On the mechanism of biosynthesis of leukotrienes and related compounds

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[10D-^{3}H; 3-^{14}C]- and [10L-^{3}H; 3-^{14}C]arachidonic acids were incubated with human polymorphonuclear leukocytes and with human platelets. Leukotriene B_4 and 5(*S*),12(*S*)-dihydroxy-6*trans*,8*cis*,10*trans*,14-*cis*-eicosatetraenoic acid (5,12-DHETE) were isolated and the $^3H/^{14}C$ ratios determined. It could be concluded that the 10D (*pro-R*)-hydrogen is eliminated in the conversion of 5(*S*)-hydroperoxy-6*trans*,8-*cis*,11*cis*,14*cis*-eicosatetraenoic acid into leukotriene A_4 whereas in the conversion of arachidonic acid into 5,12-DHETE the 10L (*pro-S*)-hydrogen is lost. Incubation of the doubly labeled arachidonic acids with human platelets confirmed and extended previous data on the stereochemistry of the hydrogen removal from C-10 during the conversion into 12(*S*)-hydroperoxy-5*cis*,8*cis*,10*trans*,14*cis*-eicosatetraenoic acid, i.e., the 10L (*pro-S*)-hydrogen is eliminated and the 10D (*pro-R*)-hydrogen retained.

Leukotriene A4 *5*(S),*12*(S)-*dihydroxy-6,8,10,14-eicosatetraenoic acid*
12(S)-*hydroperoxy-5,8,10,14-eicosatetraenoic acid* *Stereospecific hydrogen removal* *Isotope effect*

1. INTRODUCTION

Two reactions are involved in the formation of leukotriene A_4 (LTA_4) from arachidonic acid:

(1) A lipoxygenase reaction by which arachidonic acid is transformed into 5(*S*)-hydroperoxy-6*trans*,8*cis*,11*cis*,14*cis*-eicosatetraenoic acid (5-HPETE) [1];

(2) A dehydrase reaction in which the hydroperoxide is cyclized into 5(*S*)-*trans*-5,6-oxido-7*trans*,9*trans*,11*cis*,14*cis*-eicosatetraenoic acid (LTA_4) [2].

Several compounds are formed by further transformation of LTA_4; i.e., leukotriene B_4 (LTB_4, 5(*S*),12(*R*)-dihydroxy-6*cis*,8*trans*,10*trans*,14*cis*-eicosatetraenoic acid), 5(*S*),12(*R*)-dihydroxy-6*trans*,8*trans*,10*trans*,14*cis*-eicosatetraenoic acid, and 5(*S*),12(*S*)-dihydroxy-6*trans*,8*trans*,10*trans*,14*cis*-eicosatetraenoic acid as well as the amino acid containing leukotrienes LTC_4, LTD_4, and LTE_4 [2]. Human polymorphonuclear leukocytes have also been found to produce 5(*S*),12(*S*)-dihydroxy-6-*trans*,8*cis*,10*trans*,14*cis*-eicosatetraenoic acid (5,12-DHETE) [3,4]. This dihydroxy acid, although isomeric with LTB_4, is not formed from LTA_4 but by double dioxygenation of arachidonic acid. The compound is therefore not included in the leukotriene family.

This work is concerned with the stereochemistry of the hydrogen removal from C-10 of arachidonic acid during the biosynthesis of leukotrienes and of 5,12-DHETE.

2. MATERIALS AND METHODS

Human polymorphonuclear leukocytes (HPMNL) were isolated from leukocyte concentrates obtained from blood as in [5]. [10L-^{3}H; 3-^{14}C]arachidonic acid was prepared as in [6] (see fig. 1). [10D-^{3}H; 3-^{14}C]arachidonic acid was obtained in a similar way except for the use of (+)α-phenylethylamine for preparation of 3L-hydroxytridecanoic acid (cf. [7]; see fig. 1). The yield of labeled arachidonic acids from labeled

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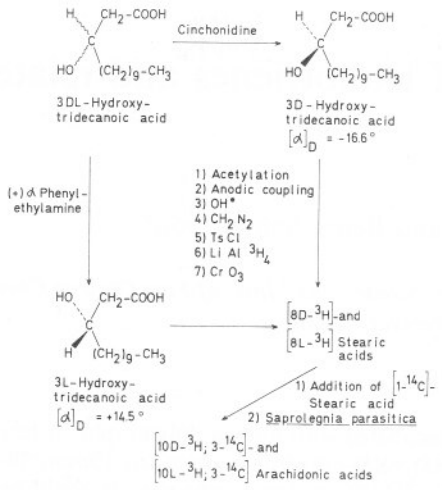

Fig. 1. Reactions used to prepare [10D-^{3}H; 3-^{14}C]- and [10L-^{3}H; 3-^{14}C]arachidonic acids; 'Ts', *p*-toluenesulfonyl.

stearic acids following incubation with the fungus, *Saprolegnia parasitica*, was 0.5–2%.

The cell preparation (~100 × 10^6 HPMNL/ml; contaminated with platelets) was stirred for 10 min at 37°C in the presence of ionophore A 23187 (5 μM) and the doubly labeled arachidonic acids (150 μM, 0.05–0.5 μCi of ^{14}C). The incubations were stopped by the addition of 1.5 vol. of methanol and the diethyl ether extracts were subjected to silicic acid chromatography (column, 1 g of silicic acid CC-4 obtained from Mallinckrodt). Elution was performed stepwise with diethyl ether–hexane 1:9 (v/v), diethyl ether–hexane 4:6 (v/v), and ethyl acetate. The material eluted with ethyl acetate was subjected to reversed-phase and straight-phase high-performance liquid chromatography essentially as in [3]. Here, the methyl esters of 5,12-DHETE and LTB_4 were identified and collected.

The doubly-labeled arachidonic acids were also incubated with suspensions of human platelets [6]. The products, i.e., 12-HETE, 12-HHT and thromboxane B_2 (TXB_2) [8] were isolated in form of their methyl esters by thin-layer chromatography.

$^3H/^{14}C$ ratios of the incubated arachidonic acids as well as of the products formed in leukocytes and platelets were determined with a Packard TriCarb model 3375 liquid scintillation spectrometer using Instagel® as scintillation fluor.

Table 1

Relative retention of ^3H in LTB_4 and 5,12-DHETE observed upon incubation of [10D-^{3}H; 3-^{14}C]- and [10L-^{3}H; 3-^{14}C]arachidonic acids with HPMNL

20:4 incubated $^3H/^{14}C$ (%)	LTB_4 $^3H/^{14}C$ (%)	5,12-DHETE $^3H/^{14}C$ (%)
[10D-^{3}H; 3-^{14}C]20:4		
100	22	128
100	27	149
100	–	139
[10L-^{3}H; 3-^{14}C]20:4		
100	98	9

3. RESULTS AND DISCUSSION

3.1. *Incubation with leukocytes*

[10D-^{3}H; 3-^{14}C]- and [10L-^{3}H; 3-^{14}C]arachidonic acids were incubated with suspensions of HPMNL as above. $^3H/^{14}C$ ratios of 5,12-DHETE and LTB_4 relative to that of the corresponding precursor acid are given in table 1.

As seen, during the conversion of the 10D-tritio arachidonic acid into LTB_4 tritium was largely lost. On the other hand, there was no loss of ^3H during the formation of 5,12-DHETE. Instead a certain enrichment of tritium was observed (table 1). LTB_4 formed from the 10L-tritio arachidonic acid retained the ^3H label whereas 5,12-DHETE lost most of the tritium.

These data show that the 10D (*pro-R*)-hydrogen is lost during the formation of LTA_4 from 5-HPETE whereas the 10L (*pro-S*)-hydrogen is lost upon formation of 5,12-DHETE from arachidonic acid. The enrichment of tritium in 5,12-DHETE observed when formed from [10D-^{3}H; 3-^{14}C]arachidonic acid suggests the presence of isotope effects in the conversions of the 10D-tritio arachidonic acid. A likely explanation for the enrichment involves the presence of an isotope effect in the conversion of [10D-^{3}H; 3-^{14}C]5-HPETE into [3-^{14}C]LTA_4. 5-HPETE remaining unconverted will thus be enriched with respect to tritium. Dioxygenation at C-12 does not involve elimination of the 10D-tritium ([6]; table 2). Therefore the resulting [10-^{3}H; 3-^{14}C]5,12-DHETE should be enriched with tritium. In order to study this question

Table 2

Relative retention of ^{3}H in 12-HETE, 12-HHT and TXB_2 observed following incubation of [10D-^{3}H; 3-^{14}C]- and [10L-^{3}H; 3-^{14}C]arachidonic acids with human platelets

20:4 incubated $^{3}H/^{14}C$ (%)	12-HETE $^{3}H/^{14}C$ (%)	12-HHT $^{3}H/^{14}C$ (%)	TXB_2 $^{3}H/^{14}C$ (%)
[10D-^{3}H; 3-^{14}C]20:4			
100	95	2	98
100[a]	96	–	–
[10L-^{3}H; 3-^{14}C]20:4			
100[a]	9	–	–

[a]Indomethacin (10 μg/ml) was added

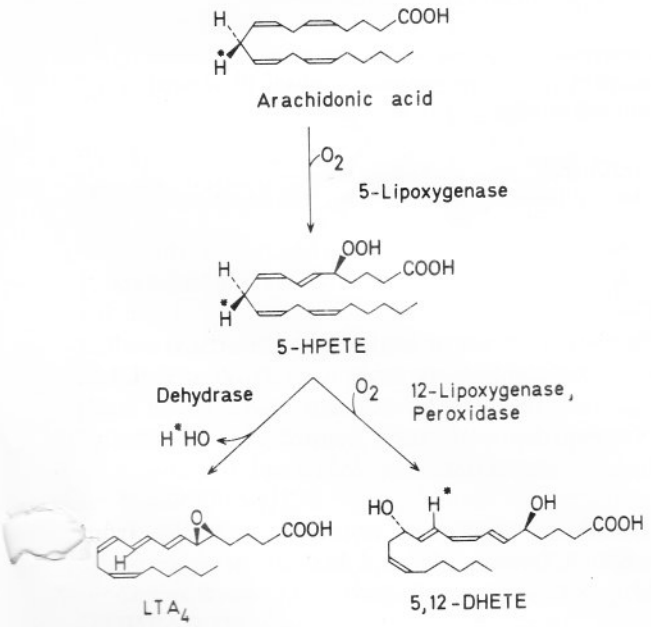

Fig. 2. Scheme of transformation of arachidonic acid into LTA_4 and 5,12-DHETE; (*) 10D (*pro-R*)-hydrogen of arachidonic acid and 5-HPETE.

in more detail, a separate experiment was carried out in which 5,12-DHETE, as well as 5-HETE were isolated and analysed following incubation of [10D-^{3}H; 3-^{14}C]arachidonic acid. In agreement with the interpretation discussed above (fig. 2) 5,12-DHETE as well as 5-HETE (reduction product of 5-HPETE) were found to be enriched with respect to tritium (130% and 167% relative to precursor, respectively). It thus appears that 5,12-DHETE may be formed by the sequence arachidonic acid → 5-HPETE → 5,12-DHETE (fig. 2). However, these data do not exclude the possibility of simultaneous formation of 5,12-DHETE by the alternate sequence of reactions; i.e., arachidonic acid → 12-HPETE → 5,12-DHETE.

3.2. *Incubation with platelets*

[10D-^{3}H; 3-^{14}C]- and [10L-^{3}H; 3-^{14}C]arachidonic acids were incubated with suspensions of human platelets as in [6,8]. Table 2 gives the relative retentions of ^{3}H in the products.

It has been found that tritium is lost during the conversion of [10L-^{3}H; 3-^{14}C]arachidonic acid into 12-HETE [6]. This result was confirmed here. Furthermore, the 10D-tritio arachidonic acid was found to retain its ^{3}H label upon conversion into 12-HETE (table 2). Also, as would be expected [8], TXB_2 retained the ^{3}H label when formed from [10D-^{3}H; 3-^{14}C]arachidonic acid, whereas 12-HHT was essentially devoid of ^{3}H (table 2).

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REFERENCES

[1] Borgeat, P., Hamberg, M. and Samuelsson, B. (1976) J. Biol. Chem. 251, 7816–7820; see also correction (1977) J. Biol. Chem. 252, 8772.
[2] Samuelsson, B. (1982) in: Leukotrienes and other lipoxygenase products (Samuelsson, B. and Paoletti, R., eds) Advances in Prostaglandin, Thromboxane and Leukotriene Research, vol. 9, pp. 1–17, Raven, New York.
[3] Lindgren, J.-Å., Hansson, G. and Samuelsson, B. (1981) FEBS Lett. 128, 329–335.
[4] Borgeat, P., Picard, S., Vallerand, P. and Sirois, P. (1981) Prostaglandins Med. 6, 557–570.
[5] Bøyum, A. (1976) Scand. J. Immunol. suppl. 5, 5, 9–15.
[6] Hamberg, M. and Hamberg, G. (1980) Biochem. Biophys. Res. Commun. 95, 1090–1097.
[7] Stoffel, W., Caesar, H. and Ditzer, R. (1964) Hoppe-Seylers Zeitsch. f. physiol. Chemie 339, 182–193.
[8] Hamberg, M. and Samuelsson, B. (1974) Proc. Natl. Acad. Sci. USA 71, 3400–3404.