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STEREOCHEMISTRY IN THE FORMATION OF 9-HYDROXY-10,12-OCTADECADIENOIC ACID AND 13-HYDROXY-9,11-OCTADECADIENOIC ACID FROM LINOLEIC ACID BY FATTY ACID CYCLOOXYGENASE

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Summary

9-Hydroxy-10,12-octadecadienoic acid and 13-hydroxy-9,11-octadecadienoic acid are formed from linoleic acid upon incubation with the microsomal fraction of homogenates of the sheep vesicular gland (Hamberg, M. and Samuelsson, B. (1967) J. Biol. Chem. 242, 5344—5354. This communication is concerned with the stereochemical aspects of the conversion.

The ratio between the 9- and 13-hydroxy isomers was 77:23. Steric analysis of the individual isomers showed that the hydroxyl group of both isomers had mainly the L configuration, i.e. 9L:9D, 79:21 and 13L:13D, 78:22. Incubation of [11L-^{3}H; 1-^{14}C]linoleic acid led to the formation of 9- and 13-hydroxyoctadecadienoates which had largely lost the tritium label (6% and 7% retention of tritium relative to precursor, respectively) showing that the hydrogen which is removed from C-11 during the conversion has the L (pro-*S*) configuration.

The 'fatty acid cyclooxygenase' [1] or 'prostaglandin endoperoxide synthetase' [2], which catalyzes the formation of prostaglandins G_2 and H_2 from arachidonic acid, in addition catalyzes oxygenation of certain dienoic acids into monohydroxy acids [3—5]. Thus linoleic acid was converted in high yield into a mixture of 9-hydroxy-10,12-octadecadienoic acid and 13-hydroxy-9,11-octadecadienoic acid [3, 5]. Similarly, 11,14-eicosadienoic acid afforded 11-hydroxy-12,14-eicosadienoic acid [4]. This hydroxy acid was subsequently shown to have the L configuration [6].

Abbreviations: prostaglandin G_2, 15-hydroperoxy-9α,11α-peroxidoprosta-5,13-dienoic acid; prostaglandin H_2, 15-hydroxy-9α,11α-peroxidoprosta-5,13-dienoic acid; prostaglandin E_1, 11α,15-dihydroxy-9-ketoprost-13-enoic acid.

During studies on the absolute configuration of 9- and 13-hydroperoxy-octadecadienoates formed from linoleic acid by action of plant lipoxygenases [7] and during studies on the stereochemistry of the hydrogen removal in the conversion of 8,11,14-eicosatrienoic acid into 15L-hydroperoxy-8,11,13-eicosatrienoic acid [8] and prostaglandin E_1 [9] data on the stereochemistry of the oxygenation of linoleic acid by fatty acid cyclooxygenase were accumulated. Recent work on the stereochemistry of lipoxygenase catalyzed oxygenations of linoleic acid by Egmond et al. [10] and Van Os et al. [11] prompted us to report our data on the stereochemistry of the fatty acid cyclooxygenase catalyzed oxygenation of linoleic acid.

[1-^{14}C]linoleic acid (55 Ci/mol) was purchased from The Radiochemical Centre, Amersham, and diluted with unlabeled material to give a specific radioactivity of 0.1 Ci/mol. [11L-^{3}H;1-^{14}C]Linoleic acid was obtained during preparation of [13L-^{3}H; 3-^{14}C] 8,11,14-eicosatrienoic acid from [11L-^{3}H; 1-^{14}C]stearic acid as previously described [9]. A sample was diluted with unlabeled linoleic acid to give a specific radioactivity of 0.064 Ci of ^{3}H/mol and 0.02 Ci of ^{14}C/mol.

Homogenates of the sheep vesicular gland were prepared as previously described [5]. The microsomal fraction obtained from 10 g of vesicular gland was suspended in 25 ml of 0.1 M Tris-HCl buffer pH 7.4 containing 1 mM reduced glutathione and incubated with 2.5 mg of labeled linoleic acid at 30° C for 30 min. The product was extracted with diethyl ether (conversion into monohydroxy acids, 50—70%) and treated with diazomethane. Positional and steric analysis of methyl 9- and 13-hydroxyoctadecadienoates obtained from [1-^{14}C]linoleic acid was carried out as previously described [7]. The mixture of methyl 9- and 13-hydroxyoctadecadienoates obtained following incubation of [11L-^{3}H; 1-^{14}C]linoleic acid was subjected to catalytic hydrogenation using 5% palladium-on-carbon. The methyl 9- and 13-hydroxystearates thus formed were separated by thin-layer chromatography using diethyl ether/light petroleum (3:7, v/v) as the solvent system. ^{3}H/^{14}C-ratios were determined using a Packard Tri-Carb model 4322 liquid scintillation counter.

The results are given in Table I. As seen, 9L-hydroxy-10,12-octadecadienoic acid was the major hydroxyoctadecadienoic acid isomer formed from linoleic acid. This was in agreement with the mechanism postulated for the biosynthesis of prostaglandins [9, 4] in which the first step consists of formation of an 11L-peroxy derivative of the C_{20} precursor acid. Interestingly, the composition of hydroxyoctadecadienoic acid isomers (Table I) was similar to that found following incubation of linoleic acid with lipoxygenase-2

TABLE I

STEREOCHEMISTRY IN THE FORMATION OF HYDROXYOCTADECADIENOATES FROM LINOLEIC ACID BY ACTION OF FATTY ACID CYCLOOXYGENASE

Positional and steric analysis			Percentage retention of tritium* in	
9:13	9L: 9D 13L:13D	9L: 9D:13L:13D	9-OH-18:2	13-OH-18:2
77:23	79 :21 78 :22	61 :16 :18 : 5	6	7

*[11L-^{3}H; 1-^{14}C] Linoleic acid was used as the precursor.

from soybeans and peas at pH 9.0 [11].

The 9- and 13-hydroxyoctadecadienoates formed from [11L-^{3}H; 1-^{14}C]-linoleic acid by action of fatty acid cyclooxygenase had largely lost the tritium label (Table I). This finding taken together with the result of the steric analysis made it apparent that an antarafacial relation between hydrogen abstraction and oxygen insertion existed in the case of the fatty acid cyclooxygenase catalyzed oxygenation of linoleic acid, i.e. abstraction of the 11L-hydrogen resulted in the formation of 9- and 13-hydroxyoctadecadienoates both of which had the L configuration. Such a relationship between the stereochemistry of hydrogen removal and oxygen insertion has earlier been found in the oxygenation of 8,11,14-eicosatrienoic acid by soybean lipoxygenase (predominantly lipoxygenase-1) [8] as well as in the oxygenation of linoleic acid by soybean lipoxygenase (predominantly lipoxygenase-1) and corn lipoxygenase [10]. Also in the biosynthesis of prostaglandin E_1 from 8,11,14-eicosatrienoic acid there is an antarafacial relation between the hydrogen removal from C-13 and introduction of oxygen at C-11 and C-15 [9].

Studies on the stereochemistry of the platelet lipoxygenase catalyzed oxygenation of arachidonic acid [12] is in progress and will be reported at a later date.

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