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L13. HOW NMR SPECTRA OF NATURAL AND DERIVATIZED HUMIC SUBSTANCES PROVIDE COMPLEMENTARY INFORMATION CONCERNING THE NATURE OF THEIR ALIPHATIC CONSTITUENTS

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Humic substances are operationally defined polymeric and polydisperse natural organic materials and they are characterized by a distribution of their average molecular weight, aggregate size, functional groups and NMR longitudinal and spin spin relaxation times. A molecular irregularity and heterogeneity of humic materials is recognized as a reasonable assumption and prerequisite to explain their key ecological function as an important transient of considerable persistence against chemical and microbial decomposition within the global carbon cycle in soil, aquatic and marine environments.

Despite extensive research not much reliable information is accessible about the structures of the aliphatic constituents of humic substances. One dimensional NMR spectra of humic substances are comprised of heavily overlapping sets of resonances; an assignment of these spectra on the basis of chemical shift alone will remain severely ambiguous and requires to be supplemented by other analytical data. Notably, connectivity information obtained from homo- and heteronuclear 2D NMR spectra significantly enhances the reliability of the resonance assignments in NMR spectra of underivatized humic substances.

The combination of chemical derivatization with NMR active labels and one and two dimensional NMR spectroscopy is an independent new method for the identification and quantification of exchangeable protons in polymeric and humic materials. It combines structural information from two different NMR spectra with the high sensitivity of proton detection. Functional groups are relevant constituents of humic substances and decisively affect their physico-chemical properties and ecological efficacy. Derivatization of humic substances substitutes acidic protons with less polar groups and therefore will decrease the tendency of aggregation. This will increase the relaxation times and will provide enhanced resolution in one and two dimensional NMR

spectra useful to investigate both the functional groups and the backbone of humic substances.

Amino acid side chains, methylated and normal carbohydrates and terpenoid hydrocarbons comprise the bulk of the more easily recognizable aliphatic constituents of humic matter. They are supplemented by highly oxidized chains and by a broad range of aliphatic (and aromatic) carboxylic acids. The analysis of derivatized humic materials reveals a vast array of branched aliphatic units with a near statistical distribution of α -, β -, and γ -branching.

L14. SPIN-PROBE STUDY OF PLANT DESICCATION TOLERANCE

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Plant organs such as seeds and pollens are to a large extent tolerant to an almost complete removal of water. Even a few whole (resurrection) plants have this capacity. During dehydration, the cellular content becomes increasingly viscous, and the chance for molecular interaction increases. We have used spin probes of different polarity to follow, in situ, what happens in cells during dehydration [1,2]. After insertion of amphiphilic probes, such as TEMPO or TEMPONE, the volume reduction drives the probe into oil bodies and membranes, completely out of the aqueous cytoplasm. This was derived from the changes in shape of the EPR spectra. If endogenous amphiphiles follow the same trend during dehydration, membrane structure and function may be impaired. We have evidence that this is the case, and that this is dealt with in desiccation-tolerant systems [2].

Desiccation-tolerant organisms have ample amounts of di- and oligosaccharides that form glasses upon loss of the water. Polar spin probes (e.g. 3-carboxy-proxyl) inserted in the cytoplasm are trapped in the glassy matrix on dehydration, where they give solid-like spectra [3]. The glass transition temperature T_g can be derived from the sudden decrease in $2A_{zz}$ during heating. In an attempt to estimate the rotational correlation time τ_R , ST-EPR was applied using glycerol as a reference material [3,4]. The estimated values of τ_R were around 10^{-3} s in a number of dry systems.

Molecular mobility and ageing rates appear to be linked. The more mobile the spin probe is under a certain set of temperature and water content, the shorter the life-span. This relationship allowed for estimates of longevity at sub-zero temperatures for which determinations are impractical due to the extremely slow ageing involved [5]. Survival times of more than 40,000 years appear possible for seeds at -20°C at water contents giving minimal mobility (not always the driest condition). It was found, also with ST-EPR, that glasses in cells differ from those of pure sugars. The collapse temperature of the cytoplasmic glass is at least 50°C above T_g , whereas for sugar glasses this interval is much less. This stems from the presence of proteins in the cytoplasmic glass. For the dry organisms this implies that above T_g there is a moderate increase in mobility as compared to a huge increase in amorphous sugars.

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