Uptake of Humic Acids by Wheat Plants: Direct Evidence Using Tritium Autoradiography

Natalia A. Kulikova, Gennady A. Badun[a], Viktor I. Korobkov[a], Violetta Yu. Pozdnyakova[a], Irina V. Perminova[a]

Department of Soil Science, Lomonosov Moscow State University, 119992 Moscow, Russia; [a]Department of Chemistry, Lomonosov Moscow State University, 119992 Moscow, Russia

knat@darvodgeo.ru

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1. INTRODUCTION

Humic substances (HS) are the natural organic compounds comprising 50 to 90 % of the organic matter of peat, lignites, sapropels, as well as of the non-living organic matter of soil and water ecosystems (1). In spite of numerous studies on the biological effects of HS, the mechanism of their action remains unclear. Problem of bioavailability of HS and their penetration into organisms is even more sophisticated due to difficulties in HS determination in the presence of other organic substances. The most convenient way is the of labeled compounds, and the availability of isotope-labeled HS is an important prerequisite to elucidate the fate of the heterogeneous organic matter in complex environments. Labeling of HS can be accomplished by the addition of a labeled precursor to an unlabeled soil sample during composting (*2*) or by the synthesis of model polymeric compounds under defined conditions. The latter are synthesized either by enzyme mediated (usually initiated by adding H_2O_2 in the presence of horseradish peroxidase) oxidative polymerization of phenolic compounds (3), or by their spontaneous polymerization in the presence of oxygen or other oxidants, usually at alkaline pH (4). If polymerized with other, nonaromatic precursors (e.g., proteins, peptides, amino acids, and several carbohydrates and amino sugars peptides or carbohydrates), the resulting preparations possess an astonishing resemblance to natural HS (3). By the above-described ways both ^{14}C and ^{15}N labeled HS can be produced. Usage of those techniques, however, does not allow producing labeled preparation identical in their properties to the native preparations. On the other hand,

labeling of samples of native HS cannot be accomplished. Some methods of the direct labeling of HS were therefore developed including labeling of HS with ^{125}I (5) and ^{3}H (6). The main advantage of the direct labeling of HS is an opportunity to produce a broad spectrum of isotope-labeled native humics varying significantly in both their origin and properties. Given high structural heterogeneity and irregularity of HS, the latter is of great importance as the structure-properties relationship for HS can be established using such a set of humics.

The objective of the study was to obtain a direct evidence of HS uptake by higher plants.

2. MATERIALS AND METHODS

Preparation of tritium-labelled humic substances: Leonardite humic acid (HA) was a commercially available preparation Powhumus (Humintech, Germany) desalted using dialysis before the experiments. Elemental analysis of HA was determined with a Carlo Erba Strumentazione analyzer and showed that HA contained (on ash-free and moisture-free bases) 45.9 % C, 3.4 % H, and 1.6 % N. Ash content was 1.1 %. Molecular weight of HA was determined using size-exclusion chromatography (SEC) analysis according to (7) and was 10 kD.

Sample of tritium-labeled HA was prepared as described in (6). The obtained ^{3}H-HA sample was dissolved in a phosphate buffer (0.028 M, pH = 6.8) and purified by dialysis during a month at 4 ºC. It allowed to eliminate exchangeable tritium of OH^{-}, $COOH^{-}$, and NH_n groups of HS. A dialysis membrane with cut-off of 3 kD (Merk, Germany) was used.

Plant cultivation and uptake experiments: Plants of wheat *Triticum aestivum* L. (var. Inna) were used for the experiments. Wheat seeds were germinated at 24 °C in the dark for 72 h followed by transferring seedlings into the 0.5 l tanks containing Knopp nutrition solution (KH_2PO_4 0.14 $g \cdot l^{-1}$, KCl 0.1 $g \cdot l^{-1}$, KNO_3 0.14 $g \cdot l^{-1}$, $MgSO_4 \times 7H_2O$ 1.42 $g \cdot l^{-1}$, $Ca(NO_3)_2 \times 12H_2O$ 4.88 $g \cdot l^{-1}$, $FeCl_3 \times 6H_2O$ 0.05 $g \cdot l^{-1}$, pH 5.5). After another 72 h plants were transferred into the vials containing HA at concentration 50 $mg \cdot l^{-1}$ with specific radioactivity 0.02 $Curie \cdot l^{-1}$. After 96 h plants were harvested, weighted and subjected to autoradiography. To estimate HA uptake by plants, radioactivity of HA solutions before and 96 h of plant growing was measured using liquid scintillation method.

Autoradiography protocol: the harvested plants were divided into roots and shoots sections, dried at 200 °C under press for ca. 5 min, and then subjected to autoradiography analysis using tritium sensitive X-ray film Retina XBM (Fotochemische Werke GmbH, Germany). Exposure time was determined experimentally and was 4.5 h and 56 days for roots and shoots, respectively. Film developing, fixing, and washing were performed according to procedure recommended by the manufacturer.

3. RESULTS AND DISCUSSION

The calculated amount of HA adsorbed by plants was 0.24±0.02 $mg \cdot g^{-1}$ of wet weight. At that, adsorbed HA were mainly allocated in the wheat roots while only small amounts of HA were found in the shoots (Fig. 1). The amount of HA adsorbed by plants estimated on the basis of the registered autoradiograms ca. 300 times exceeded that for shoots. The amount of adsorbed HA was therefore recalculated to the wet weight of roots and was 0.71±0.03 $mg \cdot g^{-1}$.

Figure 1: Fragments of autoradiograms of wheat roots (a) and shoots (b) subjected to ^{3}H coal HA. Exposure time was 4.5 h and 56 days for roots and shoots, respectively.

As it can be seen from the picture, HA were intensively adsorbed by wheat plants. The roots of the plants subjected to ^{3}H HA were characterized with homogeneous distribution of signal intensity. Distribution of HA within the shoots was also relatively homogeneous except for the tips of the leaves where local increase of signal density was observed. The autoradiograms presented in Figure 1 allowed concluding that HA could be taken up through plant roots and moved al least in the xylem with transpiration stream to areas of new growth. Symplastic translocation of HA within the plant in the phloem is however questionable and requires additional experiments.

4. CONCLUSIONS

A new useful tool for investigation of HS uptake by plants has been developed. The obtained results are evident for HA uptake by higher plants through roots and HA movement al least in the xylem with transpiration stream to areas of new growth.

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