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STEROLS AND FATTY ACIDS OF AN ANTARCTIC SEA ICE DIATOM, *STAURONEIS AMPHIOXYS*

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Abstract—Unialgal clonal cultures of the diatom *Stauroneis amphioxys* Gregory, isolated from sea ice of the Indian Ocean sector of the Southern Ocean, were grown at 3° and 20°. The relative abundances of fatty acids, sterols and phytol for the two cultures are comparable. The two sterols observed [24-methylcholesta-5,22E-dien-3β-ol (79%) and cholesterol (21%)] did not vary with culture temperature. The major fatty acid composition is typical of most diatoms. A pronounced change of ratio with temperature occurred with the pair 16:4 Δ6, 9, 12, 15 and 16:3 Δ6, 9, 12 followed by 18:4 Δ6, 9, 12, 15:18:3 Δ9,12,15 and 20:5 Δ5,8,11,14,17:20:4 Δ8,11,14,17; thus the relative abundances of 16:4, 18:4, 20:5 and 22:6 increase at the lower growth temperature. The total amounts of unsaturated acids do not change with temperature suggesting an effect on the final desaturase step. No cryoprotective role for such changes in lipid composition was inferred.

INTRODUCTION

The existence of an algal microflora associated with the sea ice in Antarctica was first reported by J. D. Hooker [1] who noted diatoms associated with brash and pancake ice. Taxonomic studies on the diatoms found in the ice were performed by Hendy [2] and Hart [3] and primary productivity measurements were reported by Bunt and Wood [4] and Bunt [5]. Our own work (McFadden, G. and Wetherbee, R., unpublished results) has identified several diatom species in the sea ice capable of growth over very broad temperature and salinity ranges. Interestingly, growth studies indicated that cell division rates were not enhanced at the higher temperatures under the light conditions used in this study.

Previous reports [7–9] indicating increased unsaturation in the fatty acids of various organisms at lowered growth temperatures, suggested that a study of the lipids of an Antarctic sea ice diatom at two growth temperatures would provide insight into a possible cryoprotection mechanism operable in the membranes of such algae. In this report, we analyse in detail the fatty acids and sterols of the diatom, *Stauroneis amphioxys* at growth temperatures of 3° and 20°.

RESULTS AND DISCUSSION

The relative abundances of fatty acids, sterols and phytol for two cultures of *S. amphioxys* differing only in growth temperature are shown in Table 1. The values reported are similar to those reported for other diatoms in studies by Orcutt and Patterson [10] (sterol: fatty acid *ca* 0.02:1.0) and Volkman *et al.* [11] (phytol: sterol: fatty acid *ca* 0.02:0.03:1.0), although the abundance of phytol is appreciably higher than that in the latter study. No studies on *S. amphioxys* were available for comparison. All the lipid fractions examined in this report were obtained by solvent extraction. No sterols and only a trace of fatty acids (less than 0.2% solvent extractable fatty acids) were obtained by saponification of the residue left after solvent extraction, indicating complete lipid extraction. This contrasts with the results of Dubinsky and Aaronson [12] who found incomplete extraction of certain algal lipids using our solvent system.

Only two major sterols were evident by capillary GC, although the small quantity of lipid available precluded detection of components with abundances less than 0.5% of the total sterols. The sterol structures were identified by computerised capillary GC/MS. The two cultures gave almost identical distributions, having *ca* 79% of 24-methylcholesta-5,22E-dien-3β-ol as the major component. This sterol is found in many diatoms: it was found in seven of the 11 species analysed by Orcutt and Patterson [10], being the predominant sterol in five (*Nitzschia closterium, N. ovalis, N. frustulum, Navicula pelliculosa* and *Phaeodactylum tricornutum.*) Similarly, it has been observed to be a major sterol in a mixed diatom culture from an intertidal sediment taken from S.E. Victoria, Australia [13], and in total plankton samples from temperate and tropical waters ([14] and Gillan, F. T. and Johns, R. B., unpublished results). NMR studies on this sterol [10, 15] in other diatoms have indicated that the 24-methyl group has the *S*(α) configuration in contrast to the 24-methyl sterols of green algae which have the *R* configuration. It is probable that the 24-methyl group also has the *S* configuration in *S. amphioxys*, though our techniques were unable to distinguish the two epimers.

Table 1. Relative abundances of various lipid classes in *Stauroneis amphioxys*

Growth temperature	Fatty acids	Sterols	Phytol
3°	1.0	0.015	0.05
20°	1.0	0.010	0.05

The only other sterol identified was cholest-5-en-3β-ol (cholesterol) (21%) which is also common in diatoms. The sterol analysis of *S. amphioxys* is thus quite similar to that of *N. frustulum* (42% cholest-5-en-3β-ol, 54% 24-methylcholesta-5,22E-dien-3β-ol [10]) and shows some similarities to other *Nitzschia* species. In contrast, the sterol composition of *Amphora*, *Fragilaria*, *Thallasiosira* and *Skeletonema* spp. [10, 22] is completely different. The taxonomic significance of sterols in diatoms has not been adequately explored.

The fatty acid analyses of the two cultures are reported in Table 2. The use of a polar capillary column has allowed the resolution and tentative identification of several polyunsaturated fatty acid isomers not commonly reported in diatoms (e.g., 16:2 Δ7,10). The major acids observed are typical of most diatoms [10, 11, 16, 17] although the level of 16:4 Δ6,9,12,15 was unusually high in the 3° culture, and 16:3 Δ6,9,12 was quite abundant in the 20° culture. The similar quantities of 20:5 Δ5,8,11,14,17 in the two cultures suggest that growth phase effects on the fatty acid distribution, as observed by Volkman *et al.* [11], were not operative in this work. Both cultures were harvested during logarithmic growth phase. Thus, the observed changes in fatty acid composition should result from temperature effects alone.

Table 2. Fatty acids of *Stauroneis amphioxys*

Fatty acid	3° Culture	20° Culture
12:0	0.47	0.48
13:0	TR*	TR
14:0	4.94	5.60
14:1 Δ7	TR	0.09
15:0	0.68	0.26
16:0	11.00	10.78
16:1 Δ3TR	1.14	1.08
16:1 Δ7	0.57	0.28
16:1 Δ9	20.60	20.31
16:2 Δ6, 9	0.57	0.34
16:2 Δ7, 10	0.06	0.08
16:2 Δ9, 12	6.01	5.14
16:3 Δ6, 9, 12	3.35	11.05
16:3 Δ7, 10, 13	0.11	0.07
16:4 Δ6, 9, 12, 15	6.59	1.31
17:0	0.21	0.11
18:0	2.39	1.38
18:1 Δ9	2.00	1.49
18:1 Δ11	0.67	0.70
18:2 Δ9, 12	1.05	1.07
18:3 Δ6, 9, 12	0.12	0.50
18:3 Δ9, 12, 15	0.86	2.22
18:4 Δ6, 9, 12, 15	4.98	2.79
20:0	0.11	0.06
20:1 Δ11	0.11	0.08
20:1 Δ13	0.05	0.08
20:4 Δ5, 8, 11, 14	TR	1.88
20:4 Δ7, 10, 13, 16	0.12	0.36
20:4 Δ8, 11, 14, 17	0.13	0.63
20:5 Δ5, 8, 11, 14, 17	24.20	26.06
22:0	0.18	0.11
22:5 Δ7, 10, 13, 16, 19	TR	0.16
22:6 Δ4, 7, 10, 13, 16, 19	3.23	1.91
23:0	0.13	0.10
24:0	0.08	0.09
25:0	0.07	0.11
26:0	TR	0.14
27:0	TR	0.12
28:0	ND†	0.12
29:0	ND	0.03
30:0	ND	0.10

*TR: <0.05%.
†ND: Not detected (<0.01%).

The most pronounced change in fatty acid ratios occurred with the biosynthetically related pair 16:4 Δ6,9,12,15:16:3 Δ6,9,12 (Table 3), the ratio changing from 1.97 at 3° to 0.12 at 20° although the total amount of the two acids is virtually constant at *ca* 10% of the total acids. Analyses of other diatoms have yielded ratios between 0.0 [18] and 1.4 [11], but the amount present was typically less than 4%. The 18:4 Δ6,9,12,15:18:3 Δ9,12,15 and 20:5 Δ5,8,11,14,17:20:4 Δ8,11,14,17 ratios were also observed to change markedly. The changes in these cases were only by a factor of *ca* 4.6 (cf. × 16 above). The almost identical chain length distribution (Table 4) indicates that the chain elongase enzymes are not markedly affected by temperature, only specific desaturases show marked effects. In all cases, the desaturases affected are those involved in the final desaturation step leading to the most unsaturated acid of the particular chain length. Thus the relative abundances of 16:4 Δ6,9,12,15; 18:4 Δ6,9,12,15; 20:5 Δ5,8,11,14,17 and 22:6 Δ4,7,10,13,16,19 are increased at lower growth temperature, compared to the corresponding precursors. It is of interest to note that the calculated iodine numbers (INs) for the fatty acid Me ester mixtures are the same (within experimental error) (IN = 103) for the two growth temperatures. The absence of increased unsaturation indicates that temperature-sensitive desaturases are probably not important in the thermoprotection of this diatom species. The calculated INs are similar to those calculated for other diatom species: for example, *Asterionella japonica* IN = 107 (Gillan, F. T. and Johns, R. B., unpublished results) and *Biddulphia sinensis* IN = 80 [11]. It is probable that the diatom lipids are sufficiently unsaturated to maintain membrane fluidity over a wide temperature range without modification of the unsaturate distribution. Thermal tolerance limits presumably derive from some other biochemical feature.

Most of the wide range of unsaturated acids reported in these analyses are readily explicable in terms of a Δ9-desaturase, a Δ ± 3 desaturase (causing desaturation at a position ±3 from an already present double bond) and a chain elongase with low activity for mono-unsaturates. The small quantities of Δ7-C_{16} isomers cannot, however,

Table 3. Ratios of biosynthetically related fatty acids in *Stauroneis amphioxys*

Ratios	3° Culture	20° Culture
16:4 Δ6, 9, 12, 15: 16:3 Δ6, 9, 12	1.97	0.12
18:4 Δ6, 9, 12, 15:18:3 Δ9, 12, 15	5.79	1.26
20:5 Δ5, 8, 11, 14, 17:20:4 Δ8, 11, 14, 17	186	41.6
22:6 Δ4, 7, 10, 13, 16, 19:22:5 Δ7, 10, 13, 16, 19	large	15.9

Table 4. Chain length distributions (as a %) of fatty acids of *Stauroneis amphioxys*

Culture temperature	20°	3°
Σ14*	5.46	4.94
Σ16	49.21	50.00
Σ18	9.89	12.07
Σ20	28.52	24.72
Σ22	1.97	3.41

* Σn = total percentage of fatty acids of carbon number = n.

be explained by this process. These isomers are typical of Chlorophyceae [17, 19, 20] and have been used as specific markers for green algae in sediments [21]. This result suggests some caution should be exercised in such interpretations. The major C_{16} isomers are formed by Δ9-desaturation followed by desaturation at Δ9 + 3 or Δ9 − 3 followed by further Δ ± 3 desaturation. Whether one or more desaturase enzymes is involved or not is not known, though Moreno *et al.* [18] suggested at least six separate desaturases in the biosynthesis of unsaturated acids in the diatom *Phaeodactylum tricornutum*. The C_{18} mono-unsaturates presumably arise from direct desaturation (18:1 Δ9) and desaturation of 16:0 and subsequent chain elongation (16:0 → 16:1 Δ9 → 18:1 Δ11) as previously proposed for *Biddulphia sinensis* [11]. This analysis further extends the number of reports of vaccenic acid (18:1 Δ11) in microscopic algae.

EXPERIMENTAL

Uni-algal clonal cultures of *S. amphioxys* Gregory were isolated from sea ice of the Indian Ocean sector of the Southern Ocean (67° 30'S, 60° 51'E) during February 1979. Experimental material was grown under identical conditions, one at 3° and another at 20°. Cells were illuminated with 1000 lx standard white fluorescent illumination for a 16:8 hr light: dark cycle in "*f*/2" media [9] and were harvested by millipore filtration in prewashed glass fibre filters (GF/C, Whatman) during log-phase growth. The cells were extracted ×2 with $CHCl_3$–MeOH (2:1) to produce a total solvent extract, and the residue from extraction was saponified (5% KOH in MeOH (aq.) (80%)). The solvent extract was partitioned with H_2O and the organic layer concd to a small vol. An aliquot was saponified (as above) and the neutral lipids were extracted ×2 with $CHCl_3$–*n*-heptane (1:4) from the basic soln. After acidification, the acidic compounds were extracted ×2 with $CHCl_3$–*n*-heptane (1:4). The neutral lipids were converted to TMSi ethers (with BSTFA) and analysed by capillary GC on a SCOT SE-30 column (45m × 0.5mm N_{eff} = 60000, SGE Australia) (carrier gas: He at 20 cm/sec, 140–290°, 2.5°/min). The acids were converted to Me esters with BF_3–MeOH [23] and analysed on the non-polar column (above) and on a capillary SIL-47-CNP column (see [24]). The sterol structures were confirmed by GC/MS.

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