

Redox Properties and Antiradical Activity of Humic Acids under Exposure to UV and Visible Light

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Abstract—Changes in the redox and antiradical properties of humic acids upon exposure to monochromatic UV light of an excilamp and polychromatic light of a helium lamp were studied.

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Humic substances (HSs) are natural organic formations widely occurring in soils and peats, coals and shales, marine and lake deposits, and water of rivers and mineral springs. Humic substances occupy a central place in the composition of the organic matter of soils by performing accumulative, transport, protector, and trophic functions [1–3]. On the whole, it can be said that HSs control the stability of ecosystems. Their effect on living organisms has become a subject of extensive studies comparatively recently. In [4], a conclusion was made on the basis of an analysis of a large number of published sources that the resistibility of animal and human organisms is enhanced under the action of HSs and their favorable influence on the growth and development of plants was demonstrated, depending on external conditions.

The reactivity of HSs is attributed by some researchers to presence of free radicals and paramagnetic centers in their structure [5–8]. This is indicated by changes in paramagnetic properties of humic acids (HAs) upon UV irradiation [9]. It was demonstrated in [10] that free radicals are formed in HAs under irradiation with UV and visible light because of the appearance of active forms of oxygen (AFO). In recent years, the important role of triplet states of HAs and fulvic acids in intermolecular interactions and photoinduced transformations has been

revealed [11–13]. An analysis of active species formed under irradiation of aqueous solutions of HAs with light with a wavelength of 365 nm was made in [14]. Finally, it should be noted that some researchers attribute an important role in the formation of AFO to reduced Fe³⁺ bound to HAs in the form of Fe²⁺ [15].

With the role of HAs in the biosphere and their permanent exposure to solar radiation taken into account, it seems appropriate to further study formation of AFO in HAs under various conditions (especially on exposure to light with varied spectral composition), determine mechanism of possible formation of free radicals, and make a general evaluation of the antioxidant activity of humic substances.

A promising method for determining the redox properties of HAs is voltammetry, which is highly sensitive to presence of oxygen in both aqueous and nonaqueous media. As a model reaction has been used the oxygen electroreduction (ER) by the mechanism similar to that in oxygen reduction in a living cell [16, 17].

To find the role played by HAs in the oxidation process, we chose the method of chemiluminescence (CL) from luminol whose fluorescence is due to the formation and transformations of free radicals [18,

19]. The sensitivity of this technique enables its use for determining the formation and consumption of free radicals. The CL method determines the rate of the reaction in which radicals are formed, rather than their concentration. The CL intensity is proportional to the rate of radical formation.

Luminol solutions thoroughly purified to remove trace amounts of heavy metals, such as copper, vanadium, and manganese, yield no fluorescence even if hydrogen peroxide is used as the oxidizing agent. As active catalysts for chemiluminescence processes serve some natural compounds, e.g., hemin, glucose, peroxidase. The antiradical activity of a particular substance can be judged from deceleration or acceleration of the oxidation reaction [20].

The goal of our study was to examine the redox properties and the kinetics of the antiradical activity of humic acids under excitation with UV and visible light.

EXPERIMENTAL

As objects of study served humic acid from brown coal (humate-fulvate complex, HFC) (Aldrich brand with Pcode: 22709097 53680-50G) and HAs recovered from high-moor sphagnum peat from the Temnoe deposit in Tomsk oblast, with a degree of decomposition, $R = 5\%$. Humic substances were recovered from peat by extraction with a 0.1 N NaOH solution. HAs were precipitated from the resulting extract by acidification with HCl to pH 2. The precipitate was separated by centrifugation, washed with water, and dried. The working concentration of HFC and HAs in solutions was $1 \times 10^{-3} \text{ g l}^{-1}$ at pH 10–11.

^{13}C NMR spectra of the HA preparations under study were recorded with a Varian VXR-400 spectrometer in the accumulation mode followed by a Fourier transform. The preparations were dissolved in a 0.1 M solution of a deuterated alkali, NaOD. The spectra were obtained in the quantitative mode precluding appearance of the Overhauser effect. The chemical shifts were calibrated against an external signal from tetramethylsilane. The relative contents of carbon nuclei in various structural groups were determined by electronic integration of the spectra. The aromaticity of HAs, f_a , was calculated as a percentage content of the aromatic carbon relative to the sum of carbon atoms in other structural groups [8].

The redox properties of the HA preparations were determined on an Antioksidant analyzer (Tomsk) by

the voltammetric method of cathodic reduction of oxygen, using a film-type mercury electrode [17]. A phosphate buffer solution served as a supporting electrolyte. A weighed portion of a substance to be analyzed was dissolved in 0.1 N NaOH, placed in an electrochemical cell, and agitated with a magnetic rabble. Voltammograms were recorded each time after keeping a solution under study at the potential of the limiting current of O_2 ER ($E = -0.3 \text{ V}$ for aqueous media) for 3 min. The voltammograms were used to plot time dependences of the function $1 - I/I_0$ in the presence of an antioxidant concentration. The linear part of the plot and the slope ratio of the tangent to this portion of the curve were used to calculate the kinetic criterion of antioxidant activity (AOA), K ($\mu\text{M min}^{-1}$), of samples by the formula

$$K = c_0/t(1 - I/I_0),$$

where c_0 is the initial oxygen concentration in solution (μM); t , duration of the interaction between the antioxidant and active oxygen radicals (min); I_0 , limiting current of O_2 ER in the absence of the substances being analyzed (μA); and I , limiting current of O_2 ER in their presence (μA); the measurement error did not exceed 10% [16, 17].

The antiradical activity of HAs before and after irradiation were analyzed in the reaction of luminol chemiluminescence on a Varian Cary Eclipse spectrofluorimeter (Australia) in “Chemiluminescence” and “Kinetics” modes. Luminol solutions (Aldrich) were prepared from a dry weighed portion in a 0.1 N NaOH solution. To 0.1 ml of luminol ($c = 0.003 \text{ M}$) was added 2.87 ml of a buffer solution of 0.01 M of sodium tetraborate (pH 9.18) and 10 μl of phthalocyanine ($c = 0.5 \text{ g l}^{-1}$) as the reaction catalyst. After a thorough agitation, a quartz cuvette ($l = 1 \text{ cm}$) with a solution was placed in the cuvette compartment of the instrument with a thermostat ($T = 25^\circ\text{C}$). Then, 10 μl of hydrogen peroxide from a 0.1 M solution was added to the system, the mixture was allowed to stay for 1 min, and after that 10 μl of HA from a solution with a concentration of 0.01 g l^{-1} was introduced into the cuvette. The total volume of a solution under study was constant (3 ml). Chemiluminescence spectra and the time dependence of the luminol fluorescence at a wavelength of 425 nm in the presence of HAs before and after irradiation were obtained.

As sources of light for irradiation of HAs served

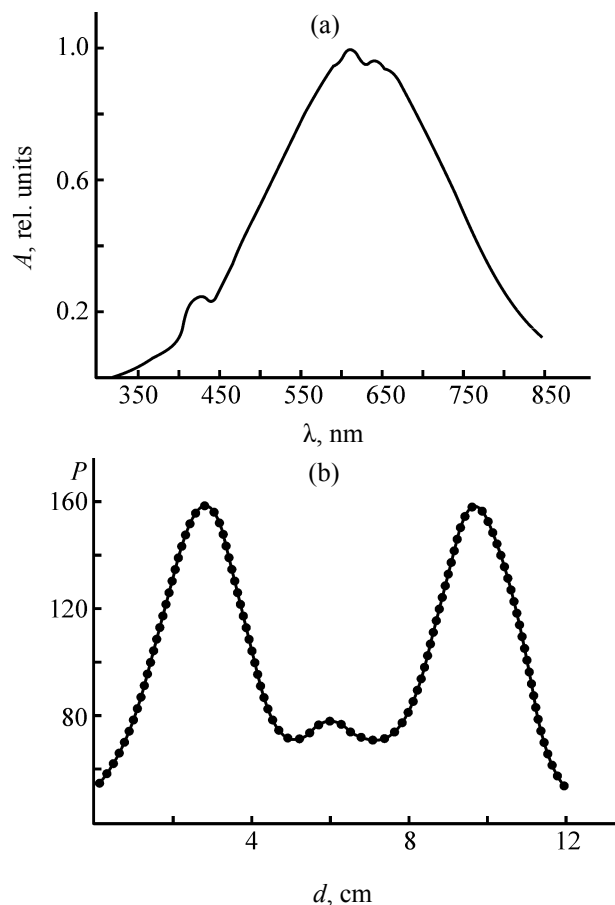


Fig. 1. (a) Emission spectrum and (b) distribution of the emission power P along the diameter d for a Solar lamp with an SZS-24 filter. (A) Absorption and (λ) wavelength.

a pulse exciplex barrier-discharge lamp operating on KrCl^* molecules, with the following parameters: $\lambda_{\text{emiss}} \approx 222 \text{ nm}$, $\Delta\lambda = 5\text{--}10 \text{ nm}$, $W_{\text{peak}} = 18 \text{ mW cm}^{-2}$, $f = 200 \text{ kHz}$, pulse width $1 \mu\text{s}$; the solar light was simulated with a Solar helium lamp with an SZS-24 filter. Figure 1 shows spectral and energy characteristics of this lamp. HA solutions (50 ml) were irradiated at room temperature in a vessel at a distance of 6–10 cm from the lamp. The irradiation durations were 2, 8, 16,

and 32 min. The optimal operation of the lamps was provided by air cooling with a ventilator.

^{13}C NMR spectroscopic data characterizing the structural specific features of the HA samples under study are listed in Table 1. HA preparations from high-moor peat are distinguished by low content of aromatic carbon and large amount of alkyl carbon C_{alk} and alkyl heterosubstituted carbon C_{alkO} . In Aldrich HFC, the fractions of carbon of oxygen-containing functional groups and condensed aromatic carbon are larger, with the degree of aromaticity (f_a) being higher, but the content of alkyl and heterosubstituted alkyl carbon is lower. According to the data obtained, HFC molecules contain a larger number of chromophore groups, compared with HA from peat.

The redox properties of HA were determined by voltammetry on the model reaction of O_2 ER. In the presence of HA at a pH of the medium 10–11, the current of O_2 ER increases, compared with the current of a supporting electrolyte without HA. The reason is that unstable active forms of oxygen are reduced in the presence of HAs to water, with the subsequent disproportionation of the reaction product and partial regeneration of the depolarizer, molecular oxygen [16]. The amount of active oxygen radicals regenerated, with HA involved, to molecular oxygen was estimated by the kinetic AOA criterion calculated from plots of the function $1 - I/I_0$ against time. Because $I > I_0$, values of the kinetic AOA criterion K are negative. The results of the calculation indicate that an unirradiated HFC sample ($K = -0.62$) exhibits a higher catalytic activity than HA from peat ($K = -0.32$) (Fig. 2). The value of the kinetic AOA criterion of HAs of varied nature and their behavior in the O_2 RE are determined by the mechanism of interaction of the substances under study with active radicals of oxygen and depend on structural features of preparations. Specific features of the molecular structure of HAs incorporating carboxy groups, phenolic hydroxides, and quinoid metal-complexes capable

Table 1. Structural parameters of the HAs according to ^{13}C NMR spectroscopic data

Sample	Content of carbon in indicated structural fragments, %					
	COO^- ; CO^- ; C=O	C_{aromO}	$\text{C}_{\text{aromC, H}}$	C_{alkylO}	C_{alkyl}	f_a
peat HA	12.1	2.3	18.5	31.9	35.1	24
HFC	18.7	9.3	30.5	16.9	24.6	40

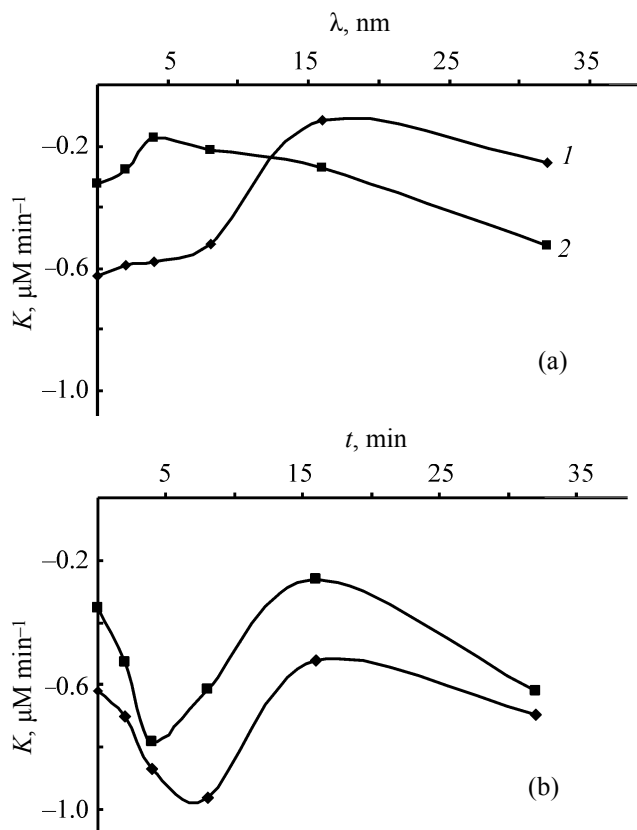


Fig. 2. Effect of the wavelength λ and irradiation time t on the AOA criterion K for (1) HFC and (2) peat HA. (a) KrCl excilamp and (b) Solar lamp with a filter.

of forming free radicals in single-electron reduction determine their behavior in O_2 RE to a greater extent. When being ionized, carboxy groups and phenolic hydroxyls create a negative charge on the surface of

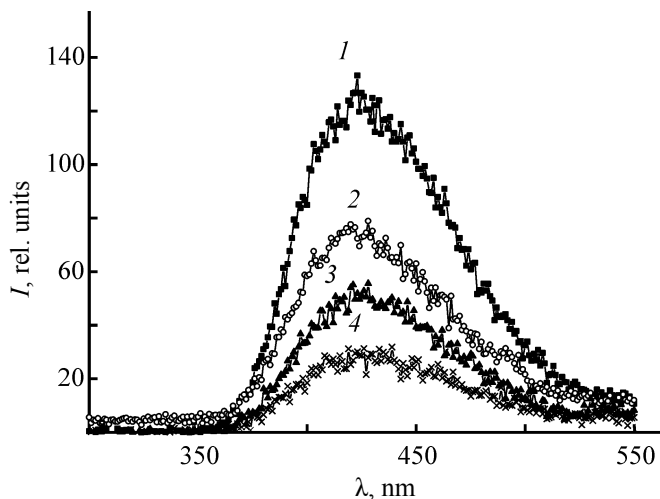


Fig. 3. Chemiluminescence spectra of 3 mM of luminol (1) taken alone and in the presence of (2) unirradiated HFC and that irradiated for (3) 2 and (4) 32 min with a KrCl excilamp. (I) Chemiluminescence intensity and (λ) wavelength.

HA macromolecules. The catalytic activity of HAs in an alkaline medium is attributed to presence of radicals of the semiquinone type in their structure. Quinoid structures determine the course of redox reactions and the shift of equilibrium in the quinone–semiquinone–hydroquinone system [5, 21–24].

Figure 2 shows how the kinetic AOA criterion of HA samples in O_2 ER depends on the spectral range and irradiation duration. When exposed to light from both lamps, HA solutions, similarly to unirradiated samples, increase the current I in O_2 ER, compared with the background current I_0 . Treatment with light with $\lambda \approx 222$ nm of peat HA for 7 min and HFC for 15 min

Table 2. Chemiluminescence time of luminol at various reaction mixture compositions

Reaction mixture composition	Fluorescence duration, min			
	Aldrich HFC		Peat HA	
	KrCl	Solar	KrCl	Solar
Luminol	0.6			
Luminol + unirradiated HA	0.7	0.7	0.3	0.3
	1.5	1.5	1.6	1.6
Luminol +(HA + 2 min of irradiation)	0.15	–	–	–
	1.6	1.2	1.8	–
Luminol +(HA + 8 min of irradiation)	1.7	1.7	1.3	1.7
Luminol +(HA + 16 min of irradiation)	–	0.8	0.9	–
	1.6	1.3	1.3	1.4
Luminol +(HA + 32 min of irradiation)	0.15	0.6	–	–
	2	1.2	1.6	1.4

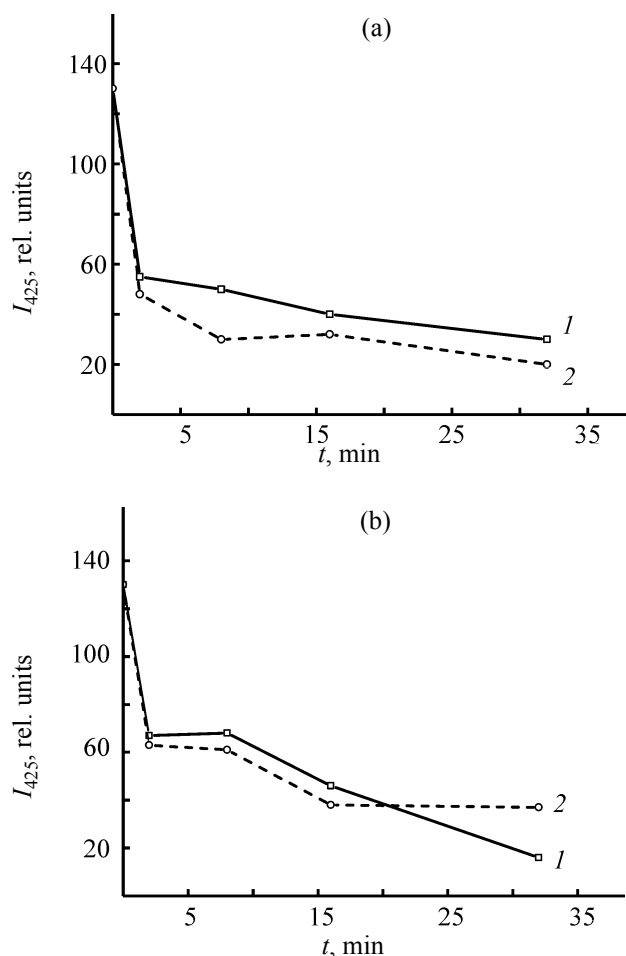


Fig. 4. Variation of the chemiluminescence intensity from luminol, I_{425} , at the peak of the band at 425 nm in the presence of (a) HFC and (b) peat HA, in irradiation with (1) KrCl excilamp and (2) Solar lamp. (t) Irradiation duration.

leads to a decrease in their catalytic activity (Fig. 2). Further increase in the time of UV irradiation of HA to 32 min results in that the kinetic AOA criterion becomes larger. Presumably, the irradiation with UV light from the KrCl excilamp more strongly affects the structure of peat HAs, compared with HFC, which enhances their catalytic properties in O_2 ER (Fig. 2a).

The manner in which the catalytic properties of HAs vary with the time of exposure to light of the visible spectral range is the same for both samples (Fig. 2b). The maximum rise in the kinetic AOA criterion for these samples was observed upon irradiation with a lamp simulating the solar light for 3–7 min.

The antiradical activity of HAs was evaluated by the intensity of the chemiluminescence signal formed in the reaction of luminol oxidation. The intensity of chemiluminescence from luminol markedly decreases

even in the presence of unirradiated HA samples, irrespective of their structure. This can be attributed to active interaction of HAs with radicals involved in the chemiluminescence reaction of luminol (Fig. 3). Figure 4 shows how the intensity of chemiluminescence from luminol depends on the irradiation time for HAs of varied nature. Addition of HAs irradiated for 32 min to a system with luminol (Fig. 4) leads to further decrease in the fluorescence intensity. This may be due to UV-induced formation of free radicals interacting with reagents involved in the chemiluminescence reaction. The strongest suppression of the luminol fluorescence was observed after 32 min of irradiation with a KrCl excilamp for the system containing peat HA and upon irradiation with a Solar lamp for HFC.

The chemiluminescence kinetics in the reaction of luminol oxidation initiated by addition of H_2O_2 is described by a typical first-order exponential dependence with a fluorescence of 0.6 min (Table 2). Upon addition of HA to the system, the kinetic dependence shows, in addition to the short-lived reaction of luminol oxidation, also a component with a longer fluorescence time. Exposure of HFC to light from the KrCl lamp leads to a decrease in the fluorescence time of the short-lived component and to a longer duration of the long-lived component. It is the presence of the short-lived component that points to the antioxidant activity of irradiated HFC samples [19].

CONCLUSIONS

(1) It was shown that preparations of humic acids from high-moor peat are distinguished by low content of aromatic carbon and large amount of alkyl and alkyl heterosubstituted carbon. In humic acids from brown coal (Aldrich), the fractions of oxygen-containing functional groups and condensed aromatic carbon are larger, the degree of aromaticity is higher, but the content of alkyl and heterosubstituted alkyl carbon is lower.

(2) The redox properties of the preparations under study were determined and it was found that humic acids from brown coal exhibit a higher catalytic activity.

(3) A change in the structure of humic acids from peat under the action of UV light was observed, which enhances their catalytic properties.

(4) It was found that the kinetic criterion in irradiation with a lamp simulating the solar light has the maximum value in the case of exposure for 3–7 min.

(5) An antiradical activity of humic acid preparations was found, with its maximum values observed for a system containing humic acids from peat upon irradiation with an excilamp and for a system of brown coal upon exposure to an analog of solar light.

(6) The spectral range and duration of irradiation govern the course of redox reactions and the shift of equilibrium in the quinone–semiquinone–hydroquinone system.

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