Inhibition of Growth of the Green Alga *Chlorella pyrenoidosa* by Unsaturated Fatty Acids

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ABSTRACT

The activity of a number of unsaturated fatty acids in inhibiting the growth of the green alga *Chlorella pyrenoidosa* has been studied using the paper disk-agar plate method. Acrylic and undecylenic acids were highly active, but other ω-unsaturated fatty acids tested were only weakly active. Oleic and elaidic acids were inactive at 0.2 M, but other C_{18}-C_{22} acids with 2 to 6 double bonds were generally active down to a concentration of 0.01-0.005 M. Activity with chaulmoogric acid was noted down at 0.002-0.001 M. Diffusion rates of the acids through the agar seem to affect the size of the inhibition zones. The implications that polyunsaturated fatty acids may play a role in algal ecology are discussed.

INTRODUCTION

In a previous paper we reported that of the saturated straight chain fatty acids, those between C_6 and C_{12} were the most effective in inhibiting the growth of the green alga *Chlorella pyrenoidosa* in a paper disk assay system (1). Limited experiments with unsaturated fatty acids showed that acrylic acid was extremely active in inhibiting *Chlorella* growth and that undecylenic acid also was very active. This paper reports on further work which has been done with unsaturated fatty acids. The possible implications of unsaturated fatty acids in algal ecology also are discussed.

MATERIALS AND METHODS

The acrylic and vinylacetic acids were purchased from Aldrich Chemical Co., Milwaukee, Wisconsin. The 4-pentenoic, 5-hexenoic and chaulmoogric acids were from ICN K and K Laboratories, Plainview, New Jersey. The crotonic, dimethylacrylic, sorbic, oleic (99%), elaidic (99%), ricinoleic (99%), linoleic (99%), linolelaidic, α-linolenic (99%), γ-linolenic (99%), 11,14,17-eicosatrienoic (99%), 8,11,14-eicosatrienoic (99%), arachidonic (99%), 5,8,11,14,17-eicosapentaenoic (90%) and 4,7,10,13,16,19-docosahexaenoic (99%) acids were bought from Sigma Chemical Co., St. Louis, Missouri.

The assays were carried out by placing paper disks (6 mm [¼ inch]) sterile disk, Difco Laboratories, Detroit, Michigan), to which 20 μl of fatty acid solution had been applied, onto *Chlorella*-seeded agar Petri plates and observing zones of growth inhibition, as previously described (1). Activity was recorded as diameter of net zone of inhibition, which was obtained by subtracting the diameter of the disk (6 mm) from the total diameter of the inhibition zone (mm). The plates were seeded with *Chlorella pyrenoidosa* (strain 395 recently obtained from the Culture Collection of Algae, Department of Botany, University of Texas, Austin, Texas). This strain has been shown to behave similarly to a strain maintained at the University of New Hampshire and used in previous work (2).

RESULTS AND DISCUSSION

The results of the assays are shown in Table I. Results of disk assays undoubtedly are affected by solubility and diffusion rates through the agar. In our system the pH is 6.7, which is considerably higher than the pKa's of fatty acids (ca. 4.9). The acids would exist mostly in their anionic forms and be more soluble in an aqueous system. Diffusion rates vary inversely as the radius of the molecule (3). Although the larger acids tested all have similar molecular weights, the number and configuration of double bonds undoubtedly will have an effect on the conformation of the molecule and on micelle formation and, therefore, on their diffusion rates. With several of the acids tested (chaulmoogric, linoleic) the zones of inhibition stayed constant over a 4-fold dilution range before decreasing, indicating that diffusion probably is an important factor, especially when trying to quantitate the results. However, as long as diffusion takes place at all, inhibition or non-inhibition can be detected.

As shown previously (1) for *C. pyrenoidosa* (UNH strain), both acrylic acid and undecylenic (10-undecenoic) acid were found to be very active in inhibiting the growth of *C. pyrenoidosa* (strain 395). Since these are both ω-1 unsaturated acids (counting from the non-carboxyl end), other ω-1 acids were tested. Neither vinylacetic (3-butenoic), 4-pentenoic, or 5-hexenoic acids showed much activity, indicating that high activity was not a property of all ω-1 acids. 4-Pentenoic acid has been shown to be a biologically active compound in that it inhibits the growth of the protozoan *Tetrahymena* (4). Other short chain acids, dimethylacrylic acid and the doubly unsaturated sorbic acid, showed slight activity. Sorbic acid is used as a fungistatic agent in foods. Undecylenic acid also is a widely used fungistatic agent. It is of interest that ricinoleic acid (12-hydroxy-9-octadecenoic acid) also was quite active. Ricinoleic acid is the principal fatty acid of castor bean oil and gives rise to the highly active undecylenic acid on heating.

A number of C_{18}-C_{22} unsaturated fatty acids were tested. The *cis*-monounsaturated oleic acid and its *trans*-isomer elaidic acid both were inactive at the highest level tested (0.2 M). However, doubly and more highly unsaturated acids showed inhibition down to about 0.01-0.005 M. In comparing the *cis,cis*-9,12-linoleic acid with its *trans, trans* isomer linolelaidic acid, the latter appeared to be less active. Although the doubly unsaturated linoleic acid may have been slightly less active than the more highly unsaturated fatty acids, the presence of 3, 4, 5 and 6 double bonds did not seem to make much difference in activity. These results are in agreement with the fact that *cis* and polyunsaturated fatty acids generally have greater antimicrobial activity than *trans* and monounsaturated fatty acids (5,6). Although chaulmoogric acid never showed large zones of inhibition, inhibition was noted at lower concentrations than were observed with the other fatty acids. This may be due to a restricted diffusion of an otherwise highly active molecule. Chaulmoogric acid is a major constituent of chaulmoogra oil, which formerly was used widely in the treatment of leprosy. Certain of the unsaturated fatty acids tested in this study thus appear to have significant activity in inhibiting the growth of *Chlorella*.

Many of the marine algae of the various algal divisions are known to produce substances which have antimicrobial activity (7,8). These substances occur in several types of chemical structures: terpenes and their halogenated derivatives, other halogenated aliphatic compounds, phenols and

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TABLE I

Activity of Unsaturated Acids in Inhibiting the Growth of *Chlorella pyrenoidosa*

Name	Structure	Concentration (M)[a]							
		0.2	0.1	0.05	0.02	0.01	0.005	0.002	0.001
		(Diameter, net zone of inhibition (mm)[b])							
Acrylic	CH_2=CHCOOH	49	43	38	27	20	15	8	0
Crotonic	CH_3CH=CHCOOH*(trans)*	6	0						
Vinylacetic	CH_2=$CHCH_2$COOH	5	0						
Dimethylacrylic	$(CH_3)_2$C=CHCOOH	6	4	0					
4-Pentenoic	CH_2=$CH(CH_2)_2$COOH	6	4	0					
5-Hexenoic	CH_2=$CH(CH_2)_3$COOH	5	0						
Sorbic	CH_3CH=CHCH=CHCOOH*(trans)*	7	7	4	0				
Undecylenic	CH_2=$CH(CH_2)_8$COOH	30	27	24	19	11	10	7	0
Oleic	Δ^9-C_{18} *(cis)*	0							
Elaidic	Δ^9-C_{18} *(trans)*	0							
Ricinoleic	12–OH–Δ^9-C_{18} *(cis)*		10	10	7	5	0		
Chaulmoogric	$(CH_2)_2$CH=CH–$CH(CH_2)_{12}$COOH (ring)				4	3	4	2	±[c]
Linoleic	$\Delta^{9,12}$-C_{18} *(cis)*	8	8	9	5	3	0		
Linolelaidic	$\Delta^{9,12}$-C_{18} *(trans)*	12	2	0					
α-Linolenic	$\Delta^{9,12,15}$-C_{18} *(cis)*	26	21	4	0				
γ-Linolenic	$\Delta^{6,9,12}$-C_{18} *(cis)*				4	4	0		
$\Delta^{11,14,17}$-Eicosatrienoic (C_{20}, *cis*)					7	7	±	0	
$\Delta^{8,11,14}$-Eicosatrienoic (C_{20}, *cis*)					7	5	0		
Arachidonic	$\Delta^{5,8,11,14}$-C_{20} *(cis)*			15	10	5	0		
$\Delta^{5,8,11,14,17}$-Eicosapentaenoic (C_{20}, *cis*)					9	5	3	0	
$\Delta^{4,7,10,13,16,19}$-Docosahexaenoic (C_{22}, *cis*)					7	6	±	0	

[a]Acrylic, crotonic and vinylacetic acids were dissolved in water, the others in ethanol. Solvent had little effect.

[b]Total diameter of inhibition zone less disk diam. The figures represent a mean of all disks run on 2 or more separate occasions using Petri dishes containing 3-4 disks each. In this system standard deviations run 6-20% of the mean for means of 2-5 mm, and 1-10% of the mean for means >5 mm.

[c]± indicates net inhibition zones of <2 mm.

their halogenated derivatives, sulfur compounds, fatty acids, peptides and nucleosides. It is believed that these substances may function as allelopathic or chemical defense agents to help ensure the dominance or survival of a particular species. Reecently it has been reported that, in fresh water systems, the sequence of blue-green algal blooms during the course of a season is due to substances produced by the succession of algae (9,10).

One of the above classes of compounds with antimicrobial activity which occurs in algae is made up of unsaturated fatty acids and their photooxidation products (8 [review], 11,12). Of particular interest to this report are those which also inhibit the growth of algae. Acrylic acid, which is an effective antibiotic (13) and occurs in Rhodophyta, Chrysophyta, Phaeophyta and Chlorophyta (14,8), strongly inhibits the growth of *Chlorella* (2,1). Chlorellin, an antibiotic produced in *Chlorella* cultures which also autoinhibits the growth of *Chlorella* itself (15), is attributed to the photooxidation products of unsaturated fatty acids (16). Proctor (17) showed that linoleic acid was more effective than oleic acid in inhibiting the growth of certain algae. He also showed that the eventual domination of the green alga *Chlamydomonas reinhardi* over the green alga *Haematococcus pluviales* in a mixed culture of the 2 algae appeared to be due to substances which were lipoidal in nature and probably fatty acids. All of the algal divisions contain large amounts of highly unsaturated fatty acids (18). Noteworthy is the occurrence of large amounts of 5,8,11,14,17-eicosapentaenoic acid in the oils of most of the divisions. This acid has been shown to develop strong antibiotic activity on irradiation (19). In the present report, this acid has been found to inhibit the growth of *Chlorella*. Thus, algae produce unsaturated fatty acids, and their growth is inhibited by some of these same compounds. There is a body of circumstantial evidence which suggests that these compounds might participate in affecting algal ecology.

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REFERENCES

1. McGrattan, C.J.; J.D. Sullivan Jr. and M. Ikawa, J. Phycol. 12:129 (1976).
2. Sullivan, J.D. Jr., and M. Ikawa, J. Agric. Food Chem. 20:921 (1972).
3. Cooper, K.E., In "Analytical Microbiology," edited by F. Kavanaugh, Academic Press, New York, 1963, p. 1.
4. Liang, T.; G.J. Rangl and J.J. Blum, J. Protozool. 23:186 (1976).
5. Nieman, C., Bacteriol. Rev. 18:147 (1954).
6. Kabara, J.J., In "The Pharmacological Effect of Lipids," edited by J.J. Kabara, American Oil Chemists' Society, Champaign, IL, 1978, p. 1.
7. Aubert, M.; J. Aubert and M. Gauthier, In "Marine Algae in Pharmaceutical Science," edited by H.A. Hoppe, T. Levring and Y. Tanaka, Walter de Gruyter, Berlin, 1979, p. 267.
8. Glombitza, K.-W., Ibid., p. 303.
9. Keating, K.I., Science 196:885 (1977).
10. Keating, K.I., Ibid. 199:971 (1978).
11. Akhunov, A.A.; S.D. Gusakova, T. Taubaev and A.V. Umarov, Khim. Prir. Soedin (3):1978; Chem. Abstr. 89:144944.
12. Gauthier, M.J.; P. Bernard and M. Aubert, Ann. Microbiol. (Paris) 129B:63 (1978); Chem. Abstr. 89:144943.
13. Sieburth, J.M., Science 132:676 (1960).
14. Sieburth, J.M., Develop. Indust. Microbiol. 5:124 (1964).
15. Pratt, R.; T.C. Daniels, J.J. Eiler, J.B. Gunnison, W.D. Kumler, J.F. Oneto, L.A. Strait, H.A. Spoehr, G.J. Hardin, H.W. Milner, J.H.C. Smith and H.H. Strain, Science 99:351 (1944).
16. Spoehr, H.A.; J.H.C. Smith, H.H. Strain, H.W. Milner and G.J. Hardin, In "Fatty Acid Antimicrobials from Plants," Carnegie Institution of Washington (Publication 586), Washington, DC, 1949.
17. Proctor, V.W., Limnol. Oceanogr. 2:125 (1957).
18. Pohl, P., and F. Zurheide, In "Marine Algae in Pharmaceutical Science," edited by H.A. Hoppe, T. Levring and Y. Tanaka, Walter de Gruyter, Berlin, 1979, p. 473.
19. Pesando, D., Rev. Intern. Oceanogr. Med. 25:49 (1972).

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