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Inhibitory Effect of Solar Radiation on Amino Acid Uptake in Chesapeake Bay Bacteria

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The effect of solar radiation on a natural bacterial population from the Chesapeake Bay was evaluated from measured changes in numbers of organisms engaged in amino acid uptake. From July through May, freshly collected water samples were exposed in quartz containers to 3.5 h of total sunlight both with and without UV-absorbing filters. Water samples were subsequently incubated with tritiated amino acids, and the uptake-active bacteria were assayed by microautoradiography-epifluorescence microscopy. The survival index, defined as the fraction of the uptake-active population that remained active after the exposure to sunlight, ranged from 0.93 to 0.20. Decreased survival was correlated with increased solar intensity. The inhibition of amino acid uptake was attributed not only to the UV-B component of the solar spectrum (280 to 320 nm), but also to longer UV and visible wavelengths.

Concern over the destruction of the ozone layer has prompted studies on the impact of increased solar UV light on aquatic ecosystems (4, 22). UV light that reaches the earth is classified into the two components, UV-A (400 to 320 nm) and UV-B (320 to 280 nm). The latter is considered the most biologically detrimental portion of the solar spectrum.

In natural bodies of water, various factors influence the depth of light penetration. UV-B is absorbed within a few centimeters by suspended inorganic material, humus, and microorganisms (11). However, even with rapid attenuation, UV-B that does penetrate surface water is lethal to a variety of aquatic microorganisms (4). Using light-absorbing filters, Grigsby and Calkins (8) observed that UV-A and visible radiation had lethal effects on coliforms in secondary effluent from a sewage treatment facility. Earlier studies with filtered sunlight (20) showed that *Serratia marcescens* was sensitive to the spectral range of 281 to 480 nm. The longer UV and visible wavelengths inactivate various mechanisms, e.g., excision repair, recombination (17), and active transport (19, 23), whereas shorter UV wavelengths cause lethal DNA lesions (16, 23). Exposure to total sunlight is the most detrimental because of the probable synergistic action of combined wavelengths (4, 24).

Resistance to sunlight in a natural, mixed bacterial population is heterogeneous. Thomson et al. (22) exposed a continuous culture of estuarine bacteria to simulated sunlight with supplemented UV-B. They found an initial decrease in the total viable count, followed by a gradual increase in the percentage of pigmented cells and an increase in heterotrophic respiration, all of which were attributed to the survival and proliferation of organisms resistant to solar radiation. Bacteria isolated from different depths in a lake gave a range of responses to artificial UV-B (4). Some organisms were destroyed, others were resistant, and some were inhibited but were able to resume normal growth after a prolonged lag phase. Allochthonous microorganisms from limnetic and terrestrial sources, and pathogens of enteric origin in particular, have been shown to be solar-radiation sensitive in estuarine or marine waters (7). Kapuscinski and Mitchell (12) determined that *Escherichia coli* isolated from coastal waters sustained damage to the catalase system during sun exposure, which resulted in sensitivity to peroxide formation.

Although the lethal effects of solar radiation have been well documented, quantitative studies of natural bacterial populations are limited (22, 25). Microautoradiography combined with epifluorescence microscopy (14, 21) can be a useful tool to investigate the effects of an environmental parameter on metabolically active organisms. The method offers several advantages over conventional techniques that use growth as a criterion for viability. A nonselective radiolabeled substrate is incorporated into a large percentage of the total population where a variety of aquatic bacteria have diverse nutritional requirements (10). Thus, a reliable estimate of the metabolically active fraction can be obtained from the determination of substrate uptake by single

cells. The addition of only trace concentrations of substrates with a high specific activity needed to observe the active organisms imposes a minimal modification of in situ conditions. Microautoradiography-epifluorescence microscopy was used in this study to determine whether solar radiation, including the total spectrum, total UV, and UV-B components, significantly inhibits its amino acid uptake in a population of Chesapeake Bay bacteria.

MATERIALS AND METHODS

Sampling. Samples were taken from the Chesapeake Bay, 4 km off Chesapeake Beach, Md. (38°41'N, 76°30'W). The water depth was approximately 10 m, and there was strong tidal mixing. The salinity ranged from 11 to 22‰ during the experimental period (Table 1). Samples were collected monthly from July 1980 through May 1981 at approximately 10:00 a.m. Samples were drawn from a depth of 8.5 m with a hand vacuum pump through Teflon tubing and collected in 1-liter Teflon bottles. Surface samples were drawn at an average depth of 6 mm by suction through a hole in the underside of a floating rubber tube closed at the end and attached to a 1-liter Teflon bottle. All bottles and tubing were clean and sterile. Water temperatures were recorded, and salinities were determined with a refractometer. Samples were returned to the laboratory in an insulated container within 40 min.

Experimental design. A 100-ml volume of sample was introduced into sterile quartz tubes (35 cm by 2.2-cm inside diameter). Tap water was circulated through an outer quartz jacket (37 cm by 4.2-cm inside diameter) to prevent overheating during exposure and to maintain the temperature as close as possible to the in situ temperature (Table 1). The tubes were placed against a blackened plywood background at an angle approximately perpendicular to the sun and exposed to sunlight from 11:30 a.m. to 3:00 p.m. on a bright sunny day. Samples incubated in quartz tubes wrapped in aluminum foil served as unexposed controls. For samples taken during February, March, April, and May, additional surface and 8.5-m samples were exposed to sunlight filtered by Mylar film (0.13 mm; Du Pont Co., Wilmington, Del.) to absorb wavelengths of <320 nm (screening out UV-B) and by a UF-4 Plexiglas sheet (3.2 mm; Rohm and Haas Co., Philadelphia, Pa.) to absorb wavelengths of <400 nm (screening out all UV light).

Incident solar UV-B measurements. During the sun exposure period, solar UV-B was measured with a Robertson sunburn ultraviolet meter (3) connected to a recorder. The measurement was expressed as sunburn units (see Fig. 1), a biologically weighted measure of UV-B (3). One sunburn unit is approximately equal to the amount of sun necessary to cause erythema in a fair-skinned Caucasian within 1 h. This meter provides a practical method for measurement of the most biologically damaging component of solar radiation (4). Although UV-B can vary independently of other components (UV-A and visible light), the UV-B intensity is generally observed to be correlated to the total solar intensity (1, 5). The total intensity was not measured in this study.

Microautoradiography-epifluorescence microscopy. After 3.5 h of exposure to sunlight, duplicate 10-ml samples of Chesapeake Bay water were removed from each tube and pipetted into sterile 250-ml Erlenmeyer flasks. The samples were then treated with a labeled substrate, 0.1 μl of ^{3}H-amino acid mixture (35.87 mCi ml^{-1}; Amersham Corp., Arlington Heights, Ill.), to give a final activity of 0.1 μCi ml^{-1} and a final concentration of 2.8 μg liter^{-1}. The samples were incubated for 2 h at the in situ temperature on an orbital shaker (100 rpm) and then fixed with 0.4 ml of filtered (0.2-μm pore size) Formalin. Samples treated with the labeled substrate immediately upon return to the laboratory (T_0) served as controls to determine activity in the Chesapeake Bay at the approximate time of sampling and to determine activity loss due to experimental handling. Surface and 8.5-m samples were also fixed immediately with 0.4 ml of filtered Formalin for 1 h and then treated with the labeled amino acid mixture to estimate abiotic absorption and to ensure that all cells were inactivated at the Formalin concentration used. After fixing, all samples were stored overnight at 3°C.

For the first three monthly samples, the microautoradiographic technique of Meyer-Reil (14) was used. One-milliliter aliquots of labeled samples were diluted to 10 ml in filtered (0.2-μm pore size) artificial seawater adjusted to the appropriate salinity. Diluted samples were filtered through 25-mm Nuclepore polycar-

TABLE 1. Environmental parameters in the Chesapeake Bay

Date	Depth (m)	Salinity (‰)	Temp (°C)	
			In situ	Experimental
1980				
July	0.006	12.4	23.3	25.0
	8.5	13.9	23.1	
August	0.006	11.6	28.3	29.2
	8.5	13.4	27.2	
September	0.006	17.1	24.8	25.0
	8.5	18.4	24.6	
October	0.006	17.5	12.6	24.0
	8.5	17.5	13.6	
December	0.006	18.1	ND[a]	10.0
	8.5	20.2	4.9	
1981				
February	0.006	22.2	−0.8	10.0
	8.5	21.4	−0.9	
March	0.006	17.6	5.0	10.0
	8.5	17.6	5.5	
April	0.006	11.2	12.2	16.0
	8.5	12.7	12.3	
May	0.006	11.2	16.0	20.0
	8.5	11.5	16.0	

[a] ND, Not determined.

bonate membranes (0.2-μm pore size) prestained with irgalan black (9). The filters were rinsed twice with 10 ml of artificial seawater to remove extraneous label. The filters were cut in half, and each half was mounted cell-side-up onto a gelatin-coated slide. After drying, each slide was dipped in diluted (1:3) Kodak NTB-2 liquid photographic emulsion, dried, and placed in a darkened container for 3 days of radiographic exposure. Development followed a procedure described previously (21).

For the last six monthly samples, filters were mounted according to two modified procedures described by Tabor and Neihof (21). In the first modification, the filters were mounted onto gelatin-coated slides cell-side-down, air dried for 30 min, and then slowly peeled off the slides, leaving the cells embedded in the gelatin. The slides were then covered with liquid photographic emulsion, dried, and exposed for 3 days. In the second modification, no gelatin coating was used on the slides, and the filters were applied cell-side-down directly onto a slide coated with liquid photographic emulsion. After drying, radiographic exposure, developing, and staining, the filters were removed, leaving the cells embedded in the emulsion. These modifications, especially mounting the filter directly onto the emulsion, yielded up to 10 to 20% higher counts of labeled organisms and total counts than did the Meyer-Reil method. This was due to the improved visual clarity of the final preparation and the elimination of subjective elements associated with microautoradiographic counts. A detailed comparison of the different methods has been made by Tabor and Neihof (21).

Developed autoradiograms were stained in filtered acridine orange solution (1:2,500 in citrate buffer, pH 6.6 [14]). To minimize background fluorescence during the subsequent microscopic examination, all slides were destained for ca. 6 min each in filtered citrate buffers at pH 6.6, 5.0, and 4.0 consecutively, followed by a distilled water rinse. The timing was adjusted to destain the filter and gelatin or emulsion without excessive destaining of the cells themselves. Filters that had been applied directly onto the emulsion were removed after staining and destaining.

All slides were examined by both epifluorescence and transmitted light microscopy. A Zeiss microscope (Standard 14) was fitted with an XBO 150 W xenon burner, a red suppression filter (BG 38), a Calflex heat filter, a 510-nm reflector, and a 520-nm barrier filter (Zeiss filter combination no. 487709BI). A Planachromat 100/1.25 objective and KPL ×12.5/20 eyepieces gave a ×1,250 magnification. Under the epifluorescence illumination, red-orange cells were seen against a greenish-gray background. Cells were evenly distributed across the filter, and about 400 cells, or 6 to 10 fields of a 14- by 14-division graticule (sample area, 70 by 70 μm), were counted.

Activity determination. The total count was obtained by enumeration of the fluorescing bacteria. Under bright-field illumination, the same field showed aggregations of silver grains around the bacteria which were active in uptake of labeled amino acids. Percent activity was determined from the ratio of cells with associated silver grains to the total count. Background grains, determined from the water samples fixed before being treated with the labeled substrate, were subtracted from all grain counts but were less than 2% of the total number of active cells and so did not constitute a significant correction.

RESULTS AND DISCUSSION

Effects of solar radiation on amino acid uptake activity. In all samples, bacteria in the exposed tubes consistently exhibited lower activity than did the dark controls. Activity in the dark controls was considered comparable to activity in the Chesapeake Bay at the time of sampling, since the dark controls at 3.5 h after collection did not show a significant change from T_0 samples, except for the May surface sample ($P < 0.05$; Table 2).

Seasonal trends in the percentages of active organisms were observed for both dark controls and exposed samples. Activity reached a maximum of 87.1% in July (8.5 m), declined in December to 8.7% (8.5 m), and rose again in the spring (Table 2).

Percent activity in the exposed samples was significantly lower than that in the dark controls, except in December and February, which apparently had lower percent activity but not at the significance level of $P < 0.05$. To determine the fraction of the active population that remained active in amino acid uptake after 3.5 h of sun exposure, the ratio of the exposed and dark control activities was computed and termed the survival index: % activity in exposed/% activity in dark control = survival index. Survival indices from July to May and UV-B intensities expressed in sunburn units for the 3.5-h exposure during each monthly experiment are presented in Fig. 1. Solar intensity peaked at 7.5 sunburn units in July, and the survival index reached the lowest values of 0.21 (surface) and 0.10 (8.5 m) in August. As sunburn units decreased to minimum values during the winter months, the survival index rose to maximum values of 0.93 and 0.84 for February surface and 8.5-m samples, respectively. Based on these results, survival appeared to be a function of UV-B intensity. Since both parameters were random variables and subject to measurement error, a model II linear regression analysis was employed to test the negative relationship (2, 18). The regression coefficients for both depths are significant at the level of $P < 0.05$.

A comparison of the seasonal activity (T_0, Table 2) and the survival index suggests that the pattern of response to solar stress in the experiment may have also been due to the physiological status of bacteria in the Chesapeake Bay at the time of sampling, as well as to the changing solar intensity. Laboratory studies have shown that organisms are more sensitive to UV light during active stages of growth (6, 15). In an estuarine system, transient ameliorative or detrimental effects caused by unmeasured parame-

TABLE 2. Total counts, counts of uptake-active organisms, and percent activity for Chesapeake Bay bacteria exposed to sunlight

Date	6 mm[a]					8.5 m[a]				
	T_0[b] % activity	Exposure	Total count[c]	Active bacteria[c]	% Activity	T_0[b] % activity	Exposure	Total count[c]	Active bacteria[c]	% Activity
1980										
July	60.0	Dark	9.5	6.0	63.0	76.8	Dark	8.5	7.4	87.1
		Exposed	5.6	1.0	18.0		Exposed	5.3	1.7	32.1
August	10.0	Dark	8.4	1.1	13.0	59.0	Dark	7.1	4.0	56.3
		Exposed	7.4	0.2	2.7		Exposed	5.6	0.3	5.4
September	ND[d]	Dark	13.6	5.7	41.9	72.8	Dark	19.9	15.6	78.4
		Exposed	11.7	1.3	11.1		Exposed	17.9	10.2	57.0
October	36.3	Dark	19.7	8.0	40.7	46.0	Dark	17.4	9.3	53.5
		Exposed	19.5	2.9	14.9		Exposed	18.6	4.1	22.2
December	5.6	Dark	12.9	1.0	7.8	5.7	Dark	12.7	1.1	8.7
		Exposed	12.9	0.6	4.7		Exposed	11.3	0.6	5.3
1981										
February	20.7	Dark	7.5	2.1	28.0	27.5	Dark	4.9	1.5	30.6
		Exposed	4.9	1.3	27.0		Exposed	4.3	1.1	25.6
March	34.2	Dark	6.7	2.1	31.3	39.8	Dark	5.8	2.2	37.9
		Exposed	8.2	1.8	22.0		Exposed	5.4	0.9	16.7
April	21.7	Dark	4.3	1.0	23.3	47.8	Dark	5.2	2.8	53.9
		Exposed	4.2	0.20	4.8		Exposed	5.2	1.1	21.2
May	65.0	Dark	8.6	3.7	43.0	58.0	Dark	6.3	3.0	47.6
		Exposed	5.7	0.6	10.5		Exposed	5.7	0.3	5.3

[a] Sampling depth.
[b] Percent activity in the Chesapeake Bay at the time of sampling.
[c] Expressed as 10^6 counts ml^{-1}.
[d] ND, Not determined.

ters, e.g., freshwater influx, tidal flushing, and vertical mixing, would affect the composition and activity and hence the solar resistance of the bacterial population at the time of sampling.

No correlation between the depth of sample and the survival index was found; however, active microorganisms were not always homogeneously distributed in the water column. T_0 and dark control surface samples frequently showed a lower percent activity than did 8.5-m samples (Table 2). Kjelleberg and Håkansson (13) attributed the inhibition of surface organisms to the enrichment of photooxidation products refractory to bacterial degradation.

Effect of UV-absorbing filters on survival. Figure 2 shows the effect of light-absorbing filters on the survival index. In February, the protective effect of the filters was negligible because the solar intensity was low. In March, April, and May, the bacteria protected from UV-B by the Mylar filter maintained a higher survival index than did organisms exposed with no filter, demonstrating that UV-B had a detrimental effect. In addition, the decline in the Mylar survival index from February to May indicated that wavelengths of >320 nm were increasingly detrimental. The highest survival index was observed for

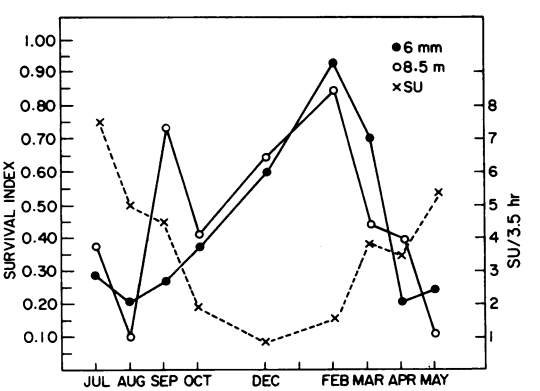

FIG. 1. Comparison of survival index and UV-B intensity measured in sunburn units (SU).

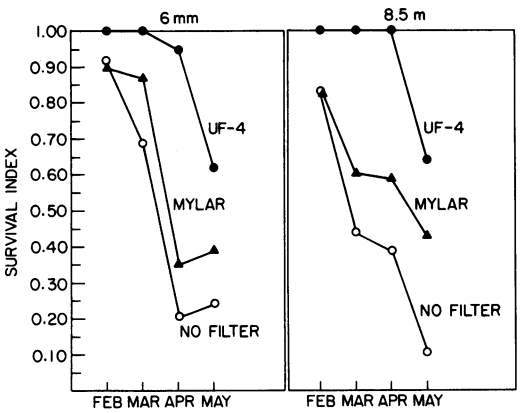

FIG. 2. Effect of light-absorbing filters on survival index. Mylar absorbs wavelengths of <320 nm, and UF-4 absorbs wavelengths of <400 nm.

samples protected by the UF-4 filter. In May the UF-4 survival index dropped, providing further evidence that visible as well as long UV wavelengths were increasingly inhibitory as the total solar intensity approached a maximum at the summer solstice. Previous studies with *E. coli* (19) demonstrated that long UV and visible wavelengths affect nonspecific membrane permeability and also damage active uptake systems for specific amino acids.

Numerous cell processes are affected by sunlight, and the measurement of amino acid uptake has been shown to be a sensitive method for detecting solar stress. When extrapolating the results of this study to the field, certain considerations should be taken into account. In the Chesapeake Bay, high productivity and continuous mixing promote a turbidity which reduces UV-B to 1% of the surface value at a depth of 28 cm (4), and turbulence allows a minimal exposure time at the surface. The relatively prolonged exposure under the experimental conditions resulted in a possible overestimate of the photoinhibition of individual organisms than would occur in the Chesapeake Bay. The experiment more closely approximated conditions in a less turbulent marsh environment. Our results indicate that solar radiation is a factor influencing metabolic activity in natural aquatic bacterial populations.

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