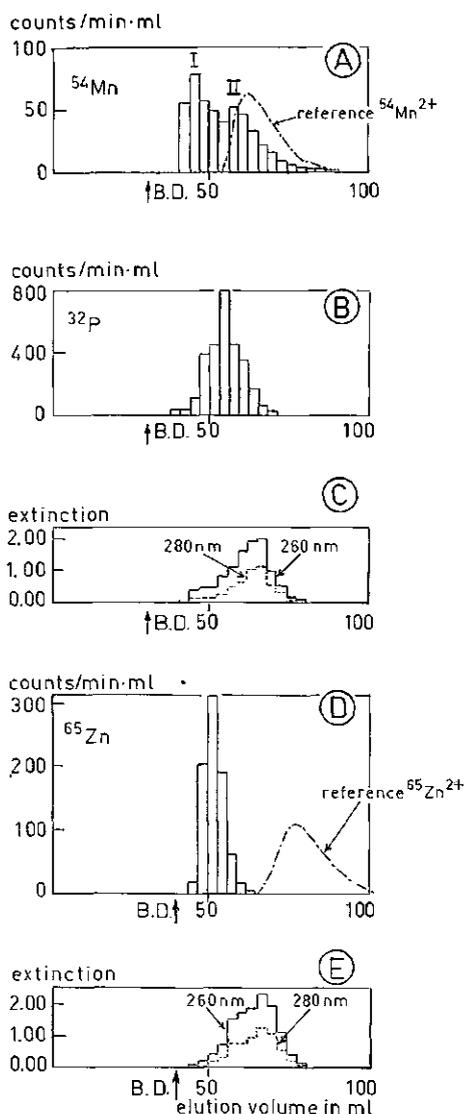


Figure 4. Same as Figure 2 but a Sephadex G25 column. (A) determination of ^{54}Mn ; (B) determination of ^{32}P ; (C) determination of ultraviolet absorption corresponding to (A) and (B). (D) determination of ^{65}Zn ; (E) determination of ultraviolet absorption corresponding to D. \uparrow B.D. = blue dextran "2000".

consideration a molecular weight in the range of 1000–5000 appears to be a reasonable estimate.

The picture for zinc differs in that almost all zinc is bound in organic compounds. This is apparent from Figure 2D, where only a small peak is present at the place of ionic zinc. Also almost all Zn has a negative charge, as is evident from Figure 1B. Another indication for this is that only one peak resulted on the Sephadex columns G15 and G25 (Figures 3A and 4D). In the case of the zinc it can be concluded that the molecular weight of the organic compound lies

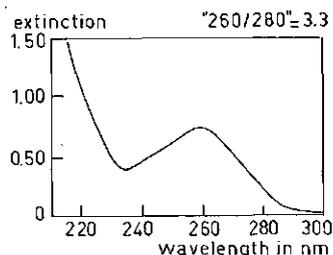


Figure 5. UV-spectrum of a fraction of phloem exudate of *Ricinus* containing ^{65}Zn . The phloem sap was separated in fractions on Sephadex G10, eluted with tris-HCl buffer and the fraction(s) containing maximum ^{65}Zn radioactivity was (were) subjected to UV-spectroscopy.

between 1000 and 1500, because fractionation takes place on Sephadex G15 and G25.

It is difficult to give more information about the chemical identity of the manganese and zinc complex. At the place of the peaks on the electropherogram and on the chromatograms there is some radioactivity from ^{32}P (Figures 1A and 4B). Only on G10 the peaks coincide (Figure 2B). An explanation can be that the peak of ^{32}P in Figure 4B consists of a mixture of compounds of which the cation-binding compound may be one. Another indication of the identity of the complexes can be found in the curves for the ultraviolet absorption as given in Figures 2C, 2E, 3B, 4C, 4E. For the G10 and G15 columns the maximal radioactivity coincides with a maximum absorption at 260 nm; this is not the case with G25, but also here there is an absorption at 260 nm at the place of the radioactivity peak. The spectrum in Figure 5 shows a peak at 260 nm. The spectral data combined with the overlap of ^{32}P -peaks and cation peaks would indicate the presence of polynucleotides. In any case they are not pure, because the 260/280 ratio is 3.3 at the point of the zinc-peak. For pure nucleotides this ratio is about 2.

The authors are indebted to Mr. J. W. Dalenberg for his contribution to the development of the radioactive methodology.

References

- Höfner, W. 1970. Eisen- und manganhaltige Verbindungen in Blutungssaft von *Helianthus annuus*. — *Physiol. Plant.* 23: 673–677.
- Milburn, J. A. 1971. An analysis of the response in phloem exudation on application of massage to *Ricinus*. — *Planta (Berl.)* 100: 143–154.
- Steiner, A. A. 1961. A universal method for preparing nutrient solutions of a certain desired composition. — *Plant Soil* 15: 134–154.
- Tiffin, L. O. 1972. Translocation of micronutrients in plants. — *In* *Micronutrients in Agriculture* (J. J. Mortvedt, P. M. Giordano and W. L. Lindsay, eds.), pp. 199–229. — *Soil Sci. Soc. Am. Inc.*, Madison, Wisc.
- Van Goor, B. J. 1974. Distribution of mineral nutrients in the plant in relation to physiological disorders. — *Proc. XIXth Int. Hortic. Congr.*, Warszawa, 1974, 7: 217–227.