The Fatty Acids of *Rhodopseudomonas* Particles

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(Received 19 January 1962)

Bloch *et al.* (1961) have shown in measurements of the mono-unsaturated fatty acids of several micro-organisms that vaccenic acid is the major acid in anaerobic growth, whereas oleic acid predominates in the aerobes.

Polyenoic fatty acids were found by Bloch *et al.* (1961) only in bacteria that used oxygen as the terminal electron acceptor. This suggested that such acids could be produced only in the presence of oxygen.

J. Lascelles & W. Bartley (unpublished work) have shown that most of the fatty acids of the particulate fraction of the photosynthetic bacterium *Rhodopseudomonas spheroides* are octadecenoic acids when the bacterium is grown aerobically in the dark or anaerobically in the light. This paper is concerned with the identity of the octadecenoic acids formed under these two conditions of growth, since under anaerobic conditions vaccenic acid might be expected and under aerobic conditions oleic acid.

EXPERIMENTAL

Gas chromatography. Fatty acid mixtures as methyl esters were analysed in a Pye argon gas chromatograph on an Apiezon L column or on glycol adipate at 200°.

Rhodopseudomonas spheroides N.C.I.B. 8253 was grown aerobically as described by Lascelles (1959). The anaerobic cells were grown in the same medium in completely filled glass-stoppered bottles incubated in the light as described by Lascelles (1956). The bacteria were harvested in a Sharples centrifuge and washed by suspending in a buffer, pH 7.65 (2.42 g. of tris and 8 ml. of 2N-hydrochloric acid made up to 500 ml.) and centrifuging again. The washed bacteria were again suspended in the buffer (500 ml.) and irradiated in a MSE-Mullard 500W ultrasonic generator for 30 min. The suspension was centrifuged at 20 000g for 15 min. to remove cell walls and intact cells. The supernatant was centrifuged at 100 000g for 75 min. and the deposit was suspended in water (10 ml.) and heated at 60° for 2 hr. with 10N-potassium hydroxide (20 ml.), water (30 ml.) and methanol (50 ml.). Water (400 ml.) was then added and the mixture was extracted with light petroleum (b.p. 40–60°; 3 × 100 ml.). The aqueous layer was acidified with 50% (v/v) sulphuric acid (25 ml.) and again extracted with light petroleum. These extracts were dried (magnesium sulphate) and evaporated to dryness at 20° under reduced pressure. The residue (38 mg. from the anaerobic and 47 mg. from the aerobic bacteria) was shown to contain 70–80% of an octadecenoic acid by methylation of a small portion of the material with diazomethane and gas chro-

matography of the esters. The other major constituents were stearic acid, palmitic acid, palmitoleic acid, myristic acid and lauric acid. The proportions of these acids were similar in the chromatophores of the aerobically and the anaerobically grown bacteria.

Oxidation of the fatty acids. The mixture of acids (30 mg.), water (40 ml.), potassium carbonate (300 μmoles), sodium metaperiodate (800 μmoles) and potassium permanganate (27 μmoles) was shaken at 20° for 20 hr. (Lemieux & Rudloff, 1955). The mixture was then acidified with 50% (v/v) sulphuric acid (1 ml.) and extracted with chloroform (10 ml.). The extract was washed with water (10 ml.), dried (magnesium sulphate) and evaporated to dryness under reduced pressure at 20°. The residue was treated with an excess of ethereal diazomethane, evaporated to dryness as before and examined on the gas chromatograph.

RESULTS

The oxidation products of vaccenic acid are undecane-1,11-dioic acid and heptanoic acid; those from oleic acid are nonoic acid and azelaic acid. With both aerobically and anaerobically grown cells, the gas chromatograms of the methyl esters of the octadecenoic acids and of the methyl esters of the oxidation products of these acids were very similar (Table 1). Four new compounds appeared on oxidation, indicated by two large and two small peaks present in the oxidized and absent from the unoxidized samples. The two large peaks were shown to be identical with dimethyl undecane-1,11-dioate and methyl heptanoate by comparison with authentic samples. The two small peaks were

Table 1. *Fatty acids of the particulate fraction from Rhodopseudomonas spheroides*

The fatty acids were separated and determined by gas chromatography. The values are for each peak area of the chromatogram expressed as a percentage of the total area of all peaks on the chromatogram.

Acid	Aerobic (%)	Anaerobic (%)
Decanoic	2.0	0.5
Lauric	2.5	1.5
Myristic	8.5	6.0
Palmitic	4.0	7.5
Hexadecenoic	2.0	2.0
Stearic	2.0	10.0
Octadecenoic*	79.0	72.0

* These figures include linoleic acid.

similarly shown to be methyl nonoate and dimethyl azelate.

Comparison of the areas under the peaks indicated that the octadecenoic acid in the anaerobically grown bacteria was approximately 90 % of vaccenic acid and 10 % of oleic acid, the corresponding proportions for the aerobically grown bacteria being 97 and 3 %.

The ratios of vaccenic acid to oleic acid arrived at by comparison of the areas of the dimethyl azelate and dimethyl undecane-1,11-dioate peaks were consistently lower than those obtained from comparison of the methyl nonoate and methyl heptanoate peaks. This was probably due to the presence of linoleic acid, which would give azelaic acid on oxidation. When the methyl esters of the unoxidized fatty acids of the anaerobically grown bacteria were chromatographed as described above, but with glycol adipate as the stationary phase, a peak corresponding to methyl linoleate (5 %) was obtained.

DISCUSSION

Bloch *et al.* (1961) showed that the biosynthesis of olefinic fatty acids in some micro-organisms was by two separate pathways. In most aerobes a direct desaturation of a fatty acid occurred to give the unsaturated fatty acid of the same chain length with the double bond invariably at C-9. Direct desaturation of the fatty acids greater than C₁₀ in chain length did not appear possible under anaerobic conditions. The anaerobic pathway was considered to involve the degradation of a hydroxyacyl-CoA intermediate to a β -unsaturated olefin, which could then be lengthened by C₂ units, as in the normal pathway of fatty acid synthesis, without a shift in the double bond with respect to the methyl end of the molecule. The fatty acid being lengthened therefore starts with the double bond at C-3, so that the lengthening of C₁₀ to C₁₈ results in the double bond's being at C-11 (vaccenic acid). It is only if C₁₂ is lengthened that oleic acid (double bond at C-9) can result.

The particulate material from *Rhodopseudomonas* is convenient for the study of the synthesis of monoenoic acids, since it contains large amounts of such acids. This enables the identification and measurement of the acids to be carried out without the complications of first isolating the monoenoic acids.

It might have been expected from the work of Bloch *et al.* (1961) that under conditions of anaerobic growth vaccenic acid would be the main product, whereas oleic would be formed under aerobic conditions. It is also arguable that growth in the light is equivalent to growth under aerobic conditions. It was therefore somewhat unexpected

to find that under both conditions of growth vaccenic acid was the product in approximately the same quantity. Presumably *Rhodopseudomonas* is a bacterium corresponding with *Escherichia coli* and *Pseudomonas fluorescens* (Bloch *et al.* 1961), which retains the anaerobic pathway of fatty acid synthesis when utilizing oxygen as final acceptor. However, a proportion of oleic acid was also found in both conditions of growth. This acid would presumably arise by the lengthening of an unsaturated C₁₂ unit as suggested by Bloch *et al.* (1961) or because under both anaerobic and aerobic conditions a desaturation of saturated fatty acids can occur. The latter view is supported by the finding of a small (5 %) amount of a polyenoic acid corresponding in its behaviour on the gas chromatograph to linoleic acid in anaerobically grown cells. On the basis of their investigations Bloch *et al.* (1961) suggested that the polyenoic acids would be formed only under aerobic conditions in micro-organisms.

SUMMARY

1. The lipids of the particulate fraction of *Rhodopseudomonas* contain largely (72–79 %) octadecenoic acid under both aerobic and anaerobic conditions of growth. Of the octadecenoic acids 90 % was vaccenic acid and 10 % was oleic acid under aerobic conditions of growth; anaerobically the values were 97 and 3 % respectively.

2. The fatty acids of the lipids of the anaerobically grown cell particles contained about 5 % of linoleic acid.

3. These results do not support the idea that the formation of monoenoic acids is by a different metabolic pathway under aerobic from that under anaerobic conditions. Nor do they support the idea that the synthesis of polyenoic fatty acids in micro-organisms requires the direct participation of oxygen.

We should like to thank Dr June Lascelles for growing the bacteria and for helpful criticism, and Professor Sir Hans Krebs, F.R.S., for advice and interest in the work. The work was aided by the Rockefeller Foundation and the National Institutes of Health, United States Public Health Service Grant no. A3369 to the Department of Biochemistry, University of Oxford. A.R.H. thanks the Medical Research Council for financial support.

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