Radiochemical Approach for Studying Properties of Humic Substances

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1. INTRODUCTION

Study of physical and chemical properties of humic substances (HS) is a main question of modern investigations because they play a central role in industry, medicine and ecology. No doubt, that radioactive label might be a good instrument for the investigations of HS properties and their behavior in different systems.

Choice of radioactive isotope and radiolabeling technique is one of the main questions in such researches. Radiolabeled HS are required to be identical with the initial material. Thus, only limited number of elements is preferred for labeling. Previously 14C-labeled HS were produced by addition of a labeled precursor to a soil sample during composting or by the synthesis of model polymeric compounds under defined conditions. However, these techniques did not allow producing labeled HS identical to the native ones. Some methods of direct labeling of HS were therefore developed including labeling of HS with 125I, 99Tc and 111In. 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.

Since hydrogen is one of the basic elements in HS, tritium is expected to be the most useful for the investigation of HS properties and HS behavior in different systems including biological ones. Among the methods of tritium introduction into organic molecules, tritium thermal activation method (the bombardment of solid targets by atomic tritium) is the only that allows introducing ^{3}H-label in any structural fragments of macromolecule. This work focuses on novel integration of several opportunities to advance studying of hydrophobicity and biological activity of humic materials by means of radioactive label. Thus, this work has two critical important goals. The first relates to introduction of radioisotope into HS molecule without lose of initial properties of HS. The second deals with the investigation HS behavior in different systems.

2. MATERIALS AND METHODS

Humic materials used in this study were humic (HA) and fulvic acids (FA) isolated from soil and peat and also included preparations of HA and hymatomelanic acids (HMA) from brown coal. All the HS were characterized by methods of elemental analysis, size-exclusion chromatography (SEC) and ^{13}C NMR spectroscopy.

Preparation of tritium-labeled HS. In the present study, we applied a new developed technique for radioactive labeling of HS using thermal activation method (1). The method implied bombardment of the target HS with atomized tritium followed by equilibrium dialysis to purify the labeled product from labile tritium and low molecular weight fractions. To prove the developed technique of HS labeling with tritium, parent HS and obtained samples of ^{3}H-HS was analyzed by SEC with radioactivity and UV detection.

Characterization of hydrophobicity and surface activity of HS. To characterized hydrophobic and surface activity properties of HS under study, the obtained ^{3}H-HS samples of corresponding initial humic materials were used. Hydrophobic properties of HS used were characterized using common logarithm of octanol-water coefficient $\lg K_{OW}$ determined directly following the distribution of HS between octanol and water phase by a scintillation phase method according to (2). To characterize surface activity of target HS, a parameter reflecting maximum adsorption of HS in the toluene-water interface Γ_{max} was used as described in (ibid.).

Study of HS interaction with bacteria. Experiments on ^{3}H-HS interaction with bacteria were conducted by growing *E. coli* cells overnight at 37°C in M9 medium supplied with labeled HS at concentrations of 5-50 mg l^{-1} After overnight cultivation, the cells were harvested by centrifuging (5000 rpm, 30 min), and the radioactivity of supernatant was measured. The value obtained was assigned to the equilibrium concentration of HS. Then the supernatant was replaced with the equal amount of M9 media, the cells were re-suspended, and radioactivity of the suspension was measured. That value corresponded to the amount of HS taken up by the cells, i.e. a sum of HS adsorbed on the surface of the cells and HS penetrated into the cells. To determine the amount of HS penetrated into the cells, cell lysis was performed using chloroform followed by centrifugation (5000 rpm, 30 min) to separate debris. The radioactivity of the supernatant was assigned to the amount of HS penetrated the cells. Bioconcentration factor (BCF) was calculated as a slope of the HS taken up by *E. coli* cells vs. HS equilibrium concentration.

Study ob HS interaction with higher plants. 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. After another 72 h plants were transferred into the vials containing HS at concentration 5-50 mg l^{-1}. After 24 h plants were harvested, weighted and subjected to autoradiography. To estimate HS uptake by plants, radioactivity of HS solutions before and 24 h of plant growing was measured using liquid scintillation method.

3. RESULTS AND DISCUSSION

Preparation of tritium-labeled HS and comparative analysis of parent and ^{3}H-HS. Obtained results demonstrated that developed approach allowed synthesis of ^{3}H-HS of high specific radioactivity and yield. For the studied HS value of specific radioactivity varied from 0.14 to 0.63 TBq g^{-1}; yield ranged from 23 to 87% depending HS labeled.

Parent HS and obtained samples of ^{3}H-HS were subjected to SEC analysis with radioactivity and UV detection. It was demonstrated that parent and labeled HS were characterized with the similar UV SEC profiles what was evident for the absence of significant alteration of HS structure due to either tritium introduction or dialysis. On the other hand, similarity of UV and radioactivity SEC profiles of ^{3}H-HS confirmed that introduced tritium was uniformly distributed among HS fractions of different molecular sizes. Therefore, the developed technique of the radioactive labeling of HS with tritium by thermal activation method can be used for the labeling of a broad spectrum of HS of different origin and composition.

Hydrophobicity and surface activity of HS. Determined values of partition coefficients of HS in octanol – water system lgKow for the studied HS varied from -2.98 to -1.95 indicating slight hydrophobicity of HS. At that soil FA were characterized with the lowest hydrophobic properties whereas peat HA possessed the highest values of lgKow. It should be noted that direct determination of HS hydrophobicity was performed for the first time.

Surface activity of HS also depended greatly on HS origin. Among studied HS the most surface active HS was obtained to be peat HA and FA whilst soil FA was showed to possessed negligible surface activity.

The preformed experiments demonstrated clearly that ^{3}H-HS are useful in study of both HS hydrophobicity and surface activity.

Study of HS interaction with bacteria. Obtained results indicated that HS adsorption onto bacterial surfaces can be extensive, and that the sorption behavior depended on a preparation used. HS adsorption onto bacterial surfaces was the highest in case of peat HA, and decreases to negligible amounts of adsorption in case of soil FA. Observed values of bioconcentration factor of HS by E. coli varied in the range 0.9-13.1 l kg^{-1}

depending considerably on the HS preparation. Performed experiments demonstrated also that HS could penetrate into the bacterial cells. Measured amounts of HS penetrated into the cells were 23-167 mg kg^{-1} what were at least 20% of totally sorbed HS and reached maximum of 100% in case with FA. Therefore, the obtained 3H-HS were demonstrated to be a promising approach for study of bacteria-HS interaction.

Study of HS interaction with higher plants. HS uptake by wheat seedlings was characterized by smooth, nonsaturating curves for all the studied HS. The obtained curves of concentration-dependent HS accumulation by wheat seedlings were fitted to Michaelis-Menten kinetics equation allowing determination of Michaelis constant Km and rate of accumulation Vmax. Among the HS studied, the highest Michaelis constant was observed for HMA derived from coal, which was characterized with the lowest molecular weight and the highest contents of quinoic and ketonic groups. On the other hand, a minimum value of Km was also recorded for coal HMA possessing the lowest C/N ratio. Rate of accumulation for the studied HS varied in the range 6-54 mg kg^{-1} h^{-1} and had its maximum in case of peat HA with the highest observed molecular weight.

Performed autoradiography analysis of wheat seedlings treated with ^{3}H-HS demonstrated that roots of the plants were characterized with homogeneous distribution of signal intensity. Distribution of HS within the shoots was also relatively homogeneous except for the tips of the leaves where local increase of signal density was observed. It could be concluded that HS could be taken up through plant roots and moved al least in the xylem with transpiration stream to areas of new growth.

4. CONCLUSIONS

The developed technique of the radioactive labeling of HS with tritium by thermal activation method can be used for the labeling of a broad spectrum of HS of different origin and composition. The obtained labeled HS may be useful for the study of HS behavior in the environment and biological systems.

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REFERENCES

1. Badun G.A., Pozdnyakova V.Yu. et al. 2003. Bulgarian J. of Ecol. Sci. 2(3-4), 26-27.
2. Badun, G.A., Chernysheva M. G. et al. 2005. Radiochemistry. 47(6), 584-588.