Chemical Forms of Nickel and Cobalt in Phloem of *Ricinus communis*

By

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Abstract

Investigations were performed to study the chemical form in which nickel and cobalt are transported in sieve tubes. *Ricinus communis* plants were used since they allow easy collection of relatively pure phloem sap. In the experiments labelled nickel or cobalt were given to the nutrient solution. The tapped phloem exudate was separated by gel filtration on Sephadex or by paper electrophoresis. Nickel and cobalt were bound to complexing organic compounds with a molecular weight in the range of 1000–5000. The overall charge of the nickel complex was negative, and the bulk of cobalt also appeared to complex to a compound with negative overall charge.

Introduction

Increasing pollution with heavy metals necessitates the study of their uptake and distribution within plants. As the varying distribution within the plant is governed by the modes of transport, this has to be investigated. Little work has been done on the determination of the chemical forms in which heavy metals are transported in plants, especially in phloem. If the compounds which chelate with these metals during translocation are known, there may be a possibility to alter the mechanisms of translocation by altering the ratio of available nutrients.

Tiffin (1967, 1971, 1972) investigated binding forms of heavy metals in xylem exudates of plants and found that copper and zinc were present in unknown negatively charged compounds and that iron was bound to citrate. Up to a limiting concentration, nickel is translocated as a negatively charged complex, but above that concentration he also found that the excess of nickel migrated cathodically. This limiting concentration above which the cathodical migration appears depends on the plant species.

Höfner (1969, 1970) found zinc, copper, manganese and iron in the xylem exudate of *Helianthus annuus* chelated by amino acids or peptides with a molecular weight below 1500.

In preceding investigations — utilizing the phloem exudate of *Ricinus communis* — we (Van Goor and Wiersma 1976) found that the major portion of manganese was present in ionic form and the remaining portion was bound to complexing compound(s) with a molecular weight between 1000 and 5000. Almost all of the zinc was bound by (a) negatively charged compound(s) with a molecular weight between 1000 and 1500. The present investigation is intended to provide some information on the chemical form in which nickel and cobalt are translocated in the phloem of *Ricinus communis*.

Materials and Methods

Plant material

The experiments were carried out with *Ricinus communis* L. var. *gibsonii*. The plants were cultivated under the same conditions as described for earlier investigations (Van Goor and Wiersma 1976). For the translocation experiments with nickel 17.4 μg Ni $\cdot$ l^{-1}, marked with 100 μCi $^{63}NiCl_2 \cdot l^{-1}$, was added to the nutrient solution (Steiner 1961). For the cobalt translocation experiments 23.6 μg Co $\cdot$ l^{-1} marked with 200 μCi $^{58}CoCl_2 \cdot l^{-1}$ was added to the nutrient solution.

Three to 5 days after the addition of the isotopes to the solutions, tapping of phloem exudate was commenced according to Milburn's method (1971) of making an incision in the bark and draining off the sap from the incision via a glass capillary. During a period of 2 to 3 weeks the incision was renewed twice a day. For the separations we always used exudate tapped on the preceding day and stored at 4°C during the night.

Sephadex gel filtration and paper electrophoresis of the exudate

Gel filtration on Sephadex and paper electrophoresis were carried out as described previously (Van Goor and Wiersma

1976). A 'tris' buffer of pH 8.2, containing 0.05 *M* HCl and 0.1 *M* tris(hydroxymethyl)aminomethane in water, was used throughout all experiments. The pH 8.2 was chosen, because this is the pH of the phloem exudate. The reference solutions for the phloem sap contained respectively 0.2 μCi $^{63}NiCl_2 \cdot ml^{-1}$ and 0.035 μg $Ni \cdot ml^{-1}$ or 0.47 μCi $^{58}CoCl_2 \cdot ml^{-1}$ and 0.01 μg $Co \cdot ml^{-1}$ in 0.005 *M* HCl. The β-radiation of ^{63}Ni was counted by a Philips liquid scintillation counter PW 4510 and the γ-radiation of ^{58}Co in a Philips gammacounter PW 4520.

Ultraviolet spectrometry and ninhydrin colouring

The extinction of all fractions from the column chromatography was determined at 260 and 280 nm in a Zeiss spectrophotometer PMQII, using the buffer solution as a blank.

The intensity of the ninhydrin colour reaction was determined for the fractions from the Sephadex G10 column. An aliquot of each fraction was first diluted with an equal volume of a sodium acetate–acetic acid buffer of pH 4.4 (0.2 *M* sodium acetate and 0.2 *M* acetic acid in the ratio of 1 part to 2), followed by addition of ninhydrin solution (after Höfner 1969) to the diluted sample in the ratio of 1 part to 4. The mixture was heated in test tubes for 20 min in a sand bath at 105°C. After cooling, the extinction of the solution was read in a Zeiss spectrophotometer PMQII at 570 nm.

Replications

All experiments were carried out in duplicate with different plants.

Results and Discussion

The gel filtration on Sephadex G10 and G25 shows a great difference between the nickel present in the phloem exudate and the ionic nickel of the reference solution (Figures 1B and 2B). The complexed Ni of the exudate is not retained by the G10 column, while it is fractionated in the G25 column. From this data a molecular weight in the range of 1000–5000 appears to be a reasonable estimate. The organic compound binding the nickel is negatively charged as can be seen in Figure 3A.

Cobalt gave almost the same results as nickel. It is bound by compounds with a molecular weight between 1000–5000 (Figures 1C and 2C), but not all of them are negatively charged as seen in the paper electrophoretogram (Figure 3B). Initially, a 'cobalt complex' was found in the reference solution with a molecular weight above 700. After acidifying the $^{58}CoCl_2$ stock solution to a final concentration of 0.05 *M* HCl, this 'complex' could no longer be detected.

The observations do not provide sufficient information to elucidate the chemical identity. An indication of the identity of the complex(es) can be found in the curves for the

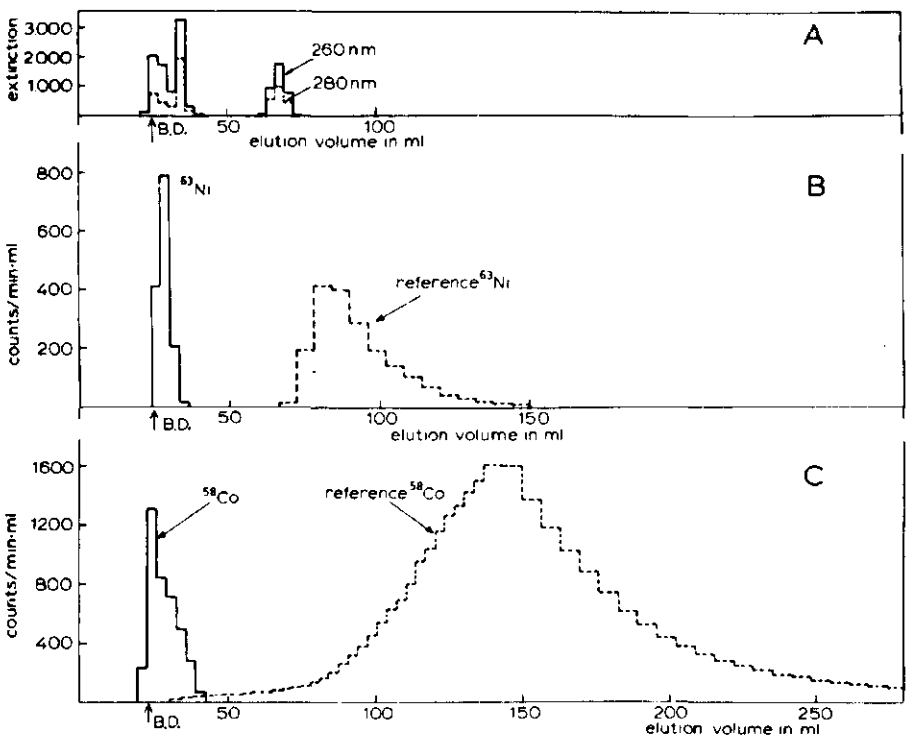

Figure 1. *Gel filtration of phloem exudate of Ricinus through a Sephadex G10 column.* Elution with tris-HCl buffer. (A) determination of ultraviolet absorption corresponding to (B) and (C); (B) determination of ^{63}Ni; (C) determination of ^{58}Co; ↑B.D. = blue dextran '2000'.

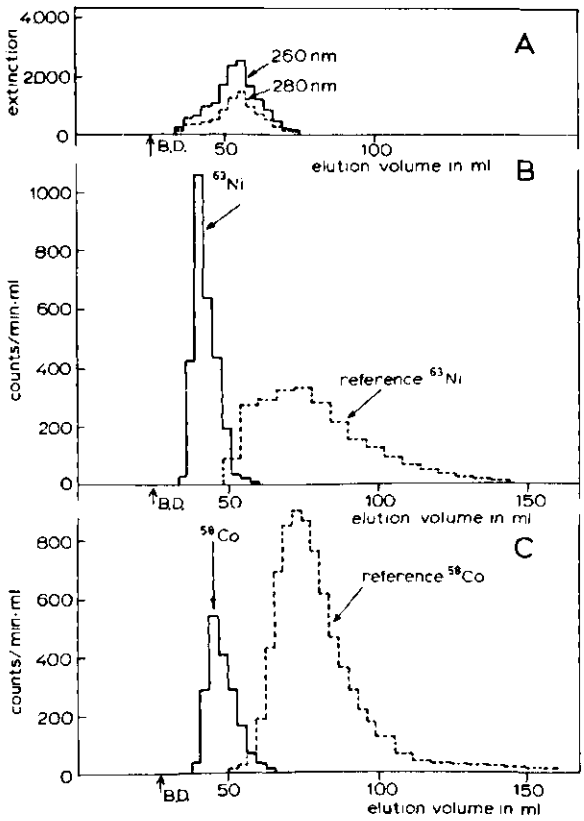

Figure 2. *Same as Figure 1 but a Sephadex G25 medium column.* (A) determination of ultraviolet absorption corresponding to (B) and (C); (B) determination of ^{63}Ni; (C) determination of ^{58}Co; ↑B.D. = blue dextran '2000'.

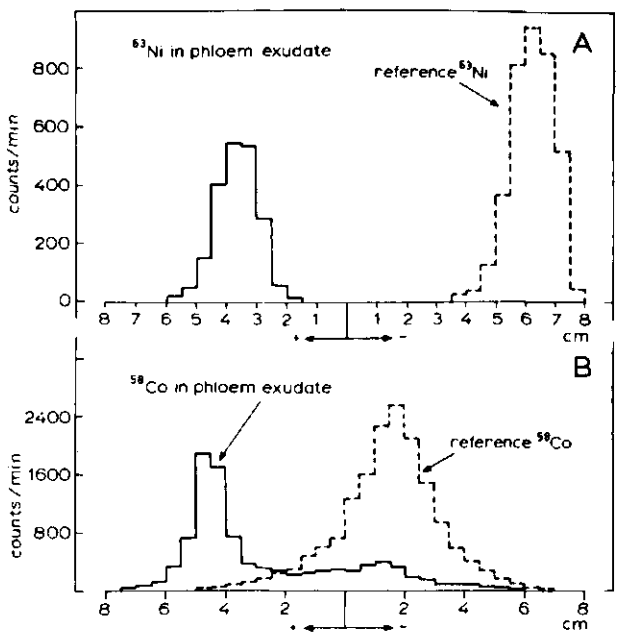

Figure 3. *Paper electrophoresis of phloem exudate of Ricinus after cultivation in a liquid medium supplied with ^{63}Ni* (A) *and ^{58}Co* (B).

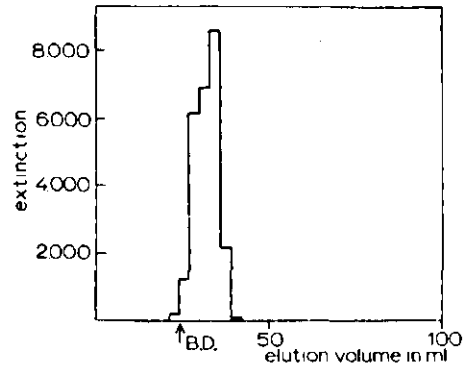

Figure 4. *Ninhydrin colouring of the fractions from a Sephadex G10 column.* The phloem exudate was separated in fractions on Sephadex G10, eluted with tris-HCl buffer. Ninhydrin reagent was added to the fractions, followed by measuring the absorbance at 570 nm.

ultraviolet absorption in Figures 1A and 2A. For the G10 column the maximum radioactivity coincides rather well with a maximum absorption at 260 nm; this is not the case with G25, but there is also absorption at 260 nm in the same region where the radioactive peak appeared. The radioactive fractions of the G10 column give a positive reaction with ninhydrin (Figure 4). These results are very similar to those reported for zinc (Van Goor and Wiersma 1976). There are some indications that polynucleotides or nucleo-proteins may be the cation-binding compounds, but as the fractions still contain mixtures of compounds further separation of the binding fractions is necessary before the chemical nature of the complex can be identified.

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