Triplet states of humic acids studied by laser flash photolysis using different excitation wavelengths

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The spectra and kinetics of short-lived intermediates formed from aqueous (0.1 *N* NaOH) solutions of the natural mixture of humic and fulvic acids (HFA) were studied by laser flash photolysis using excitation wavelengths of 337, 390, 470, and 520 nm. Laser photolysis of HFA with light of 520 and 470 nm results in the formation of triplet excited states (T_{HFA}) characterized by the broad absorption spectrum with a maximum near 630 nm and lifetimes of ~0.15 ms in deoxygenated solutions. The formation of two types of T_{HFA} with lifetimes of ~0.1 and 2 ms and absorption spectra with maxima at 570 nm is observed under photolysis with light of 337 and 390 nm. The estimation of quantum yields of T_{HFA} gives ~1 and 0.3% under photolysis with excitation wavelengths of 337 and 520 nm, respectively. The rate constants of T_{HFA} quenching by molecular oxygen are equal to $(7—8) \cdot 10^8$ L mol^{-1} s^{-1}.

Key words: humic acids, laser flash photolysis with different excitation wavelengths, triplet states, quenching by oxygen.

Humic substances (HS) represent one of the most important class of natural high-molecular compounds. Although they are absent from living organisms, the living activity of the latter is based on these substances and, hence, they have been intensely studied for more than two centuries.[1,2] Their structure is irregular and has not exactly been established up to presently. Humic substances contain residues of various monosaccharides (to 25 wt.%) and amino acids (to 10%). Humic substances can conventionally be divided into three fractions: fulvic acids soluble in water at all pH values, water-soluble humic acids at pH > 2, and humins insoluble in water.

It is of interest to study the photochemical properties of HS, because they can act as photosensitizers and favor the degradation of such organic contaminators of the environmental contaminators as phenols, amines, and heterocyclic compounds.[3—8] Humic substances contain a large set of various functional groups, such as phenol, ketyl, quinoid, amino, carboxy, and methoxy groups, which allow various photochemical processes to occur, although HS, as a whole, are stable to the solar light.

Triplet states of different HS moieties, including carbonyl and quinoid fragments, whose content in HS is ~2 and 0.1—6 mol kg^{-1}, respectively, are assumed to be active intermediate species responsible for the photosensitizing effect of HS.[1,2] The involvement of triplet states of HS in the photosensitization of contaminator decomposition was concluded from indirect data, for example, the composition of photolysis products, the role of quenchers of triplet states, and analogies with model photosensitizers.[5,6,8,9] Studies of intermediate products of HS photolysis by direct methods, *e.g.*, laser photolysis, are restricted[10,11] because of low quantum yields of intermediate species absorbing in spectral intervals accessible for measurement (the color of HS makes it possible to reliably detect the electronic absorption spectrum (EAS) of intermediate products in the wavelength region >350 nm). The measurements described in the literature used only one excitation wavelength: 308 nm.

In this work, we carried out the spectral study of the kinetic characteristics of intermediate photolysis products of a natural mixture of humic and fulvic acids (HFA) in aqueous alkaline solutions at the excitation wavelengths 337, 390, 470, and 520 nm by nanosecond laser photolysis with signal averaging over 1028 kinetic curves. Several new triplet states of HFA were detected, depending on the excitation wavelength. The rate constants of dynamic quenching of these triplet states by molecular oxygen were measured for the first time in direct observation. The data on EAS and fluorescence of HFA are presented.

Published in Russian in *Izvestiya Akademii Nauk. Seriya Khimicheskaya,* No. 2, pp. 300—304, February, 2004.

Experimental

Absorption spectra and the kinetics of formation and decay of intermediate products were recorded on a nanosecond laser photolysis technique.[12] A PRA LN 1000 nitrogen laser (pulse duration 1 ns, excitation wavelength 337 nm) or a PRA LN 102 dye laser (pulse duration 0.5 ns, excitation wavelengths 386, 470, and 520 nm) with an N_2 laser pump were used as excitation sources operating in a frequency mode of 10—20 Hz. Kinetic curves were accumulated and averaged (over 1024 laser pulses) using a Biomation 6500 high-performance analog-to-digit converter (USA) connected to a Pentium 200 personal computer. Each kinetic curve contained 1024 points, and the distance between the points was 2—10 μs, depending on the duration of the process. The data presented in the work are averaged values obtained by processing of at least 10 kinetic curves under the specified conditions.

Before measurements, the cell was thoroughly evacuated to remove dissolved oxygen to a residual pressure of 0.001 Torr. All measurements were carried out at 20 °C.

Electronic absorption spectra were measured on Shimadzu UV-VIS-NIR and Shimadzu UV-3101PC spectrophotometers. Fluorescence spectra were obtained on a Hitachi M-850 spectrofluorimeter.

Humic and fulvic acids were extracted from the valley peat of the lowland (pH 4.9) deposit of the Vasyuganskoe bog in the Tomsk District (sol content 37.6%, hydrolytic acidity 9.8 mg-equiv (100 g)$^{-1}$). The HFA concentration in the initial 0.1 *N* NaOH solution was 0.3 g L^{-1}.

Results and Discussion

The electronic absorption spectrum of HFA is a broad curve containing no maxima and descending according to an exponential law with an increase in the wavelength (Fig. 1).[13] However, although maxima are absent, the electronic absorption spectrum of HFA contains the characteristic regions of inflections, which is well seen in the plot of the corresponding derivative. The minima in the plot of the EAS derivative indicate the presence of the respective bands in the EAS. The deconvolution of the EAS of HFA into four Gaussian components with maxima at 470, 335, 270, and 210 nm is shown in Fig. 1. The presence of various bands in the EAS corresponding, most likely, to different HFA moieties suggests that the nature of the intermediate products of HFA photolysis can strongly depend on the excitation wavelength. The structureless character of the EAS in the long-wave region is assumed to be caused by various systems of conjugated double bonds in the chemical structure of HFA.[1] The absorbance remains noticeable even in the near-IR range. However, it is worth mentioning that light scattering due to large sizes of molecules should be taken into account when interpreting the long-wave region of the EAS of HFA. It is known that the prolonged photolysis of HFA results in the disappearance of the color likely due to the decomposition of the system of conjugated bonds and photofragmentation of HFA.[1] However, no changes in the electronic absorption spectrum of HFA were observed even after several dozens of thousands of pulses under the laser photolysis conditions used in this work.

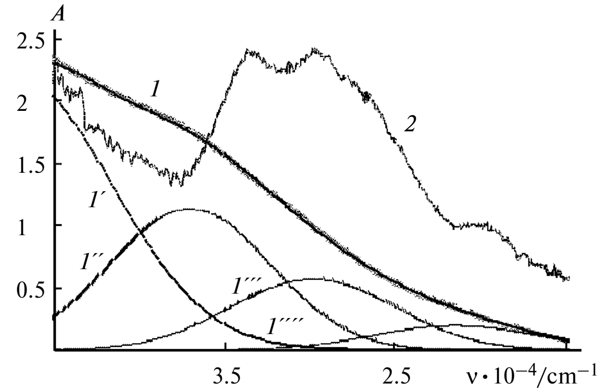

Fig. 1. Electronic absorption spectrum (*A*) of a working solution of HFA in a cell with the 1-mm optical path (*1*), its approximation by the sum of four Gaussian functions (the respective components are given under EAS lines *1*′, *1*″, *1*‴, and *1*⁗), and the EAS derivative in relative units (*2*).

The HFA structure contains different photochromes responsible for absorption in different spectral ranges, which is confirmed by analysis of the fluorescence spectra of HFA obtained at different excitation wavelengths (Fig. 2). At the same time, the fluorescence spectra show that no selective photoexcitation presumably occurs. For example, the fluorescence spectrum obtained by photoexcitation with light of 337 nm can be presented by the superposition of the fluorescence spectra obtained by excitation with light of 390, 470, and 520 nm and of the additional band with a maximum at 450 nm.

Pulse photoexcitation of aqueous solutions of HFA with light of 470 or 520 nm results in the appearance of a

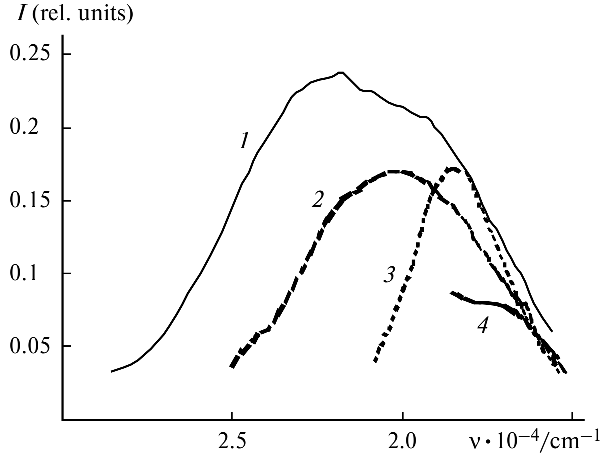

Fig. 2. Fluorescence spectra of the tenfold diluted working solution of HFA obtained using different excitation wavelengths: 337 (*1*), 390 (*2*), 470 (*3*), and 520 nm (*4*).

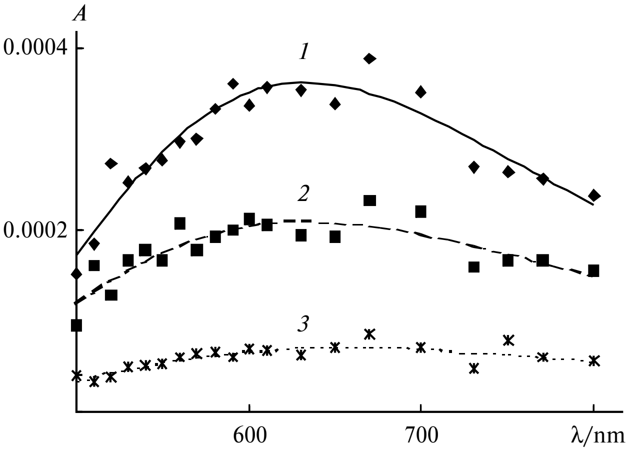

Fig. 3. Differential absorption spectra of the intermediate products obtained by the laser photolysis of HFA solutions with light of 470 nm at different time delays after the laser pulse: 1 (*1*), 100 (*2*), and 400 μs (*3*).

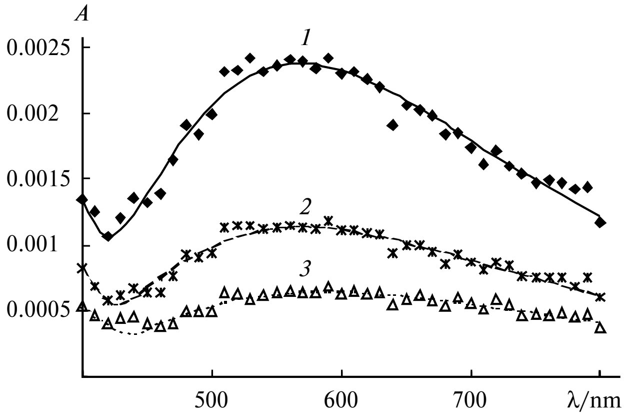

Fig. 4. Differential absorption spectra of the intermediate products obtained by the laser photolysis of HFA solutions with light of 337 nm at different time delays after the laser pulse: 1 (*1*), 200 (*2*), and 800 μs (*3*).

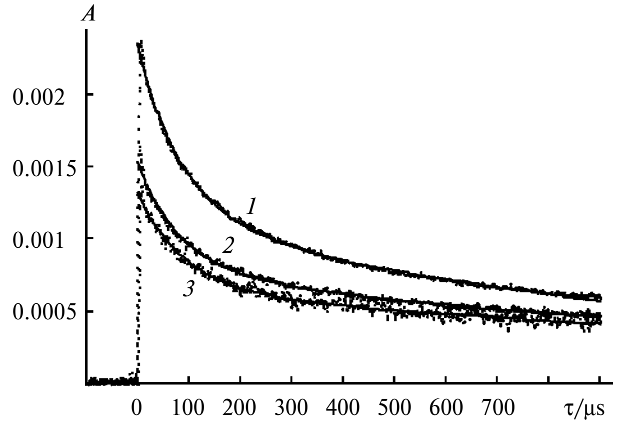

Fig. 5. Decay kinetics of the intermediate products absorbing at 570 (*1*), 750 (*2*), and 400 nm (*3*) obtained by the laser photolysis of a deoxygenated HFA solution with light of 337 nm. The curves calculated by the two-exponential approximation are superimposed on the experimental curves.

weak absorption of intermediate products characterized by a broad structureless differential spectrum with a weakly pronounced maximum at ~630 nm (Fig. 3). The ratio of quantum yields of formation of intermediate products upon photolysis with light of 470 and 520 nm is 2/1. The decay kinetics of these products is well described by a monoexponential law with a rate constant (in deoxygenated solutions) of ~$8 \cdot 10^3$ s^{-1}, which is almost independent of the wavelenght of observation, indicating that one intermediate product is observed or all intermediate products are close in nature. The introduction of air oxygen into the solutions under study is accompanied by the shortening of the lifetime of the induced absorbance. The decay kinetics in air-saturated solutions is also described by a monoexponential law with a rate constant of $2.1 \cdot 10^5$ s^{-1}, which gives a rate constant of intermediate product quenching by molecular oxygen of $7.2 \cdot 10^8$ (L mol s)$^{-1}$ (the O_2 concentration in an air-saturated aqueous solution was accepted $2.8 \cdot 10^{-4}$ mol L^{-1}).

The pulse photoexcitation of aqueous solutions of HFA with light of 337 nm results in the appearance of the absorbance of intermediate products characterized by the broad structureless differential spectrum with a maximum at ~570 nm (Fig. 4). The quantum yield of these products is 1.5-fold higher than the yield of intermediate products obtained by photolysis with light of 470 nm (the estimation assumed that the respective molar absorption coefficients were equal). The decay kinetics of these products is described by a two-exponential law with the rate constants in deoxygenated solutions about $11 \cdot 10^3$ and $6 \cdot 10^2$ s^{-1} for the fast and slow components, respectively, which are characterized by equal fractions almost independent of the observation wavelength (Fig. 5). Coincidence of the EAS of the fast and slow components (see Fig. 4) indicates that they are close in nature. However, a very substantial difference in the respective lifetimes indicates that either different intermediate products are observed, or the same intermediate product exists in different molecular environments. The introduction of air oxygen into the solutions is accompanied by the shortening of the lifetime of the induced absorbance. The decay kinetics in air-saturated solutions is also described by a two-exponential law with the rate constants $2.5 \cdot 10^5$ and $2 \cdot 10^3$ s^{-1}, respectively, and a fraction of the slow component of ~10%. The corresponding quenching rate constants are $8.5 \cdot 10^8$ and $4.3 \cdot 10^6$ L (mol s)$^{-1}$.

For photolysis with light of 390 nm, the absorption spectrum and decay kinetics of intermediate products, as well as their yield, are close to those observed for photolysis with light of 337 nm. At the same time, in the case of photoexcitation at 390 nm, the broad band in the induced absorption spectrum is somewhat shifted to the long-wave region and the band with a maximum at wavelengths shorter than 400 nm is not observed. These differences show that photolysis with light of 337—390 nm produces several intermediate products, and their relative contribution depends on the excitation wavelength.

Thus, the studies of HFA by laser photolysis using different excitation wavelengths revealed a set of relatively long-lived intermediate products, which disappear according to the first-order law and are efficiently quenched by molecular oxygen. These data suggest that these are triplet states (T_{HFA}) of different HFA moieties. The values obtained for the respective rate constants of T_{HFA} decay by molecular oxygen are somewhat lower than the typical quenching rate constants of triplet states of normal organic and inorganic molecules by molecular oxygen in aqueous solutions (usually $(2-3)\cdot10^9$ L (mol s)$^{-1}$).[14—16] This fact can be attributed to screening of the triplet chromophore by other HFA moieties. Hindered quenching by molecular oxygen of other triplet states in biomacromolecules and other complex molecular systems is a common phenomenon observed previously, *e.g.*, for dyes intercalated into DNA[17] and triplet states in the dendrimer composition.[18—20] At the same time, it should be noted that rate constant values of $\sim1\cdot10^9$ L (mol s)$^{-1}$ are often observed for quenching of unscreened triplet states of quinoid and carbonyl compounds and dyes by molecular oxygen in aqueous media.[20,21] The slow component in the decay kinetics of T_{HFA} in air-saturated solutions takes origin, most likely, from the presence of the environment in HFA molecules, because of which the latter are hardly accessible by water-dissolved oxygen.

The main part of triplet states of HFA is quenched by molecular oxygen likely *via* the energy transfer to form singlet oxygen (according to the published data, the quantum yield of $^1O_2$ for the photolysis of different HS can range from 0.05 to 5%).[13,22,23] The estimation of the quantum yield of T_{HFA} for the photolysis of HFA with light of 337 nm was performed by comparison with the respective aqueous alkaline (0.1 *N* NaOH) solution of 4-carboxybenzophenone (the quantum yield of the triplet state is 100%, the molar absorption coefficient at 545 nm is 5200 L (mol cm)$^{-1}$)[24] and gave ~1%, if the molar absorption coefficients of the triplet states of 4-carboxybenzophenone and HFA were equal. The resulting value corresponds by an order of magnitude to the expected yield of singlet oxygen.

It should be noted in conclusion that the photolysis with light of 337 and 390 nm produces, in addition to the relatively long-lived intermediate products, a hardly detectable short-lived induced absorbance at 400—800 nm characterized by a lifetime shorter than 100 ns (independent of the presence of oxygen) and a broad band with an unpronounced maximum at 700 nm. This absorbance can likely be attributed to the formation of solvated electrons, which disappear rapidly by the back recombination with holes or in reactions with other HFA moieties. Photoionization occurs, likely, in the HFA moieties containing the properly organized ensembles of moieties with a low ionization potential. It is known that the yield of solvated electrons in the UV photolysis of humic substances rarely exceeds 1% and decreases sharply with an increase in the photolysis wavelength.[25] Therefore, solvated electrons were not observed for the laser photolysis with light of 470 and 520 nm.

The authors thank L. I. Inisheva (Siberian Research Institute of Peat, Russian Academy of Agricultural Sciences, Tomsk) and N. V. Yudina (Institute of Petrochemistry, Siberian Division, Russian Academy of Sciences, Tomsk) for kindly presented HFA solutions.

This work was financially supported by the Russian Foundation for Basic Research (Project No. 03-03-32159), the Division of Chemistry and Materials Sciences of the Russian Academy of Sciences (program "Theoretical and Experimental Investigation of the Nature of Chemical Bond and Mechanisms of the Most Important Chemical Reactions and Processes"), and the Ministry of Education of the Russian Federation (Grant E02-12.2-63).

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Received November 12, 2003;
in revised form December 19, 2003