Quadrupole Relaxation Studies of Humic Acids

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In an earlier paper [1] the bonding of Rb^+ ions to a humic acid fraction was established by nuclear quadrupole relaxation studies with ^{85}Rb. The appreciable signal broadening obtained has later been confirmed for other humic acids as well, and in this paper some observations from these further studies and some possible applications of the method in the study of humic acids will be outlined.

The experiments were made as reported earlier. All comparisons were made for corresponding concentrations of the humic acids (expressed as carbon content per liter).

Results. 1. Different fractions of different humic acids from different soils (experimental methods described by Lindqvist [2]) give varying line broadening. The variations can be as large as a factor of ten in line broadening. The method can thus be used to characterize the humic acids (and their compounds with the inorganic matter in soil).

2. The characteristic broadening can also be used to study artificial humic acids and compare them with natural humic acids. An oxidation product of hydroquinone thus gave the same line broadening as a *chernozem* humic acid. (Similarities of the light absorption spectra have been reported earlier.[3])

3. The addition of hydroxide ions to the neutral solutions increases the line width to some extent and the addition of hydrogen ions eliminates almost the whole line broadening (already before the humic acid has been precipitated). Protolytic line width titrations will thus give a further characterization of humic acids.

4. The ^{35}Cl signal is not influenced by addition of humic acids, indicating that Cl^- ions are not appreciably bound to the humate ions. (This is in striking contrast to the proteins [4] which often exhibit a Cl^- ion bonding even above the isoelectric points but no Rb^+ bonding.)

5. The ^{133}Cs nuclear magnetic resonance signal is, within the experimental error (about 5 % of the corresponding ^{85}Rb broadening), not affected by addition of a humic acid. ^{133}Cs has a negligible quadrupole moment compared to ^{85}Rb but a considerably greater magnetic moment. This result thus confirms that the dominant relaxation for ^{85}Rb takes place through a coupling between the nuclear quadrupole moment and electric field gradients at the place of the nucleus and not *via* an interaction between the nuclear magnetic moment and the electron spins on the humate ions (for expressions for the relaxation rates in the two cases see, *e.g.*, Hertz [5]).

6. For one humic acid (an iron podsol A_{12}) the effect of varying the humic acid concentration at a constant RbCl concentration (0.5 M) was investigated. The results are shown in Fig. 1. The linear

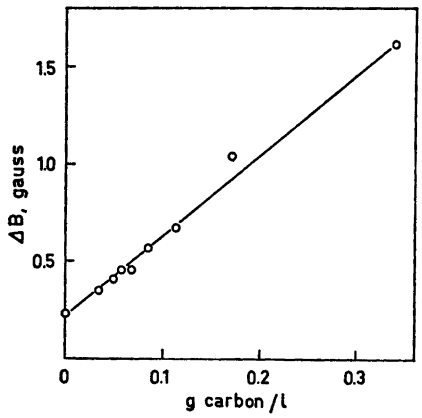

Fig. 1. The dependence of the ^{85}Rb nuclear magnetic resonance line width on the humic acid concentration (expressed as grams of carbon per liter solution) for an iron humus podsol A_{12}. The concentration of rubidium is 0.5 M. For comparison a straight line is drawn through the experimental points (*cf.* text).

dependence is in agreement with the expression valid for the limit of rapid exchange (see, *e.g.*, Ref. 6) of rubidium nuclei between the different binding sites.

$$\Delta B_{obs} = \sum p_i \Delta B_i + p_0 \Delta B_0$$

Here p_i is the probability for the rubidium ion to be located in a site i on the humate ion and ΔB_i is the ^{85}Rb line width characterizing this site. p_0 is the mole fraction of unbound rubidium ions and ΔB_0 the corresponding line width. At moderate humate concentrations p_0 is close to unity whereas the p_i's are proportional to the humate concentration. Thus if the number of rubidium ions bound to the humic acid is much smaller than the total number of rubidium ions the line width should vary linearly with the humic acid concentration.

The studies are continued along the lines presented here.

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Visible Absorption and Circular Dichroism Spectra of 5(4′-Sulfamylphenylazo)-8-hydroxyquinoline Bound to Carbonic Anhydrase and Alcohol Dehydrogenase

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Spectral studies of the binding of inhibitors and other ligands containing chromophoric groups (*i.e.* groups absorbing some kind of electromagnetic radiation) to biological macromolecules have given valuable information about the state of such macromolecular complexes, because the spectral properties of the bound ligand are changed in a characteristic way. The ligands may exhibit changes in fluorescence, microwave absorption (due to paramagnetic groups), or light absorption in the ultraviolet and visible parts of the spectrum.[1–3]

A recent example is given by the studies of azosulfonamide-carbonic anhydrase complexes by Coleman.[4] He found that the induced circular dichroism spectra of the bound chromophore disodium 2-(4 sulfamylphenylazo)-7-acetamido-1-hydroxyphthalene-3,6-disulfonate are sufficiently specific as to distinguish between the various isoenzymes from erythrocytes of human and *Macaca mulatta*. As the three-dimensional structure of human carbonic anhydrase form C (HCAC) is known to a resolution of 5.5 Å [5] and is being worked out at high resolution,[6] and as X-ray studies of the form B are in progress,[7] the carbonic anhydrase is a valuable reference system for comparative spectrochemical studies of bifunctional reversible inhibitors. This means, that many specific, reversible enzyme inhibitors containing a reporter group and an additional 4-sulfamyl-phenyl group could also be bound to carbonic anhydrase, and the spectral properties of the various enzyme inhibitor adducts could then be compared with the adduct of the inhibitor and carbonic anhydrase. In a search for bifunctional protein ligands, we have prepared and studied 5-(4′-sulfamylphenylazo)-8-hydroxyquinoline (SAPAO) and describe here visible absorption and circular dichroism spectra of complexes of human carbonic anhydrase, form B and C, and horse liver alcoholdehydrogenase with the chromophoric ligand.

Experimental. HCAB* was a gift from Dr. Per-Olof Nyman, Gothenburg, HCAC a gift from Dr. Kerstin Fridborg, Uppsala, and LADH a gift from Dr. Åke Åkeson, Stockholm. SAPAO was prepared by azocoupling of diazotized sulfanilamide with 8-hydroxyquinoline according to general procedures [8] and

* *Abbreviations.* HCAB and HCAC, human carbonic anhydrase form B and C, respectively. LADH, liver alcohol dehydrogenase. SAPAO, 5-(4′-sulfamylphenylazo)-8-hydroxyquinoline. NAD^+, NADH, nicotinamide adenine dinucleotide, oxidized and reduced, respectively.