Fatty Acids of Acanthamoeba Sp.

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The biochemistry of amebas is an essentially unexplored field. What studies there have been are almost exclusively concerned with Amoeba proteus and Amoeba chaos, neither of which is optimally suited to most biochemical investigations for they can be grown only in relatively small quantity and must feed on another organism. Acanthamoeba sp., a soil ameba, was isolated by Neff (1) and cultured in the axenic state on a soluble medium of proteose-peptone and glucose. Subsequently, it was found by Adam (2) that Acanthamoeba will grow on a chemically defined medium containing 18 amino acids and several vitamins. Since the organism can be grown in submersions culture (3), there is no limit to the quantity of cells that can be obtained.

Acanthamoeba, therefore, would seem to be ideally suited to any type of biochemical study. The amebas can be handled experimentally like, and offer many of the advantages of, other microbial organisms. Because they are animal cells, it may be anticipated that thorough study of amebas will be of particular utility in furthering our understanding of those aspects of mammalian biochemistry that appear to be unique to animals. One such area is the metabolism and function of certain polyunsaturated fatty acids.

This paper reports the composition of the fatty acids of Acanthamoeba. It has been found that the organism contains a particularly high concentration of the unsaturated fatty acids 20:1(11), 20:2(11,14), 20:3(8,11,14), and 20:4(5,8,11,14). Thus, Acanthamoeba would seem a useful organism for the study of the biosynthesis of these acids, and, hopefully, of their function, an apparently essential one in higher animals.

**EXPERIMENTAL PROCEDURE**

Culture Techniques Acanthamoeba sp. was obtained from Dr. R. J. Neff and cultured, as described by him (3), on a sterile medium of: proteose-peptone (Difco Laboratories), 15 g per liter; glucose, 15 g per liter; MgSO4, 10−4 M; CaCl2, 10−5 M; FeCl3, 10−6 M; KH2PO4, 3 × 10−3 M; L-methionine, 10−4 M; vitamin B12, 1 µg per liter; biotin, 200 µg per liter; and thiamine-HCl, 1 mg per liter, adjusted to pH 5.5 with HCl. Subcultures were maintained in 10 ml of medium in a 50-ml Erlenmeyer flask, making transfers (0.2 ml) every 2 weeks. Larger quantities of cells were grown in 500 ml of medium in a 3-liter Fernbach flask with shaking, or in deep submergence with aeration. The fatty acid composition did not seem to be influenced by the method of growth. It was not found necessary to coat the container with silicone grease (3) as the amebas did not adhere to the glass surfaces. Cultures were grown routinely at room temperature (22-24°) but were also grown at 15° and 30°.

**Isolation of Fatty Acids**—After 14 days of growth, the amebas were collected by low speed centrifugation, washed twice with 0.15 M NaCl and extracted overnight with 19 volumes of chloroform-methanol, 2:1. The mixture was filtered, and the residue was washed with chloroform. The lipid extract was evaporated to dryness on a rotary evaporator under reduced pressure at 40°, the residue was extracted with chloroform, and the solvent then removed at room temperature under a stream of nitrogen. In one experiment, approximately 100 mg of lipid were obtained from 700 mg of lipid-free dry residue.

The lipid was then saponified for 3 hours at 80° in 5% NaOH in 50% methanol. The cooled solution was extracted three times with petroleum ether. These washes were discarded. The solution was then acetylated with H2SO4 and the fatty acids extracted with petroleum ether. The solvent was removed under a stream of nitrogen at lower than room temperature, and the fatty acids were converted to their methyl esters by heating at 80° for 2 minutes in 1 ml of BF3-methanol reagent (Applied Science Laboratories, Inc.). An equal volume of water was added, and the methyl esters of the fatty acids were extracted with petroleum ether. The solution was concentrated and analyzed by gas-liquid chromatography.

The procedure just described was used to obtain all the data presented in this paper. In other instances, the amebas were saponified directly, without prior extraction of the lipids, and the rest of the procedure followed as described. Methyl esters of the fatty acids have also been prepared by transesterification of the original lipid extract in 5% H2SO4 in methanol. In all cases, the analytical results were the same as those reported in this paper.

Individual fatty acids were isolated by preparative gas-liquid chromatography. Usually, this was done with the entire mixture of methyl esters. The isolations of 20:1 and 18:2, however, were facilitated by first separating the saturated, monounsaturated, and polyunsaturated fatty acids as their mercuric acetate adducts, and then chromatographing each class separately with gas-liquid chromatography.

**Formation of Mercuric Acetate Adducts**—Adducts were prepared according to the method of Mangold (4) and separated by chromatography on silicic acid (5). Saturated fatty acids, which do not form adducts, were eluted with benzene; adducts of monounsaturated fatty acids were eluted with diethyl ether; and adducts of polyunsaturated fatty acids were eluted with 5% acetic acid in methanol. After evaporation of the solvents at
low pressure, the adducts were decomposed in 0.6 N HCl at
room temperature overnight, and the methyl esters of the fatty
acids were extracted into petroleum ether. Each fraction was
analyzed by gas-liquid chromatography.

Hydrogenation—The fatty acid methyl esters were dissolved
in heptane, PtO₂ catalyst was added, and a stream of H₂ bubbled
through the solution for 2 minutes at room temperature. The
products were analyzed by gas-liquid chromatography.

Iodine Uptake—Iodination was performed on the methyl esters
of the fatty acids in glacial acetic acid with mercuric acetate
catalyst according to the procedure of Smits (6) in which the
uptake of I₂ is determined by the decrease in absorbance at
357 mp. The molar concentration of each unsaturated fatty
acid methyl ester was calculated from the area of the peak ob-
tained on gas-liquid chromatography of an aliquot of the solu-
tion to be iodinated. The entire method was standardized with
pure methyl arachidonate. The iodine taken up by as little as
5 μg of arachidonate can be determined with accuracy.

Oxidative Degradation—Before oxidation, the individual fatty
acid methyl esters prepared by preparative gas-liquid chromato-
graphy were freed of the small amount of ethylene glycol succin-
cinate polyester with which they were contaminated. The
esters were saponified in 5% NaOH in 50% methanol, the mixture
acidified, and the fatty acids extracted into petroleum ether.
If this is not done, succinate, derived from the liquid phase of
acidified, and the fatty acids extracted into petroleum ether.

Gas-Liquid Chromatography—Analytical gas-liquid chromato-
graphy of the methyl esters of the long chain fatty acids was
performed on a column (5 ft × 6 mm) of 17% ethylene glycol succinate polyester on Chromosorb W, 80 to 100 mesh.
The temperature was 196° and the input pressure 10 p.s.i. of argon.
An argon ionization detector was used. Proportionality of peak
area to mass was confirmed with known mixtures of standard
saturated and unsaturated fatty acids (Applied Science Labora-
tories, Inc.).

Preparative gas-liquid chromatography was done on either
17% ethylene glycol succinate polyester or 20% ethylene
glycol adipate polyester on Chromosorb W, 80 to 100 mesh, in
a column (5 ft × 10 mm) at 196° and an argon input pressure
of 25 p.s.i. The effluent stream was split, approximately 1% going
to the detector and the remainder to the collecting outlet where
the methyl esters of the fatty acids were trapped on Celite
contained in a glass cartridge held in a fraction collector. The

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Relative retention time</th>
<th>Percentage composition (mass)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>15°</td>
</tr>
<tr>
<td>14:0</td>
<td>0.33</td>
<td>10</td>
</tr>
<tr>
<td>16:0</td>
<td>0.67</td>
<td>14</td>
</tr>
<tr>
<td>16:1</td>
<td>0.67</td>
<td>6</td>
</tr>
<tr>
<td>18:0</td>
<td>1.0</td>
<td>3</td>
</tr>
<tr>
<td>18:1</td>
<td>1.17</td>
<td>32</td>
</tr>
<tr>
<td>18:2</td>
<td>1.43</td>
<td>6</td>
</tr>
<tr>
<td>20:1</td>
<td>2.03</td>
<td>1.4</td>
</tr>
<tr>
<td>20:2</td>
<td>2.51</td>
<td>8.2</td>
</tr>
<tr>
<td>20:3</td>
<td>3.08</td>
<td>8.2</td>
</tr>
<tr>
<td>20:4</td>
<td>3.48</td>
<td>11.0</td>
</tr>
</tbody>
</table>

* Retention times on ethylene glycol succinate polyester (see
text) relative to 18:0.
† Cells grown at the temperatures indicated in stationary
cultures of 50 ml for 14 days.

RESULTS
A typical chromatogram of the methyl esters of the fatty
acids of Acanthamoeba grown at 20–22° is shown in Fig. 1. The
relative retention times are given in Table I. Approximately
80% of the fatty acids are unsaturated, and particularly notable
is the content of unsaturated 20 carbon acids. In another
experiment, acanthamoebas were grown at 15°, 24°, and 30°
in stationary cultures. Except for the temperature, conditions
of growth were identical in each flask; inoculums were from the
same subculture. It can be seen (Table I) that when grown at the
higher temperature Acanthamoeba contains relatively less of
the 16-0, 16:1, 18:2, and, particularly, 20:3 acids. The
relative concentrations of 18:0, 18:1, and 20:1 acids were all
appreciably greater at the higher temperature. The contrast
between the fatty acid composition of amebas grown at 24°
and 30° has been confirmed several times. Growth of amebas
was approximately the same at 24° and 30°, but only about 50%
as great at 15°.

Each of the fatty acids was characterized after isolation by
preparative gas-liquid chromatography. Except for 20:3 and
20:4, there was less than 1% contamination of the isolated fatty
acids by other fatty acids. There was approximately 4%
contamination of 20:3 by 20:4, and 20:4 was similarly con-
taminated by approximately 4% of 20:3.

The saturated fatty acids were identified by their relative
FIG. 1. Gas-liquid chromatogram of the methyl esters of the fatty acids of Acanthamoeba. Chromatography was on 17% ethylene glycol succinate polyester at 196° and 10 p.s.i. of argon.

Retention times compared to standards, by their inability to form mercuric acetate adducts (Fig. 2A), and by the identity of their retention times before and after subjection to the hydrogenation procedure. The saturated fatty acids found were 14:0, 16:0, and 18:0. A very small amount of 15:0 (cf. Fig. 2A) may have been present. Methyl esters of fatty acids shorter than 10:0 would not have been detected by gas-liquid chromatography under the conditions used.

Fatty acids 16:1(9), 18:1(9), and 18:2(9,12) (Table II) were initially identified by their retention times and by the retention times of the saturated fatty acids formed by hydrogenation. The mercuric acetate adducts of these three fatty acids behaved as expected after chromatography on silicic acid (Fig. 2B and C). The number of double bonds in each acid was confirmed by iodination (Table II). Oxidative degradation yielded only one monocarboxylic and one dicarboxylic acid, in the proper molar ratio, in each case. Malonic acid, which might be expected from carbon atoms 10 to 12 of 18:2 (9,12), is destroyed by periodate oxidation and therefore not recoverable (8, 9). Identifica-

![Retention time comparison graph](image)

**FIG. 2.** Gas-liquid chromatogram of the methyl esters of the saturated, monounsaturated, and polyunsaturated fatty acids of Acanthamoeba after separation of the classes by silicic acid chromatography of the mercuric acetate adducts. Chromatography was on 17% ethylene glycol succinate polyester at 196° and 10 p.s.i. of argon. In B, the sensitivity of the detector was increased at the arrow.

**Table II**

Identification of unsaturated fatty acids of Acanthamoeba

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Hydrogenation product</th>
<th>Is uptake</th>
<th>Products of oxidation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Monocarboxylic</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>pmole/µmole</td>
</tr>
<tr>
<td>16:1</td>
<td>16:0</td>
<td>1.7</td>
<td>Heptanoate</td>
</tr>
<tr>
<td>18:1</td>
<td>18:0</td>
<td>1.7</td>
<td>Nonanoate</td>
</tr>
<tr>
<td>18:2</td>
<td>18:0</td>
<td>1.7</td>
<td>Hexanoate</td>
</tr>
<tr>
<td>20:1</td>
<td>20:0</td>
<td>2.2</td>
<td>Nonanoate</td>
</tr>
<tr>
<td>20:2</td>
<td>20:0</td>
<td>2.2</td>
<td>Hexanoate</td>
</tr>
<tr>
<td>20:3</td>
<td>20:0</td>
<td>2.2</td>
<td>Hexanoate</td>
</tr>
<tr>
<td>20:4</td>
<td>20:0</td>
<td>4.0</td>
<td>Hexanoate</td>
</tr>
</tbody>
</table>
The unsaturated 20-carbon fatty acids were identified by a similar process. Standards were not available for 20:1, 20:2, and 20:3, but 20:4 had the same retention time as known methyl arachidonate. All four unsaturated 20 carbon acids were quantitatively converted to 20:0 upon hydrocyanation. On separation of the mercuric acetate adducts, 20:1 appeared in the monounsaturated fraction (Fig. 2B) and the other three unsaturated 20-carbon fatty acids in the polyunsaturated fraction (Fig. 2C). The number of double bonds in the three polyunsaturated acids was indicated by their relative retention times in gas-liquid chromatography and established by the results of microiodination (Table II). Upon oxidative degradation, each of the 20 fatty acid fatty acids yielded only one monocarboxylic acid and one dicarboxylic acid (Table II). Traces of glutarate were present in the oxidation products of 20:3 and traces of succinate in the oxidation products of 20:4 because of their slight contamination by each other.

The products of oxidation of the monounsaturated 20 carbon acid account for all of its carbon atoms and clearly establish its structure to be 20:1(11). The products of oxidation (hexanolate and undecanolate) of the diunsaturated 20 carbon fatty acid account for all but three of the carbon atoms, and its structure must, therefore, be 20:2(11,14). In the case of the trienoic acid, the identification of hexanolate and succinate as the only oxidation products places two of the three double bonds at positions 8 and 14. If the third double bond were at either position 10 or position 12, an equimolar amount of succinate would have been found among the products of oxidation. Therefore, the original acid must be 20:3(8,11,14). Similarly, the structure 20:4(9,8,11,14) for the tetraenoic acid is deducible from the number of double bonds in the molecule, and the presence of equimolar amounts of glutarate and hexanolate, but no succinate, in the products of oxidation. The destruction of malonic acid (8,9) accounts for the inability to recover 3,6, and 9 carbon atoms in the products of oxidation of 20:2(11,14), 20:3(8,11,14), and 20:4(5,8,11,14), respectively.

No evidence was found for the presence of either α-linolenate, 18:3(9,12,15) or γ-linolenate, 18:3(6,9,12). In the original chromatogram, there was no detectable peak in the region expected for 18:3(6,9,12). If 18:3(9,12,15) had been present, it might have overlapped 20:1(11). The products of oxidation of 20:1 contained a very small amount of azelaate, but this was most probably derived from contaminating 18:1(9) and 18:2(9,12).

**DISCUSSION**

The fatty acids of *Acanthamoeba* resemble qualitatively the fatty acids of higher animals. Specifically, the polyunsaturated fatty acids possess the divinylmethane-interrupted system of double bonds beginning 6 carbons from the methyl end of the molecule and include 20:4(5,8,11,14). The concentration of unsaturated 20 carbon acids, however, is unusually high. Although 20:4(5,8,11,14) (arachidonic acid) is of widespread occurrence, 20:1(11), 20:2(11,14), and 20:3(8,11,14) have been found only infrequently. All three are present in fish oils (10, 11), Klenk et al. (12, 13) have reported the presence of the monounsaturated acids in mammalian brain phospholipids, and Chang and Sweely (8) have identified 20:3(8,11,14) in adrenal lipids.

The composition of the fatty acids of *Acanthamoeba* is significantly altered by the temperature of growth. The total amount of saturated, monounsaturated, and polyunsaturated acids remains constant, but the concentrations of specific fatty acids change. There would appear to be no simple rationalization of these observations in terms of an adaptive advantage for the amebas.

It is also of interest that although both *Acanthamoeba* and the cellular slime mold ameba *Dicyostelium discoideum* contain a high percentage of unsaturated fatty acids, the fatty acids present in each are unrelated. *D. discoideum* (14) contains predominantly 18:1(11) rather than 18:1(9), and the unique diunsaturated fatty acids 16:2(5,9), 18:2(5,9), and 18:2(5,11). It has been suggested (15) that the cellular slime molds might have evolved from "solitary soil amebas capable of encystment," but this hypothesis is not supported by the composition of the fatty acids of the two examples studied, unless the alteration in fatty acids is related to the phylogenetic changes.

All of the fatty acids present in the *Acanthamoeba* were synthesized de novo. The medium was found to contain only traces of saturated fatty acids. It is apparent, therefore, that *Acanthamoeba*, like plants, synthesizes linoleate but, like higher animals, primarily 18:1(11) rather than 18:1(9), and the unique diunsaturated fatty acids 16:2(5,9), 18:2(5,9), and 18:2(5,11).

**SUMMARY**

The fatty acids of *Acanthamoeba* sp., a soil ameba that can be grown in axenic culture on soluble nutrients, have been characterized. The principle fatty acids are myristate, palmitate, 9-hexadecenoate, stearate, 9-octadecenoate, 9,12-octadecadienoate, 11-eicosenoate, 11,14-eicosadienoate, 8,11,14-eicosatrienoate, and 5,8,11,14-eicosatetraenoate. The relative concentrations of several of the fatty acids are significantly influenced by the temperature of growth.

**REFERENCES**

11. BAUDART, P., Bull. soc. chim. biol., 9, 222 (1942); 20, 440, 443 (1943); 11, 174 (1944).
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