A New Method for Labeling Humic Substances with Tritium: Future Prospects for Biological Studies

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Humics are well known to possess physiological activity in relation to various living organisms including bacteria, algae, fungi, plants, animals, and human beings. Both beneficial and direct toxic effects of humic substances (HS) were reported. However, mechanisms underlying biological properties of HS are still poorly understood. The primary reason for that is a lack of experimental tools for tracking uptake and distribution of natural organic mixtures in living cells and tissues, which makes it extremely difficult to link structure and functions in systems of such high complexity. Besides, predicting HS behavior in biological systems is extremely arduous, as HS are complex mixtures with a number of concurrent properties, such as polyanionic and polyelectrolyte character, hydrophilic and hydrophobic moieties, different functional groups, etc.

The main complication that arises in the study of the interactions of HS with living cells is the lack of a reliable analytical technique for determination of HS in the presence of biomolecules (e.g., proteins, lipids, and saccharides). To overcome the problem, radioactive labeling of HS is being used widely for this purpose. However, the reported studies deal predominantly with synthetic rather than with native humic materials.

A new method for labeling natural HS with the radioisotope tritium (^{3}H) has been developed using humic and fulvic acids of different origin (coal, peat, soil, and water) as the target HS. The method produced HS of high specific activity (0.14-0.63 TBq g^{-1} HS); that permited the tracing of HS at a detection limit of 0.2×10^{-12}g L^{-1} HS. Results from size exclusion chromatography indicated that the tritium label was distributed uniformly over the whole molecular size range of HS as well as no partial decomposition or polymerization of HS occurred during labeling. The performed correlation analysis revealed that there was no any significant relationship between HS properties and specific radioactivity of the obtained ^{3}H-HS. Thus universality of the developed technique for radioactive labeling of HS with tritium could be indicated.

The high specific radioactivity and radiochemical purity allowed direct determination of hydrophobicity and surface activity of humic substances, and investigation of the behavior of humic substances in bacteria, fungi and higher plants including both quantitative estimation of interaction and visualization using tritium autoradiography.

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