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ENZYMATIC FORMATION OF UNSATURATED LONG CHAIN FATTY ALDEHYDES IN *ULVA PERTUSA*

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Key Word Index—*Ulva pertusa*; Ulvaceae; marine green alga; flavour; aldehydes; fatty acids.

Abstract—(*Z*)-8-Heptadecenal, (*Z,Z*)-8, 11-heptadecadienal and 8,11,14-heptadecatrienal, were produced enzymatically from oleic, linoleic and linolenic acids respectively in the marine green alga, *Ulva pertusa*. The substrate specificity of the enzyme system for saturated fatty acids is in the order C_{14}, $C_{16} \geqslant C_{18}$. The unsaturated acids (C_{18} and C_{16}) were oxidized more readily than the saturated acids. Inhibitor studies showed that the enzyme system in the alga is a metalloprotein and similar to the α-oxidation system in cucumber fruits.

INTRODUCTION

Long chain aldehydes such as (*Z*)-8-heptadecenal (HD), (*Z,Z*)-8,11-heptadecadienal (HDD) and (*Z,Z,Z*)-8,11,14-heptadecatrienal (HDT), were first found in cucumber homogenates [1]. Recently, these aldehydes (HD, HDD, HDT) were identified in the green marine algae Ulvaceae [2].

Galliard and Matthew [3] have reported the biogenesis of saturated C_{15}, C_{14}, C_{13} and C_{12}-aldehydes from palmitic acid in cucumber fruits. However, the enzymatic formation of the unsaturated C_{17}-aldehydes (HD, HDD, HDT) has not been studied so far. This paper describes the enzymatic formation of the long chain aldehydes from fatty acids in a green alga *Ulva pertusa*.

RESULTS AND DISCUSSION

Enzymatic formation of long chain aldehydes

Long chain aldehydes such as HD, HDD and HDT are important contributors to the characteristic flavour of the essential oil of *U. pertusa*. In preliminary experiments, they were found to increase during incubations of unsaturated fatty acids with homogenates of the fronds, and acetone powder preparations from the homogenates were shown to retain this activity. Thus, enzyme solutions solubilized from acetone powders with 0.1% Triton X-100 were used as a source of the enzyme activity. The products, HD from oleic acid (OA), HDD from linoleic acid (LA) and HDT from linolenic acid (LNA), were identified by GC-MS analysis using synthetic (*Z*)-HD and (*Z,Z*)-HDD except for HDT. The aldehydes produced during the incubations were analysed by HPLC (Zorbax ODS) of the 2,4-dinitrophenylhydrazone (2,4-DNPH) derivatives quantitatively. HDD increased greatly (46 nmol from 4 nmol), when LA was incubated with the enzyme solution (10 ml) in phosphate buffer (pH 7.0) at 35° for 60 min (Fig. 1). Also HD and HDT were formed by incubations with OA and LNA, respectively (Fig. 1). With heat-treated suspensions, the increases were not observed. Tests of substrate specificity using the crude enzyme extract (Table 1) showed that LA and α-LNA were the best substrates. Saturated acids (C_{14}, C_{16}, C_{18}) were poor substrates, stearic acid being particularly resistant to attack. OA and palmitoleic acid were better substrates than the corresponding saturated fatty acids. Arachidonic acid was a poor substrate, and a particularly striking observation was that γ-LNA was a very poor substrate. From the results in Fig. 1 and Table 1, C_{n-1}-aldehydes were confirmed to be generated from C_n-fatty acids enzymatically.

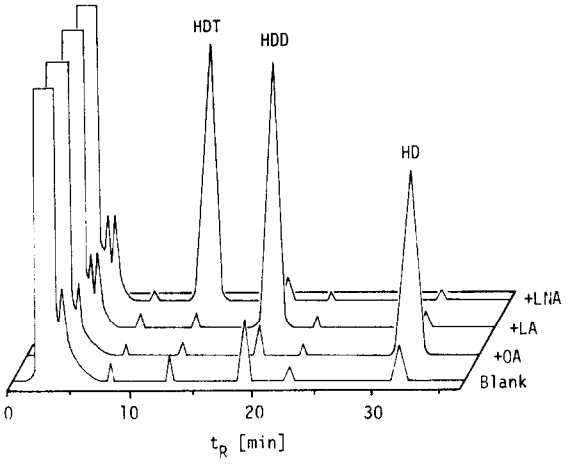

Fig. 1. Enzymatic formation of long chain aldehydes in a marine green alga *U. pertusa*. After 60 min incubations of the enzyme solution (10 ml) and substrates (20 mg each) at 35°, products were converted to the corresponding 2,4-DNPHs and were quantitatively analysed by HPLC (see Experimental).

Table 1. Substrates specificity of the enzyme preparations from *U. pertusa*

Substrate		Relative activity* [%]
Linoleic acid	(18:2)	100
Palmitoleic acid	(16:1)	67
Oleic acid	(18:1)	59
α-Linolenic acid	(18:3)	98
γ-Linolenic acid	(18:3)	12
Arachidonic acid	(20:4)	28
Myristic acid	(14:0)	43
Palmitic acid	(16:0)	42
Stearic acid	(18:0)	trace

*Activities are expressed relative in mole number to HDD obtained with linoleic acid. Enzyme activities were assayed by the same method as in Fig. 1.

Table 2. Inhibition effects on the enzyme solution from *U. pertusa* acetone powders

Additions*	Relative activity† [%]
None	100
Imidazole	43
L-Cysteine	49
KCN	19
NaN_3	20
DEDTC	34
EDTA	156
Peroxidase	26
Glucose oxidase	4

*1×10^{-4} M except for imidazole (5×10^{-4} M), peroxidase (0.05 mg/ml) and glucose oxidase (0.20 mg/ml). Enzyme activities were assayed by the same method in Table 1 except for additions using linoleic acid.

†Activities are expressed relative in mole number to HDD obtained from linoleic acid with no addition.

Properties of the long chain aldehyde-forming enzyme system

The pH optimum for aldehydes-forming activity from fatty acids was 7.0. The activity of acetone powder preparations was enhanced by addition of 0.1% Triton X-100. Under anaerobic conditions using glucose oxidase, the HDD-forming activity from LA was shown to be inhibited (*ca* 96%) as indicated in Table 2. When 0.5 mM imidazole was added to incubations of the enzyme solution and LA, a marked inhibition (*ca* 60%) was observed. Cysteine was also fairly inhibitory (Table 2). Certain heavy metal ligands such as NaCN, NaN_3 and diethyldithiocarbamate (DEDTC) showed strong inhibition (see Table 2), whereas another chelating agent, EDTA showed no inhibition. Stimulation (50%) of the oxidation was observed on addition of EDTA in phosphate buffer, as Galliard and Matthew [3] reported in the α-oxidation activity of cucumber fruits. These results suggest that this enzyme system in a green marine alga is a metalloprotein and is similar to the α-oxidation activity in cucumber fruits rather than to that formed in peanut cotyledons and pea leaves [4]. However, further purification of the enzyme system is necessary to establish whether or not the long chain aldehydes in marine algae are produced *via* a similar mechanism to α-oxidation in higher plants.

EXPERIMENTAL

Materials. *Ulva pertusa* fronds were collected from shallow water along the Aio coast of Yamaguchi, Japan, during April to June 1986.

Preparation of enzyme soln and enzymatic reaction. Fronds of *U. pertusa* (100 g) were homogenized in a mortar with 10-fold amount of Me_2CO at −20°. The homogenate was filtered and the Me_2CO discarded. The solid residue was washed with Et_2O and then dried *in vacuo*. The resulting powder (1 g) was suspended in 50 mM NaPi buffer, pH 7 (100 ml) containing 0.1% Triton X-100. After stirring for 30 min, the suspension was centrifuged at 10000 *g* for 10 min. The resulting supernatant was used as a crude enzyme soln.

Standard reaction condition. To OA, LA and LNA (20 mg each), respectively or none (blank), were added the enzyme soln and the mixtures were incubated at 35° for 60 min. The reaction mixtures were added to a hexane soln of 2,4-dinitrophenylhydrazine–HOAc (excess). The DNPHs were extracted with hexane and aliquots of the hexane soln were quantitatively analysed by HPLC: column Zorbax ODS 4.6 mm × 150 mm; solvent MeCN–H_2O–THF; 90:9:1 (v/v/v), flow rate 1 ml/min, pres. 100 kg/cm², UV detector 350 nm.

Identification of long chain aldehydes in incubations. The enzymatic reaction mixtures were subjected to steam distillation. The distillate was extracted with hexane, dried over Na_2SO_4 and then evapd *in vacuo*. The essential oils thus obtained, were analysed by GC and GC/MS.

GC was performed with a Hewlett Packard 5840A instrument, which was equipped with FID and a 0.2 mm (i.d.) × 50 m fused silica capillary column coated with SF-96. The column temp. was held at 70° for 5 min and programmed to increase 3°/min from 70 to 220°. GC/MS were recorded on a Hitachi M-80A instrument equipped with a 0.28 mm (i.d.) × 40 m fused silica capillary column coated with SF-96. The column temp. was programmed to increase from 75 to 190° at 3°/min (25 min hold at 190°) and then 190 to 210° at 3°/min. The ionization energy was 20 eV. The (*Z*)-HD and (*Z,Z*)-HDD were identified by comparison of retention times and their mass spectra with those of authentic aldehydes, which were synthesized by Wittig reaction between nonanyltriphenylphosphonium iodide or (*Z*)-3-nonenyltriphenylphosphonium iodide and 8-(2-tetrahydropyranyloxy)-octanal in THF and HMPA followed by removal of the protective group and subsequent Collins oxidation: *m/z* 248 (3.1%, M^+), 121 (12.5), 108 (41.6), 95 (60.4), 79 (100), 67 (68.8), 55 (27.1), and 41 (14.6), for HDT [1, 2, 5]; *m/z* 250 (8.3, M^+), 123 (12.5), 110 (20.8), 95 (58.8), 81 (95.8), 67 (100), 55 (47.9), and 41 (18.8) for HDD [1, 2, 5]; *m/z* 252 (3.1%, M^+), 121 (22.9), 109 (29.2), 95 (62.5), 83 (64.6), 69 (81.3), 55 (100), and 43 (41.6) for HD [1, 2, 5].

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