THE STEREOSPECIFICITY OF DESATURATIONS OF LONG-CHAIN FATTY ACIDS IN CHLORELLA VULGARIS

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Recent research in this laboratory has shown (Harris et al.,1965(a), (b), 1967) that the green alga Chlorella vulgaris effects a series of direct desaturations of stearic acid to give the family of cis-unsaturated acids typical of photosynthetic tissue; oleic (9-octadecenoic), linoleic (9,12-octadecadienoic), and α-linolenic (9,12,15-octadecatrienoic) acids. The present communication reports the use of the racemic erythro- and threo- isomers of 9,10-dideutero-stearic, 12,13-dideutero-oleic and 15,16-dideutero-oleic acids and of the D- and L- enantiomers of 9-tritio- and 12-tritio-stearic acids as substrates for Chlorella vulgaris desaturation reactions. The results indicate that cis pairs of hydrogens are removed in the enzymic formation of these double bonds and that the hydrogens removed from the 9- and 12-positions, and therefore from the 10- and 13-positions, are of the D-configuration. This corroborates and extends the work of Schroepfer and Bloch (1965) who showed that the conversion of stearic to oleic acid by Corynebacterium diphtheriae involves the specific loss of the D-9- and D-10-hydrogens from the stearic acid molecule.

SYNTHESIS OF PRECURSORS

Deuterium-labelled precursors: The reduction of olefins with deuterium-labelled hydrazine has been shown to occur stereospecifically, with cis

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addition of deuterium (Corey et al.,1961). Thus, reduction of oleate and elaidate (cis- and trans-9-octadecenoate) with deuterated hydrazine hydrate ($N_2{}^2H_4 \cdot {}^2H_2O$) in deuterated methanol ($CH_3O{}^2H$) gave, respectively, erythro- and threo-9, 10-dideuterostearate. Partial reduction with deuterated hydrazine of linoleate and cis, trans (trans, cis)-linoleate gave, respectively, erythro- and threo-12,13-dideutero-oleates, and partial reduction of cis, cis- and cis, trans (trans, cis)-9,15-octadecadienoates gave, respectively, erythro- and threo-9,15-dideutero-oleates (Morris, to be published). The desired precursors were isolated from their positional and/or geometric isomers and from residual diene and stearate by low temperature argentation-TLC as described by Morris et al.(1966, 1967).

Tritium-labelled precursors: The D- and L-enantiomers of 9- and 12-tritiostearic acids were prepared from D-9-hydroxystearic acid (Baker and Gunstone, 1963) and D-12-hydroxystearic acid (Serck-Hanssen, 1958), essentially by the procedures described by Schroepfer and Bloch (1965). To each of these stereospecifically tritiated acids was added sufficient 1-^{14}C-stearic acid to give $^3H/^{14}C$ ratios of approximately 20:1.

INCUBATIONS AND ANALYSIS OF PRODUCTS

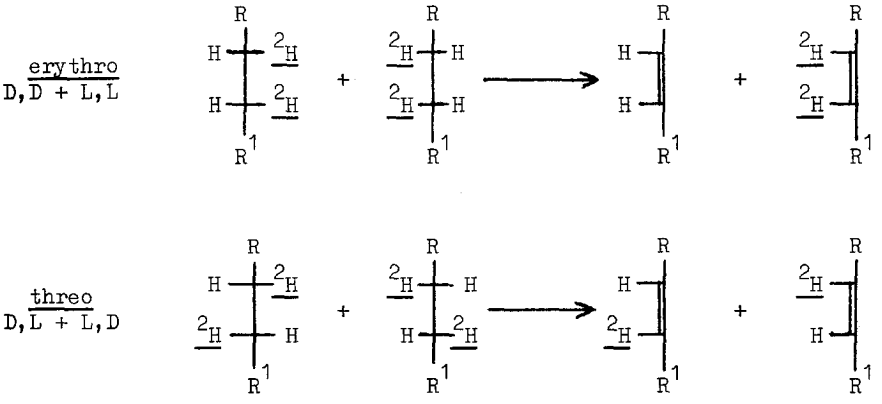
C. vulgaris cultures (strain 211/11b) were maintained, grown up and harvested as previously described (Harris et al., 1965(a),(b). For experiments on α-linolenic acid biosynthesis the cultures were grown up in the dark, otherwise light grown cells were used. Before the experiments with deuterated substrates, preliminary studies were carried out to ascertain the quantity of cells and substrates required to produce at least 2% of deuterated species in the total isolated oleate, linoleate or linolenate. This level of enrichment is desirable for accurate mass spectrometric analysis. Incubations in 0.2M phosphate buffer (pH 7.4) were carried out in the light for 5 hours (oleate or linoleate biosynthesis) or 24 hours (α-linolenate biosynthesis) and were then stopped by addition

of $CHCl_3$-MeOH (2:1). Fatty acid methyl esters were produced from the lipid extracts by transmethylation (Nichols et al., 1965).

The unsaturated methyl ester products (oleate, linoleate or α-linolenate), relevant to the labelled precursors added, were isolated and purified by preparative argentation-TLC and GLC and were then examined by mass-spectrometry or by scintillation counting, depending on the label present. The mass spectra of the products from deuterated substrates were obtained with an AEI MS12 instrument. Ten scans in each direction of the parent molecular ion region of each product were recorded, the intensities of the d_o, d_1 and d_2 parent molecular ion peaks measured, and the proportions of these species calculated. The products from tritiated substrates were counted in toluene containing 0.4% 2,5-diphenyloxazole in a Packard Tricarb Series 4000 Liquid Scintillation Spectrometer and $^3H/^{14}C$ ratios calculated.

RESULTS AND DISCUSSION

The following scheme illustrates the products obtained by stereospecific removal of cis-hydrogens from racemic erythro- and threo-dideutero precursors.



Thus, the erythro-substrate results in enrichment of dideuterated product while an enrichment of monodeuterated product is produced from the threo-substrate. Elimination of trans hydrogens from

these precursors, of course, would give the converse result.

The results of the mass spectrometric analysis of the product fatty acids are shown in Table 1, and demonstrate in each case the enrichment expected for removal of cis hydrogens. Thus, these desaturation reactions involve loss of pairs of hydrogen atoms of the same configuration on double bond formation at the 9,10-, 12,13- and 15,16- positions.

Table 1

Substrate	Product examined	%Monodeuterated species in product	%Dideuterated species in product
erythro-9,10-dideuterostearate	oleate	0.86	5.45
threo-9,10-dideutero-stearate	oleate	2.23	0.60
erythro-12,13-dideutero-oleate	linoleate	0.78	6.68
threo-12,13-dideutero-oleate	linoleate	6.80	0.80
erythro-15,16-dideutero-oleate	α-linolenate	0.68	6.20
threo-15,16-dideutero-oleate	α-linolenate	5.66	1.23

Typical results from incubations with the tritium labelled precursors are summarised in Table 2.

Table 2

Substrate	$^{3}H/^{14}C$ Ratio of substrate	Product examined	$^{3}H/^{14}C$ Ratio of product
D-9-tritiostearate	27.8	oleate	5.2
L-9-tritiostearate	31.6	oleate	25.5
D-12-tritiostearate	15.6	linoleate	2.2
L-12-tritiostearate	23.1	linoleate	21.1

The $^{3}H/^{14}C$ ratios of the products derived from the D-tritio precursors in both cases have fallen markedly, indicating almost complete loss of tritium from the D-9- and D-12-positions during the formation of the double bonds. In the case of the L-tritio precursors, however, the $^{3}H/^{14}C$ ratio has been largely maintained, showing that tritium in this configuration at these positions is retained.

It is therefore evident from this series of experiments that in the formation of the cis-double bonds of long-chain fatty acids, Chlorella

vulgaris, like Corynebacterium diphtheriae, exhibits extreme selectivity, specifically removing the D-9 and D-10 hydrogens during the formation of oleic acid and the D-12 and D-13 hydrogens during the formation of linoleic acid. Although the absolute configuration of the hydrogens lost at the 15 and 16 positions, during formation of α-linolenate, has not yet been determined, here again it has been shown that they are of the same configuration.

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