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Sequential Photochemical and Microbial Degradation of Organic Molecules Bound to Humic Acid

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We studied the effects of photochemical processes on the mineralization by soil microorganisms of [2-^{14}C]glycine bound to soil humic acid. Microbial mineralization of these complexes in the dark increased inversely with the molecular weight of the complex molecules. Sunlight irradiation of glycine-humic acid complexes resulted in loss of absorbance in the UV range and an increase in the amount of ^{14}C-labeled low-molecular-weight photoproducts and the rate and extent of mineralization. More than half of the radioactivity in the low-molecular-weight photoproducts appears to be associated with carboxylic acids. Microbial mineralization of the organic carbon increased with solar flux and was proportional to the loss of A_{330}. Mineralization was proportional to the percentage of the original complex that was converted to low-molecular-weight photoproducts. Only light at wavelengths below 380 nm had an effect on the molecular weight distribution of the products formed from the glycine-humic acid complexes and on the subsequent microbial mineralization. Our results indicate that photochemical processes generate low-molecular-weight, readily biodegradable molecules from high-molecular-weight complexes of glycine with humic acid.

Humic substances are a major reservoir of organic carbon in soils, sediments, and water (2), and their fate is relevant to carbon cycling in these environments. In soil, humic acid formation involves the enzymatic and chemical condensation of natural polyphenols, quinones, and amino compounds (24). Humic acid is stable to microbial attack in soil, as evidenced by mean residence times of about 1,000 years (8). The resistance of soil humic acid molecules to microbial degradation is ascribed to their high molecular weight and to their complex structure, both of which result from disorderly condensation and extensive copolymerization and cross-linking (25). By contrast to the resistance of humic acid to microbial degradation, its photochemical breakdown appears to occur readily and is associated with the loss of light absorbance of the high-molecular-weight fraction of humic acid (3, 11, 17). The high degree of condensation of humic substances results in chromophores that absorb light in the solar actinic range. These humic substances can be transported from soil into surface waters by erosion. Humic substances may also be leached from soils into groundwaters (21), which then move into surface waters.

The present study was designed to determine the extent to which the sequential action of photochemical and microbiological processes play a role in determining the fate of humic acid-bound organic compounds. Glycine was chosen as a model organic compound for three reasons: (i) it is a precursor in the formation of, and binds readily to, soil humic acid (1, 24); (ii) it does not absorb light in the solar actinic range and therefore is not photoreactive in sunlight; and (iii) it is easily degraded by microorganisms, so that its resistance to biodegradation would be a result of its binding to humic acid.

MATERIALS AND METHODS

Preparation of [^{14}C]GLY-HA complexes. Humic acid was prepared from Carlisle muck (Typic medisaprist) from Oswego, N.Y., by the method of Schnitzer (22). The soil was extracted with 0.5 N NaOH under a positive pressure of N_2. The alkali extract was centrifuged at 10,000 × *g* for 10 min, and the supernatant fluid was acidified to pH 2 with HCl. The humic acid precipitate was collected by centrifugation at 10,000 × *g* for 10 min. The resulting pellet was suspended in reagent grade water and dialyzed three times against reagent grade water by using a 1,000-molecular-weight-cutoff dialysis membrane. The dialyzed humic acid suspension was lyophilized. To remove metals and low-molecular-weight organic contaminants, the humic acid was subject to the following purification procedure (22). It was dissolved in 0.5 N NaOH, and the alkaline solution was passed through a glass microfiber filter (GF/C filter; Whatman, Inc., Clifton, N.J.). The filtrate was acidified with HCl to pH 2, the humic acid precipitate was collected by centrifugation at 10,000 × *g* for 10 min, and the resulting pellet was dissolved in 0.5 N NaOH. This procedure was repeated. The humic acid pellet was suspended in reagent grade water, and the suspension was placed in a 1,000-molecular-weight-cutoff dialysis bag and dialyzed three times against water and once against water containing a cation-exchange resin in the H^+ form (Chelex 100; 100/200 mesh; Bio-Rad Laboratories, Richmond, Calif.). The dialyzed suspension was lyophilized.

Humic acid (20 mg) was dissolved in 6.0 ml of 0.025 M sodium borate buffer (pH 9.3), and 25 μl of 2.1 mM [2-^{14}C] glycine (47.3 mCi/mmol; > 97% radiopurity; Du Pont, NEN Research Laboratories, Boston, Mass.) was added to the solution and mixed. The tube containing the solution was capped and placed in a water bath at 40°C, and HCl was added after 24 h to precipitate the humic acid. The reaction mixture was centrifuged at 10,000 × *g* for 10 min, the supernatant fluid was discarded, and the pellet was suspended in water adjusted to pH 2 with HCl. The suspension was centrifuged at 10,000 × *g* for 10 min. This procedure was repeated. The humic acid pellet was then dissolved in 0.025 M borate buffer (pH 9.3), and the pH was adjusted to 8. The solution was sterilized by filtration and stored frozen.

The resulting [^{14}C]glycine-humic acid ([^{14}C]GLY-HA)

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complex had a specific activity of 80,000 dpm/mg of humic acid. It contained less than 3% unreacted [2-^{14}C]glycine, as determined by thin-layer chromatography. A solution containing 10 μg of [^{14}C]GLY-HA complex per ml in 1 mM bicarbonate (pH 8.1) had an optical density of 0.586 at 330 nm measured in a quartz cell with path length 10 cm.

Ultrafiltration. Ultrafiltration was performed with a microultrafiltration system (model 8 MC; Amicon Corp., Lexington, Mass.), with either YCO5 or YM5 ultrafiltration membranes (diameter, 25 mm; Amicon) with molecular weight cutoff values of 500 and 5,000, respectively. Before use, the membranes were washed repeatedly with a filtered (pore size, 0.22 μm) 5% NaCl solution and then washed repeatedly with filtered reagent grade water. The ultrafiltration system was pressurized with N_2 at 3,500 g/cm^2, and ultrafiltration was performed with continuous stirring of the retentate.

To fractionate the [^{14}C]GLY-HA preparation, 8 ml of a dilute solution in 1 mM $NaHCO_3$ (pH 8.1) was ultrafiltered through a 5,000-molecular-weight-cutoff membrane. The retentate (1 ml; molecular weight >5,000) was washed continuously with fresh bicarbonate solution to remove material with a molecular weight of <5,000. The washed solution (1 ml; molecular weight >5,000) is referred to a F_1. The permeate (7 ml; molecular weight <5,000) was ultrafiltered through a 500-molecular-weight-cutoff membrane. The resulting retentate (1 ml, molecular weight 500 to 5,000) was washed with fresh bicarbonate solution to remove material with molecular weight of <500 and is referred to a F_2 (500 to 5,000). The permeate (7 ml; molecular weight <500) is referred to as F_3. More than 96% of the initial radioactivity was recovered at the end of this procedure.

Molecular weight distribution. The molecular weight distribution was determined by gel filtration chromatography on a column (2.0 by 57 cm) of Sephadex G-100 (Pharmacia Fine Chemicals, Piscataway, N.J.) with 0.025 M sodium borate (pH 9.3) as the eluant. According to Swift and Posner (28), the use of eluants with high ionic strength containing large anions at high pH minimizes interactions between gel and humic acid molecules, such that separation is largely a result of differences in molecular weight. The outer volume (V_o) of the gel bed was 33 ml as determined by chromatography of Blue Dextran 2000 (Pharmacia). The total available volume (V_t) was 111 ml as measured by chromatography of phenylalanine. The samples were filtered through a 0.22-μm membrane before they were added to the column, and the volume of the sample added was kept to less than 6% of the total bed volume. The eluted fractions were analyzed for UV absorption and radioactivity. Typically, between 96 and 101% of the applied radioactivity was recovered from the column. The distribution coefficient (K_{av}) of the fractions was computed from the relationship $K_{av} = (V_e - V_o)/(V_t - V_o)$, where V_e is the elution volume. The column was calibrated with [^{14}C]GLY-HA solutions fractionated by ultrafiltration. Gel filtration chromatography of the fractions yielded K_{av} values of <0.5, 0.50 to 0.80, and >0.80 for F_1, F_2, and F_3, respectively.

Photodegradation. UV-visible light absorbance was measured with a diode-array spectrophotometer (model 8450A; Hewlett-Packard Instrument Co., Palo Alto, Calif.). The integrated solar flux was measured with a UV radiometer (model TUVR; Eppley Laboratory, Inc., Newport, R.I.) fitted with a narrow-bandpass filter that limits the spectral response of the photocell to wavelengths between 295 and 385 nm.

The steady-state irradiation system consisted of a 1,000-W power supply (Schoeffel Instrument Co., Westwood, N.J.), a 1,000-W compact-arc Hg-Xe lamp (type 177B0010; Conrad-Hanovia, Inc., Newark, N.J.), and a high-intensity 0.25-m monochromator (Schoeffel). The photon flux was measured with a YSI-Kettering model 65A radiometer equipped with a model 6551 radiometer probe (Yellow Springs Instrument Co., Yellow Springs, Ohio). The radiometer was calibrated by potassium ferrioxalate actinometry (7).

Filter-sterilized solutions of [^{14}C]GLY-HA complex (approximately 10 μg/ml) in 1 mM $NaHCO_3$ (pH 8.1) were placed in 10-cm quartz cuvettes (total volume, 28 ml). The solutions were irradiated at particular wavelengths within the solar UV spectrum by using a steady-state irradiation system. Photolysis was followed by UV-visible spectroscopic analysis of the solution in the irradiation cell.

For studies of sunlight irradiation, filter-sterilized solutions of [^{14}C]GLY-HA (10 μg/ml) in 1 mM $NaHCO_3$ (pH 8.1) were placed in sterile 250-ml round-bottom quartz flasks. The solutions were exposed from 10:00 a.m. to 3:00 p.m. to full sunlight on the roof of the laboratory building in Miami, Fla. Samples wrapped with aluminium foil to prevent light penetration were exposed to the same conditions. Photolysis was followed by UV-visible spectroscopic analysis of the solutions. A single sample was analyzed at each time point.

Quantification of acidic compounds. Acidic, ^{14}C-labeled low-molecular-weight compounds were extracted by using Bond Elut SAX anion-exchange solid-phase extraction cartridges (Analytichem International, Harbor City, Calif.). These cartridges contain a strong anion exchanger (trimethylaminopropyl with a chloride counterion) bonded to silica. Before use, the cartridges were washed with methanol and then with reagent grade water. Dark-exposed and sunlight-irradiated solutions of [^{14}C]GLY-HA were subjected to ultrafiltration through 500-molecular-weight-cutoff membranes, and 4.0-ml portions of the resulting permeate (pH 8.1) were added to the cartridges, which were then washed twice with 2.0 ml of 1 mM $NaHCO_3$ solution (pH 8.1). The retained radioactivity was eluted with 2.0 ml of 1 N HCl. The radioactivities in the initial solution, the unretained fraction, the bicarbonate washes, and the fraction eluted with HCl were then determined. Approximately 80% of the initial radioactivity was recovered from the cartridge.

Analysis for the presence of ^{14}C-labeled α-keto acids in the low-molecular-weight fraction was performed by high-performance liquid chromatography (15). Glyoxylate, oxaloacetate, pyruvate, and α-ketoglutarate could be detected by this method.

Biodegradation. An irradiated solution of [^{14}C]GLY-HA was mixed in a 1:2.5 (vol/vol) ratio with a suspension of soil in buffered salts solution (15%, wt/vol). The soil suspension was prepared by shaking a mixture of Carlisle muck and salts solution for 10 min and using the liquid that passed through a glass microfiber filter. The salts solution, adjusted to pH 6.5, contained (in grams per liter) the following: $(NH_4)_2SO_4$, 0.5; KCl, 0.2; $MgSO_4$, 0.2; NaCl, 0.1; $FeCl_3 \cdot H_2O$, 0.02; $CaCl_2 \cdot 2H_2O$, 0.05; Na_2HPO_4, 0.88; and KH_2PO_4, 0.16. Amounts corresponding to 4 μg of [^{14}C]GLY-HA per ml (320 dpm/ml) were placed in 150-ml Erlenmeyer flasks, which were incubated at 30°C in the dark on a shaker operating at 200 rpm. Tests of the microbial mineralization of photolysates were unreplicated. Periodically, portions of liquid were removed for determination of the remaining radioactivity with a liquid scintillation counter (Betatrac 6895; Tracor Northern, Inc., Middleton, Wis.) by the method of Subba-Rao et al. (27). Essentially identical results were obtained if

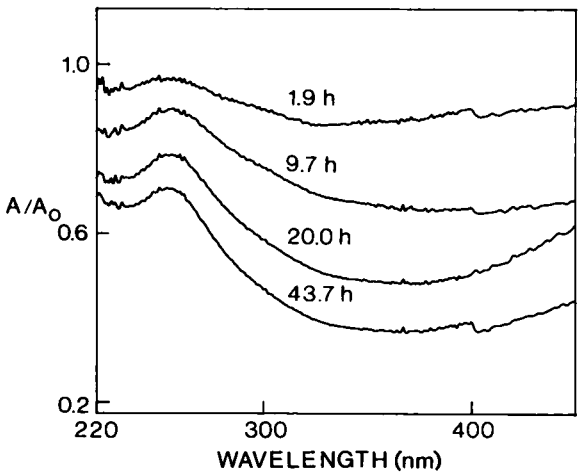

FIG. 1. Effect of sunlight irradiation on the UV-visible spectrum of a [^{14}C]GLY-HA complex solution. Numbers next to the curves indicate irradiation times.

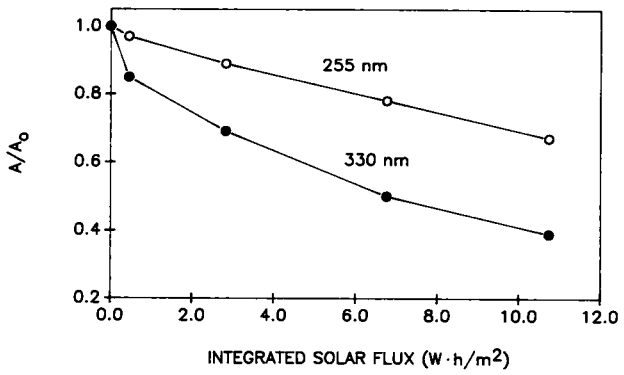

FIG. 2. Changes in A_{255} and A_{330} of a [^{14}C]GLY-HA complex with integrated solar flux.

biodegradation was measured by trapping evolved $^{14}CO_2$ or by monitoring the loss of radioactivity from solution; hence, the loss of radioactivity from solution was considered to be due to mineralization.

To measure the biodegradation of individual-molecular-weight fractions, the fractions of [^{14}C]GLY-HA obtained by ultrafiltration were mixed with a soil suspension. The relative final concentrations of each fraction of the dark-exposed complexes were 10:7:3. Portions (2.0 ml) of the mixtures were placed in glass test tubes (85 by 15 mm), and the tubes were fitted with rubber septa and incubated at 30°C in the dark on a rotary shaker operating at 100 rpm. Periodically, the reactions were stopped by injecting 3 drops of concentrated H_2SO_4 through the septum, the acidified mixture was mixed, and the evolved $^{14}CO_2$ was flushed with air and trapped with phenethylamine as described by Thomas et al. (29). The radioactivity of the removed CO_2 and the acidified solution was then determined. Three replicates of each treatment were analyzed at each time point.

Statistical analysis. The data from tests of the effects of light on mineralization by a soil suspension were analyzed by a one-way analysis of variance, and treatments were grouped according to the least significant difference of their means. The results of all tests were evaluated at the 95% confidence level.

RESULTS

Photochemical degradation. Loss of absorbance upon sunlight irradiation of the [^{14}C]GLY-HA solution was observed across the spectrum, even at wavelengths outside the solar spectrum (<300 nm) (Fig. 1). The relative loss of absorbance was lowest at 255 nm and highest between 330 and 370 nm; this pattern that was consistent throughout the irradiation period. A_{255} decreased at a constant rate with increasing solar exposure, whereas A_{330} decreased sharply during the initial stages of irradiation but decreased more slowly with increasing solar exposure (Fig. 2). At the end of the irradiation period (43.7 h, 10.7 W · h/m^2) 61.4 and 29.4% of the A_{330} and A_{255} was lost, respectively.

The radioactivity of the [^{14}C]GLY-HA complex shifted gradually to the low-molecular-weight fractions with increasing solar exposure (Fig. 3). The elution patterns following gel permeation chromatography changed from a bimodal distribution with broad peaks at distribution coefficient (K_{av}) values of 0.4 and 0.9 before irradiation to a single peak at a K_{av} of 0.9 after 43.7 h. The A_{255} of the high-molecular-weight fractions declined and the A_{255} of the intermediate-molecular-weight fractions increased with increasing solar exposure. No A_{255} was detected at K_{av} values less than 0.4 at the end of the irradiation period.

The effect of solar exposure on the radioactivity associated with different fractions is shown in Table 1. Prior to irradiation, more than 80% of the radioactivity was in the fraction containing substances with molecular weights above 500 (F_1 and F_2), mostly in the fraction with molecular weight above 5,000 (F_1). At the end of the 43.7-h irradiation period, in contrast, 56.7% of the radioactivity was in low-molecular-weight components (F_3). As radioactivity disappeared from

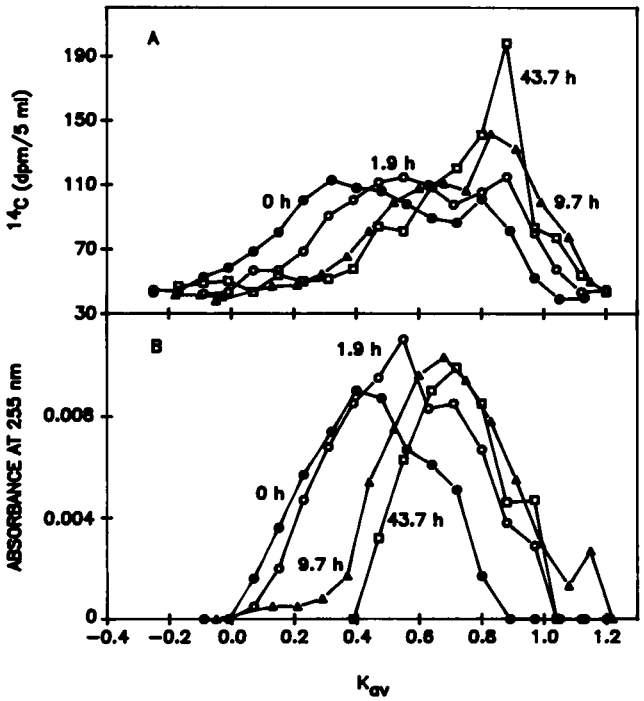

FIG. 3. Effect of sunlight irradiation on molecular weight distribution of (A) radioactivity and (B) A_{255} of a [^{14}C]GLY-HA complex as determined by gel filtration chromatography. The numbers next to the curves indicate irradiation times.

TABLE 1. Effect of sunlight irradiation on the molecular weight distribution of [^{14}C]GLY-HA complexes

Irradiation time (h)	Integrated solar flux (W · h/m^2)	Radioactivity (% of total) in[a]:		
		F_1	F_2	F_3
0	0	47.1	35.1	17.6
1.9	0.5	37.5	37.5	31.0
9.7	2.8	18.7	38.9	44.0
20.0	6.8	12.9	33.0	54.0
43.7	10.7	11.3	31.2	56.7

[a] F_1, Molecular weight >5,000; F_2, molecular weight <5,000 but >500; F_3, molecular weight <500.

F_1, it appeared in F_3, whereas only small changes were observed in the radioactivity in F_2. However, the rate of increase in radioactivity in fraction F_3 decreased with increasing solar exposure. The formation of low-molecular-weight photoproducts was better correlated with loss of A_{330} (r^2 = 0.895) than with loss of A_{255} (r^2 = 0.795).

Sunlight irradiation of a [^{14}C]GLY-HA solution almost doubled the percentage of radioactivity in the low-molecular-weight-fraction (F_1) that was retained by a strong anion-exchange column and then eluted (Table 2). The constituents thus eluted with HCl presumably consist mostly of carboxylic acids. The photolysate did not contain ^{14}C-labeled α-keto acids.

The relationship between photolysis of [^{14}C]GLY-HA complexes and the wavelength of irradiation was determined by irradiating samples with 2.5 millieinsteins, using a series of narrow spectral bands within the solar UV range (297, 303, 313, 334, and 366 nm). At all five wavelengths, irradiation resulted in a loss of absorbance that was maximal at the wavelength of irradiation and minimal at 255 nm (Fig. 4). The loss of absorbance was greatest at shorter wavelengths of irradiation.

The relationship between the wavelength of irradiation of [^{14}C]GLY/HA complexes and radioactivity in the three fractions is shown in Table 3. Irradiation was performed at a total of 2.5 millieinsteins at each wavelength. The radioactivity in F_1 was low at shorter wavelengths of irradiation, and the radioactivity in F_2 and F_3 was high at longer wavelengths. A large change in the radioactivity of all three fractions was observed following irradiation at 313 and 334 nm, corresponding to energies of 85.6 and 91.4 kcal/einstein (358.2 and 382.4 kJ/einstein), respectively.

Biodegradation. An experiment was conducted to determine the mineralization of [^{14}C]GLY-HA complexes in the various molecular weight fractions. The fractions obtained by ultrafiltration were incubated in the dark with a soil suspension. The mineralization of F_1 was linear for the 83-day incubation period (Fig. 5). The initial rate of mineralization of F_1 (0.3%/day) was lower than that of F_2 (3.1%/day) and F_3 (17.4%/day). Mineralization of F_2 and F_3 stopped after 23 days. The initial rate of mineralization was highest for F_3. The extent of mineralization after 83 days was related to the molecular weights of the components, being highest for F_3. The association of ^{14}C with particulates was measured at the end of the incubation period by passage of particulates through a 0.22-μm membrane: the values were 26.6, 20.8, and 2.8% for F_1, F_2, and F_3, respectively. The presence of ^{14}C in particulates represents ^{14}C assimilated by the cells or sorption of ^{14}C by microorganisms, or both.

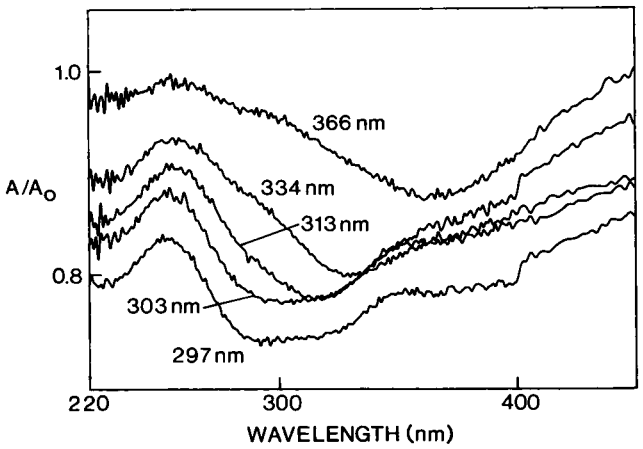

FIG. 4. Effect of irradiation wavelength on the UV-visible spectrum of a [^{14}C]GLY-HA complex solution. The numbers next to the curves designate the irradiation wavelengths.

The effects of sunlight irradiation (9.6 W · h/m^2) on the microbial mineralization of [^{14}C]GLY-HA complexes (50 μg/ml) were measured. The incubation period was 44 days, and the extent of mineralization was 47.1 and 32.3% for the irradiated and dark-exposed samples, respectively. A greater proportion of the radioactivity prior to biodegradation was present in the lower-molecular-weight fractions in the irradiated than in the nonirradiated samples (Fig. 6). On the other hand, radioactivity remaining after 44 days of biodegradation showed almost identical molecular weight distributions of ^{14}C in the dark-exposed and sunlight-irradiated samples.

To assess the effect of solar exposure on the subsequent biodegradation of the products formed photochemically, samples were taken from the preceding experiment, in which the effect of solar exposure on changes in the molecular weight distribution of [^{14}C]GLY-HA complexes had been measured. Statistical analysis showed that irradiation significantly affected the curves of mineralization of the complex. With increasing irradiation time, the susceptibility of the

TABLE 2. Retention of radioactivity from a low-molecular-weight fraction by an anion-exchange resin before and after sunlight irradiation

Treatment	Fraction	% Radioactivity
Dark exposed	Initial	26.5
	Unretained	3.8
	Retained, eluted	16.7
Irradiated	Initial	54.9
	Unretained	6.7
	Retained, eluted	31.6

TABLE 3. Effect of irradiation wavelength on molecular weight distribution of [^{14}C]GLY-HA complexes

Irradiation wavelength (nm)	Radioactivity (% of total) in:		
	F_1	F_2	F_3
Dark	49.7	35.0	15.1
366	40.0	40.4	19.6
334	35.0	34.1	20.6
313	21.2	45.7	33.2
303	18.7	50.7	30.7
297	18.8	49.9	31.2

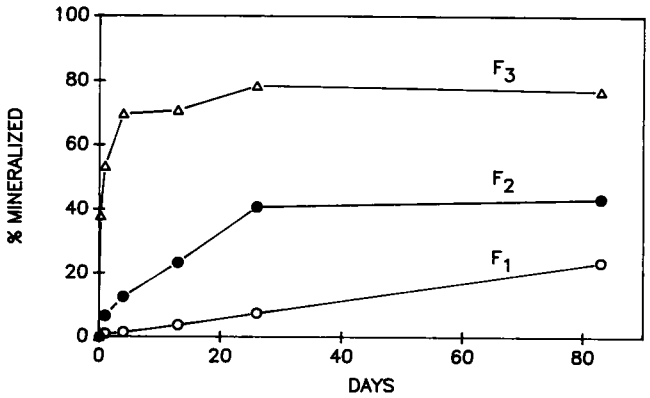

FIG. 5. Microbial mineralization of different molecular weight fractions of [^{14}C]GLY-HA complexes.

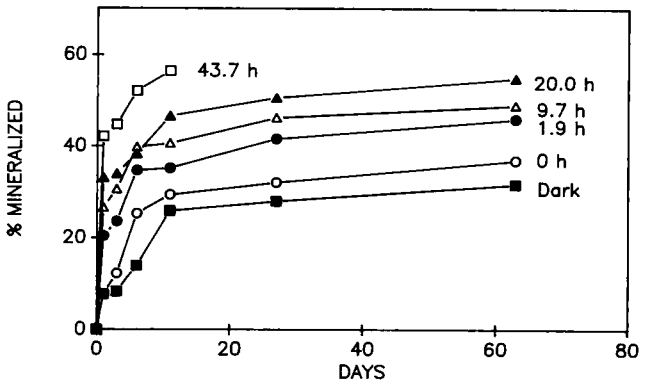

FIG. 7. Microbial mineralization of sunlight-irradiated and dark-exposed [^{14}C]GLY-HA complexes. The numbers next to the curves indicate irradiation times.

complex to biodegradation increased (Fig. 7). The differences were especially marked in the first few days. Although the rates of mineralization declined with time, the effect of the duration of solar exposure was evident even at 63 days. The differences between mineralization curves for the dark-exposed samples and the sample irradiated for 0 h may have resulted from thermal reactions such as copolymerization, which tend to occur at high pH; such reactions probably increase the resistance of the dark-exposed material to microbial attack.

The relationship between the wavelength of light used to cause photochemical degradation and the subsequent microbial mineralization of [^{14}C]GLY-HA complexes was also determined. Photolysates from studies of the wavelength dependence of the effects of light on the molecular weight distribution of the complexes were used. Except for light at 366 nm, irradiation with light of different wavelengths significantly affected the mineralization curves of the complexes. The mineralization of all irradiated samples was rapid initially and then slowed after 2 days (Fig. 8). The initial rate of mineralization of the irradiated samples was lowest for the sample irradiated at 366 nm and highest for the sample irradiated at 297 nm. The longer the wavelength, the lower the extent and initial rate of mineralization. The extent of incorporation of ^{14}C into particulates increased linearly with wavelength, ranging from 0.9% at 297 nm to 10.1% at 366 nm.

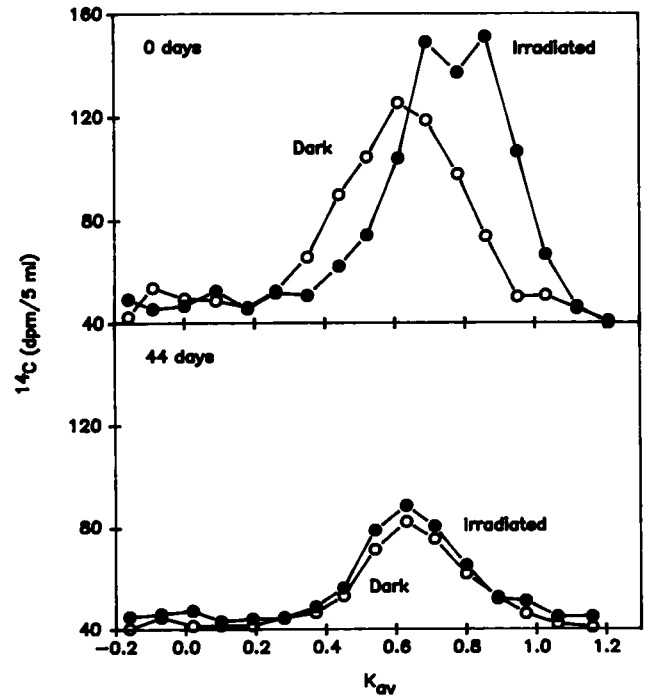

FIG. 6. Molecular weight distribution of sunlight-irradiated and dark-exposed [^{14}C]GLY-HA complexes after 0 and 44 days of incubation with a soil suspension.

DISCUSSION

Humic-acid-bound organic compounds are associated primarily with the high-molecular-weight-fractions of humic acid (19). Our results indicate that the rate and extent of mineralization of [^{14}C]GLY-HA complexes decrease with increasing molecular weight of the complexes. Similarly, *Xanthomonas* sp. strain 99 preferentially degrades the low-molecular-weight fraction of lignin (14). By contrast, the fractions of humic acid with molecular weights greater than 50,000 were attacked to a greater extent than those below 50,000 (18) by *Penicillium* spp. The observed differences in the susceptibilities of the various fractions may be the result of different physiological properties of the organisms or

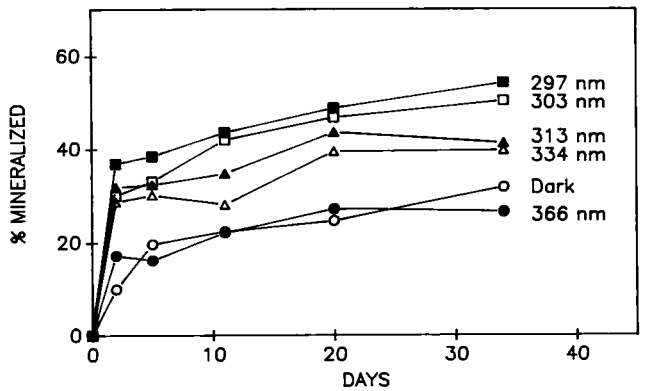

FIG. 8. Microbial mineralization of [^{14}C]GLY-HA complexes after irradiation at different wavelengths. The numbers next to the curves designate the irradiation wavelengths.

differences in the chemistry of the carbon sources whose mineralization was being measured (^{14}C from glycine versus C from humic acid).

Because of the high molecular weight of [^{14}C]GLY-HA complexes, the rate of transport of the molecules across the cell membrane may be low; hence, microbial degradation also may be slow. The rate of enzymatic attack on the complexes also may be reduced by steric hindrance. Alternatively, glycine may react with different chemical groups in different molecular weight fractions of humic acid, and the observed effect of the molecular weight may result from dissimilar degrees of resistance of different types of bonds to enzymatic cleavage; this seems unlikely in view of the report that [^{15}N]glycine binds to soil organic matter almost exclusively by forming melanoidins (4).

Changes in the absorption spectrum of humic acid upon irradiation appear to result from the destruction of chromophores in the high-molecular-weight fractions. Photolysis results in loss of absorbance and radioactivity from these fractions and an increase in radioactivity in low-molecular-weight fractions that is not accompanied by increased UV absorption. These results indicate a loss of conjugation in the humic acid, which may be interpreted as a reduction in the complexity of the [^{14}C]GLY-HA molecules. The loss of absorbance and photoproduct formation upon irradiation suggests the presence of a readily photolabile pool and a photostable pool, with molecular weights greater than 5,000 and between 500 and 5,000, respectively. These results are in agreement with a molecular-weight-dependent oxidative mechanism proposed for the photodegradation of humic acid (11). Kotzias et al. (17) observed that humic acid subjected to preliminary irradiation is less capable of generating reactive oxygen species photochemically, indicating a decrease in photoreactivity. In addition, the ability of humic acid molecules to generate ·OH radicals and alkoxy and peroxy radicals increases with molecular weight (17). Allen (3) reported that although the soluble organic fraction of lake water with molecular weights greater than 50,000 was readily photolabile, the fraction with molecular weights of 2,000 to 50,000 was essentially refractory to UV light. The present study shows that the intermediate-molecular-weight complexes remain resistant to microbial attack even after prolonged irradiation and also appear to be resistant to photochemical attack, possibly indicating similar mechanisms of resistance.

More than half of the radioactivity in the fraction containing low-molecular-weight photoproducts is associated with constituents that are presumably carboxylic acids. ^{14}C-labeled keto acids were not present in dark-exposed or sunlight-irradiated samples. Aliphatic and aromatic acids are known photoproducts of fulvic acid (9). Sunlight irradiation of dissolved organic matter in seawater has been shown to result in the formation of keto acids (16) as well as aldehydes and ketones (20).

Synthetic organic compounds and products of microbial degradation are incorporated into humic acid during humification, forming what is known as a bound residue (6, 13). It has been proposed that the formation of humic acid-bound residues be used as a means for the in situ disposal of hazardous wastes in contaminated soils (5, 23). The fate of humic acid-bound synthetic compounds is of concern because of the potential for these compounds, many of which are environmental toxicants, to be released in the free form and thus pose environmental risks. Photolysis of dissolved organic matter has previously been shown to enhance microbial degradation (10, 26). Our results, which are in agreement with these observations, further demonstrate that the loss of absorbance in the solar UV range is associated with photolysis of [^{14}C]GLY-HA complexes, which leads to enhanced biodegradation. Thus, although the microbial conversion of high- to low-molecular-weight humic acid is slow, the comparable photochemical reaction is rapid and provides substrates that microorganisms use readily.

Humic acid and humic acid-bound residues can be transported into surface water by erosion. They also may be leached from soils into groundwaters (21), which then move into surface waters. In lake water, as much as 96% of the solar radiation with the necessary energy to photolyze [^{14}C]GLY-HA complexes can penetrate through the top 1 m of the water column, depending on the degree of coloration of the water (12). Physical mixing processes can transport the photoproducts deeper in the water column, increasing the volume in which photolytic reactions can affect subsequent microbial degradation. Thus, the joint action of sunlight and microorganisms can potentially control the fate of synthetic compounds complexed to humic acid in surface waters.

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