Biochemistry of Humic-like Substances Formation

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Nowadays significant attention is given studying of the mechanism of physiological activity of humic acids and particularly to their structure and ways of biosynthesis. In this connection formation of humic substances at direct participation of copper-containing blue oxidase – laccase (monophenol, dihydroxyphenylalanine: oxygen oxidoreductase EC 1.14.18.1.) is a process of special interest. Now the most perspective producers of extra cellular laccases are basidiomycetes, calling white rot fungi. It is known, that some basidiomycetes, white rot fungi, are capable to accumulate humic-like substances in conditions of artificial culture. In our work were used and studied highly productive strains of basidiomycetes *Coriolus hirsutus, Cerrena maxima* at individual and joint cultivation on a plant substrate. Three humic-like substances synthesized by these strains, were isolated. To characterized them were used the following methods: the element analysis, IR-spectroscopy, NMR ^{13}C and size exclusion chromatography (SEC). Named humic-like substances on the physical and chemical properties were found to be similar each other and close to soil humic acids. Formation of high-molecular humic-like substances was accompanied by high laccase activity. Probably, it was caused by active degradation of a lignin-cellulose complex and co polymerization of released propylphenolic radicals and amino acids in high-molecular substances.

Biological role of mushroom laccase in formation of humic-like substances - the high-molecular polymers formed of degradation products of a wood lignin-cellulose complex was investigated. It was shown that laccase was able to form humic-like substances from low-molecular lignin derivatives and amino acids *in vitro*. According to data of the element analysis, to IR-spectra, NMR ^{13}C spectra and SEC chromatograms these synthetic polymers were close to humic-like substances of white rot fungus and to soil humic acids. The received data allow assume ability basidiomycetes strains, white rot fungus, to form humic-like substances for limited time with the given properties, and possibility to use enzyme synthesis by laccase *in vitro* in this process.

Synthesis of sulfoderivatives of humic acids and their complexing properties toward Fe(III) and Hg(II)

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The presence of wide range of functional groups (carboxylic, hydroxylic, carbonylic) in the structure of humic acids provides their ability to form the complexes with metals. As a result, humic acids play a key role in migration and bioavailability of heavy metals in the environment. The complexing properties of humic acids determine their potential for practical application as detoxifying agents and microfertilizers. To enhance the bioavailability of metals complexed with humic acids, the synthesys of sulfoderivatives of humic acids enriched with functional groups can be of use if the latter would possess higher solubility in water. The objective of this study was to sulfonate HA from the commercially relevant sources and evaluate their complexing capacity and stability constants to different metals.

For synthesys of sulfoderivatives the leonardite and humic acids isolated from the leonardite and peat were used. Concentrated sulfuric acid and chlorsulfonic acid were used as sulfonating agents. Efficiency of sulfonation was estimated as an increase in sulfur content in the obtained preparations compared to the initial ones, and as an increase in the content of strong acidic groups determined using the Ca-acetate technique. Chlorosulfonic acid turned out to be a much more efficient sulfonating agent than concentrated sulfuric acid: content of S in the sulfonated leonardite HA accounted for 5.06 and 2.94, respectively, and 2.93 and 0.79, respectively for peat HA. As follows from the above data, leonardite HA turned to be sulfonated to much higher extent than the peat HA. The titration data showed that due to sulfonation by the chlorosulfonic acid it is possible to introduce up to 50% of initial amount of strong acidic groups (2.9 mol/L in the initial leonardite humic acid and 5.0 mol/L in the sulfoderivative). The obtained derivatives were completely soluble in water.

The complexing properties of the obtained sulfoderivatives of peat and leonardite HA were studied in relation to Hg(II) and Fe(III). The above metals were selected for studying of complexing properties of sulfonated HA as a toxic and biogenic metal, respectively. The main characteristic of complexing properties of humic acids are stability constant of the complex and the amount of metal-binding sites (MBS). To determine an amount of MBS, the ability of HA was used to form insoluble comlexes with metal after the saturation of binding sites. Stability constants were determined using the competitive complexation technique with spectrophotometric detection. Tartrate-ion was used as a competetive ligand for Fe(III) and chloride-ion - for Hg(II). The results showed that sulfoderivatives had the lesser affinity for Fe(III) and Hg(II) than the initial HA: the corresponding lgK accounted for 12.01 and 11.43 L/mol MBS for initial leonardite humic acids and their sulfoderivatives, respectively. At the same tinme, sulfonation lead to essential increase in an amount of MBS in HA samples: 1-2 mmol/g in the initial humic acids to 2-4 mmol/g in the sulfoderivatives. The obtained results allow to consider sulfonation as a promising method for obtaining soluble complexes of HA.

The study was supported by the Russian Foundation for Basic Research (grant # 01-03-32664) and by the BMBF (Germany), grant # 143/97-RUS. The grant of the Research Center for Environmental Protection and Helath (GSF, Neuherberg, Germany) is deeply appreciated.