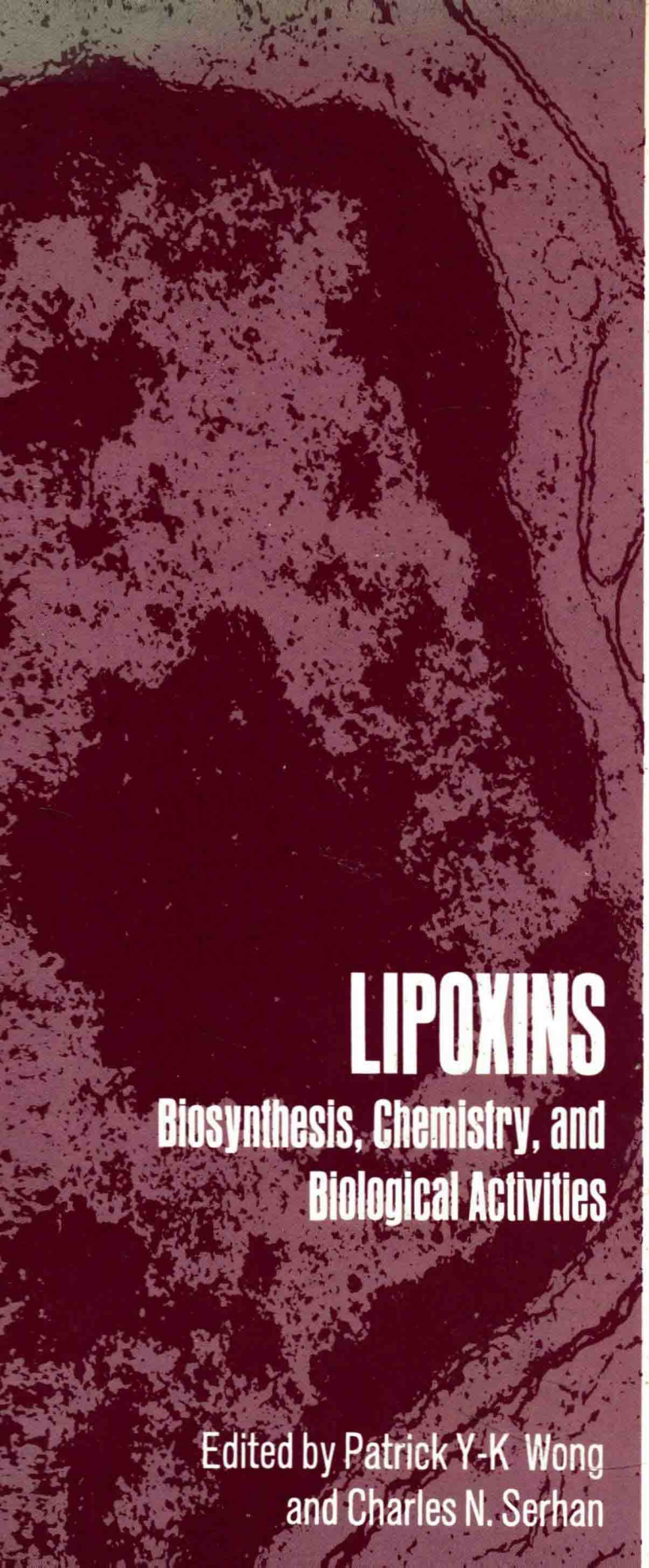

ADVANCES IN EXPERIMENTAL MEDICINE AND BIOLOGY

Volume 229

LIPOXINS

Biosynthesis, Chemistry, and Biological Activities

Edited by Patrick Y-K Wong and Charles N. Serhan

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Biosynthesis, Chemistry, and Biological Activities

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PLENUM PRESS • NEW YORK AND LONDON

Library of Congress Cataloging in Publication Data

Lipoxins: biosynthesis, chemistry, and biological activities.

(Advances in experimental medicine and biology; v. 229)
Proceedings of a FASEB symposium entitled "Lipoxins: biosynthesis and pharmacology," held March 29–April 3, 1987, in Washington, D.C.
Includes bibliographies and index.
1. Lipoxins—Congresses. I. Wong, Patrick, Y-K II. Serhan, Charles N. III. Federation of American Societies for Experimental Biology. IV. Series. [DNLM: 1. Lipoxygenases—congresses. W1 AD559 v.229 / QU 140 L764 1987]
QP752.L52L56 1988 574.19'247 88-2544
ISBN 0-306-42819-9

Proceedings of a FASEB symposium on Lipoxins: Biosynthesis and Pharmacology, held March 29–April 3, 1987, in Washington, D.C.

A Division of Plenum Publishing Corporation
233 Spring Street, New York, N.Y. 10013

Printed in the United States of America

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LIPOXINS: Biosynthesis, Chemistry, and Biological Activities
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PREFACE

The discovery of new structures which display biological activities is always an exciting event in biomedical research. In recent years, advances in this area have occurred at a rapid pace. This is particularly evident in the field of eicosanoid research because of the close interactions between chemists and biomedical researchers. The lipoxins are a new class of lipid-derived oxygenation products, discovered in 1984, which can originate from either arachidonic acid or eicosapentaenoic acid. It is now clear that these compounds can be generated by sequential lipoxygenation of either arachidonic acid or eicosapentaenoic acid within various cells or during cell-cell interactions. Continued research on the total synthesis of these and related compounds, their biosynthesis, biological roles and mechanism(s) of action may contribute to the development of new therapeutic agents.

This volume contains chapters from lectures given at the first symposiums devoted to this area held at the 1987 FASEB Meeting in Washington, D.C. entitled "Lipoxins: Biosynthesis and Pharmacology". In addition to chapters from these presentations, several other chapters are included by other investigators who have contributed to this rapidly growing area. It is our intention that this volume represents a complete and up-to-date collection of the most recent information regarding the Lipoxins.

The Editors

ACKNOWLEDGEMENTS

We wish to express our gratitude to the American Society of Pharmacology and Experimental Therapeutics for their advice and assistance during the organization of this Symposium. And also, we wish to acknowledge with thanks the generous financial support from the following pharmaceutical companies in the U.S.A. They are Ciba Geigy Inc., The Upjohn Company, S.K.F. and Beckman Inc., Ortho Pharmaceutical Corp., Lederle Inc. and W.W. Diagnostic Inc.

The organizers of this Symposium hope that the chapters of this volume will serve as a guided reference to stimulate further studies and new developments in this area.

The Editors

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PART III. ACTIONS OF LIPOXINS OF THE 4- AND 5- SERIES

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LIPOXINS: A NEW SERIES OF EICOSANOIDS

(BIOSYNTHESIS, STEREOCHEMISTRY, AND BIOLOGICAL ACTIVITIES)

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ABSTRACT

The oxygenation of arachidonic acid and other polyunsaturated fatty acids by a wide variety of cell types results in the formation of several structurally distinct classes of biologically active compounds[1,2]. These compounds include the prostaglandins, thromboxanes, leukotrienes, and other oxygenated derivatives of polyunsaturated fatty acids. A most recent addition to this family of biologically active compounds is the lipoxins (Figure 1). Leukotrienes and lipoxins are formed by mechanisms which involve initial oxygenation of free fatty acids by lipoxygenases. In general, lipoxygenase products display a wide range of actions and appear to be involved in immunity, the regulation of inflammation, and other physiological and pathophysiological processes. In this chapter we describe results of recent studies on the isolation, biosynthesis, stereochemistry and biological activities of this new series of compounds (lipoxins).

Isolation of the Lipoxins

Since lipoxygenation of arachidonic acid results in the formation of products of importance both in normal and pathological events (reviewed in refs. 1,2) bioregulation and interactions along these enzymatic pathways are of considerable interest. Whereas the biosynthesis of leukotrienes is initiated at the C-5 position of arachidonic acid, results from many studies suggested that initial lipoxygenation at the C-15 position can lead to the formation of compounds that may be of biological interest[2]. In particular, 15(S)-hydroxy-5,8,11-cis-13-trans-eicosatetraenoic acid (15-HETE) has been identified as a major product of arachidonic acid metabolism in both normal and asthmatic human lung tissue[3]. This lipoxygenase product has also been observed in large amounts in bronchoalveolar lavage fluids from patients with chronic stable asthma following antigenic challenge$_4$. Taken together these findings suggest that products derived from the action of a 15-lipoxygenase on arachidonic acid may play a role in human pathophysiology.

Although the 15-lipoxygenase activity is a major route of arachidonic acid metabolism in a wide variety of mammalian tissues (reviewed in ref. 5), the receptor-mediated activation of this enzyme system and general physiological role of its products remains a subject of considerable interest. Our initial studies on the metabolism of [1-^{14}C]-arachidonate in suspensions of mixed human leukocytes (i.e., neutrophils, eosinophils, basophils, etc.) indicated that a large percentage of the label material was transformed and associated with polar compounds which had not been previously described. To mimic cellular events and the reaction sequence(s) which may have given rise to these polar compounds, as well as to study interactions between the lipoxygenase pathways, we prepared both 15(S)-hydroperoxy-5,8,11-cis-13-trans-eicosatetraenoic acid (15-HPETE) and 15-HETE and studied the products formed upon incubation of these materials with human leukocytes. These experiments led to the isolation of a new series of biologically active oxygenated derivatives of arachidonic acid which contain a conjugated tetraene structure as a characteristic feature of the group. Since these compounds arose via interaction(s) between lipoxygenase pathways, we proposed the name lipoxins (lipoxygenase interaction products) for this group[6,7].

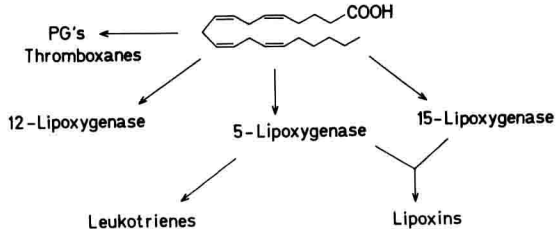

Fig. 1. Transformations of arachidonic acid.

Formation of these compounds was increased when the ionophore A23187 was added along with 15-HPETE incubations of human leukocytes. Following purification by silicic acid chromatography and thin layer chromatography, a fraction containing several unidentified tetraene-containing compounds was obtained from leukocyte suspensions. Samples of these materials were esterified, separated by thin layer chromatography, and analyzed by reversed-phase high pressure liquid chromatography. The basic structures of two main compounds of this series were elucidated by physical methods which included ultraviolet spectrometry, gas chromatography - mass spectrometry (utilizing several derivatives), and oxidative ozonolysis. One compound was identified as 5,6,15 L-trihydroxy-7,9,11,13-eicosatetraenoic acid, and the other as 5D,14,15-trihydroxy-6,8,10,12-eicosatetraenoic acid[2,6-9]. Addition of these biologically derived materials to either human neutrophils or human natural killer (NK) cells provoked selective responses different than those obtained with either leukotrienes or other eicosanoids. Hence the compounds were termed lipoxin A_4 (LXA_4) and lipoxin B_4 (LXB_4) respectively[7-9].

Stereochemistry and Biosynthesis

Next, it was of importance to determine both the complete stereochemistry of these compounds and their naturally occurring isomers as well as explore their route(s) of biosynthesis. To this end, human leukocytes in the presence and absence of ionophore were exposed to either 15-HPETE or 15-HETE and the trihydroxytetraene compounds were isolated and characterized (a schematic summary is presented in figures 2 and 3). Here, exposure to 15-HPETE alone led to both activation of a 5-lipoxygenase activity and to consumption of 15-HPETE to form tetraene-containing compounds. Addition of 15-HETE to cells exposed to either the ionophore A23187 or the chemotactic peptide f-met-leu-phe led to the formation of these compounds and reduced the appearance of products formed by non-enzymatic degradation of 15-HPETE which can be formed under similar conditions[7,9]. Human leukocytes incubated with 15-HETE in the absence of various stimuli did not generate lipoxins suggesting that activation of these cells is required for the utilization and subsequent transformation of 15-HETE to form lipoxins[9-12].

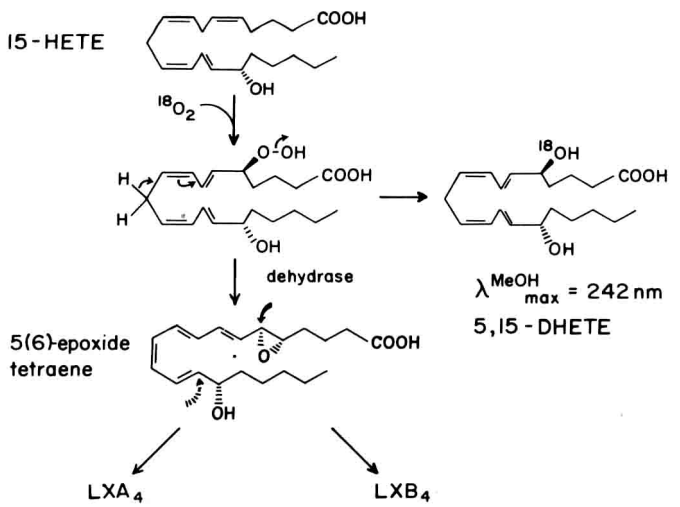

Fig. 2. Scheme of formation of lipoxins. When activated, human leukocytes convert 15-HETE by a 5-lipoxygenase activity to 5(S)-hydroperoxy-15(S)-hydroxy-6,13-trans-8,11-cis-eicosatetraenoic acid which can be further transformed to a 5(6)-epoxidetetraene intermediate[11,12]. In the presence of isotopic oxygen each of the compounds carried an ^{18}O atom at the carbon 5 position[10]. The 5(6)-epoxide tetraene (one proposed intermediate is 15(S)-hydroxy-5,6-epoxy-7,9,13-trans-11-cis-eicosatetraenoic acid) can be enzymatically converted to lipoxin A_4 by the action of an epoxide hydrolase (black arrow) or by attack of the carbon-14 position (hatched arrow) with the generation of an 8-cis double bond to form lipoxin B_4.

Strict criteria and synthetic materials prepared by total synthesis were employed to establish the complete stereochemistry of these and related compounds. The synthetic and biologically-derived materials were both subject to analysis by ultraviolet spectroscopy, HPLC (isochromatography in several systems), gas chromatography-mass spectroscopy of several derivatives, and bioassay[11-13]. Comparisons with several synthetic 5,14,15-trihydroxyeicosatetraenes prepared by Dr. J. Morris (The Upjohn Company, Kalamazoo, Mich.) showed that biologically derived LXB_4 is 5S,14R,15S-trihydroxy-6,10,12-trans-8-cis-eicosatetraenoic acid. The two naturally occurring isomers of LXB_4 were shown to be 5S,14R,15S-trihydroxy-6,8,10,12-trans-eicosatetraenoic acid (8-trans-LXB_4) and 5S,14S,15S-trihydroxy-6,8,10,12-trans-eicosatetraenoic acid (14S-8-trans-LXB_4)[10,11].

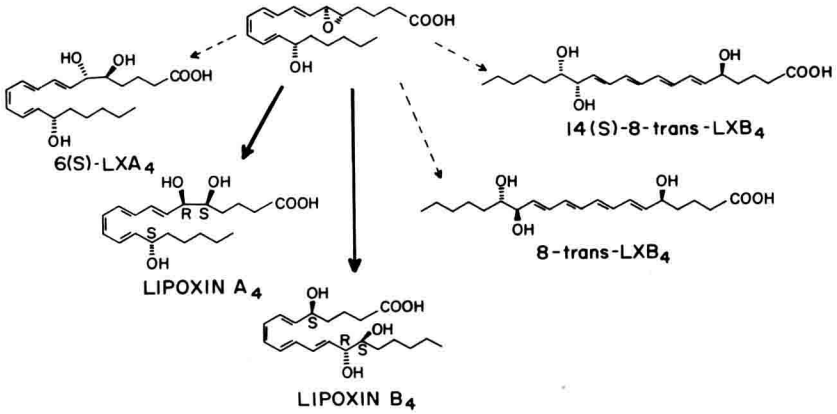

Fig. 3. One biosynthetic pathway for lipoxin formation via a 15(S)-hydroxy-5(6)-oxido-7,9,13-trans-11-cis-eicosatetraenoic acid. The stereochemistry of all of the compounds shown has been determined[11,12].

A synthetic approach was also undertaken to establish the complete stereochemistry of the biologically derived lipoxin A_4[12]. In collaboration with Prof. K.C. Nicolaou and his colleagues Dr. S.E. Webber and Dr. C.A. Veale of the University of Pennsylvania, studies with several synthetic 5,6,15-trihydroxyeicosatetraenoic acids demonstrated that the biologically derived LXA_4 is 5S,6R,15S-trihydroxy-7,9,13-trans-11-cis-eicosatetraenoic acid. The 6S isomer of LXA_4 (6S-LXA_4) was also identified from leukocyte extracts as well as two all-trans isomers which were designated 6S-11-trans-LXA_4 and 11-trans-LXA_4. The results of further studies indicated that these all-trans isomers arise, at least in part, by isomerization upon isolation and workup of LXA_4 and its epimer[10,12].

To shed light on possible routes of biosynthesis we examined the origins of oxygen in these compounds[10]. Here, incubations were performed under an atmosphere enriched in isotopic oxygen with activated human leukocytes exposed to either 15-HPETE or 15-HETE. LXA_4, LXB_4 and 5,15-DHETE, as well as the other tetraene-containing isomers, were isolated and analyzed (Figs. 2-5). Results from these studies demonstrated the incorporation of ^{18}O into each of the compounds and established that they each carried an ^{18}O atom at the carbon-5 position. In addition, they showed

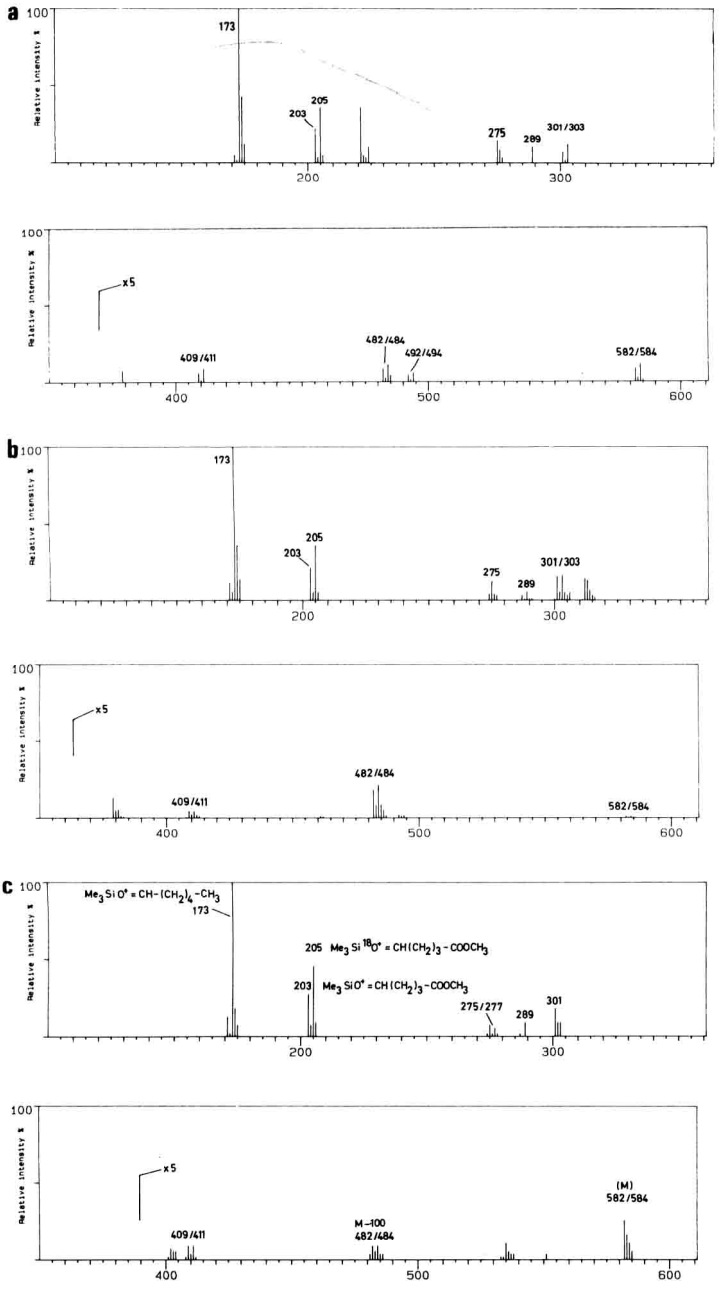

Fig. 4. Mass spectra of the Me_3Si derivatives of the methyl esters of LXB_4, 14S-8-trans-LXB_4, and 8-trans-LXB_4 obtained from human leukocytes exposed to 15-HETE (50 μM) and A23187 (2.5 μM) incubated in an atmosphere rich in $^{18}O_2$. ^{18}O-labeled Me_3Si derivative of the methyl ester of A) 14S-8-trans-LXB_4, B) 8-trans-LXB_4, C) LXB_4. The prominent ions and their positions are indicated. The ion m/e 203, which contains carbons originating from C-1 through C-5 positions, were in each case shifted to m/e 205 indicating the incorporation of ^{18}O at carbon 5. In addition, the oxygen atoms at carbon 14 positions were not derived from $^{18}O_2$, since the prominent ions showed shifts of M+2 rather than M+4.

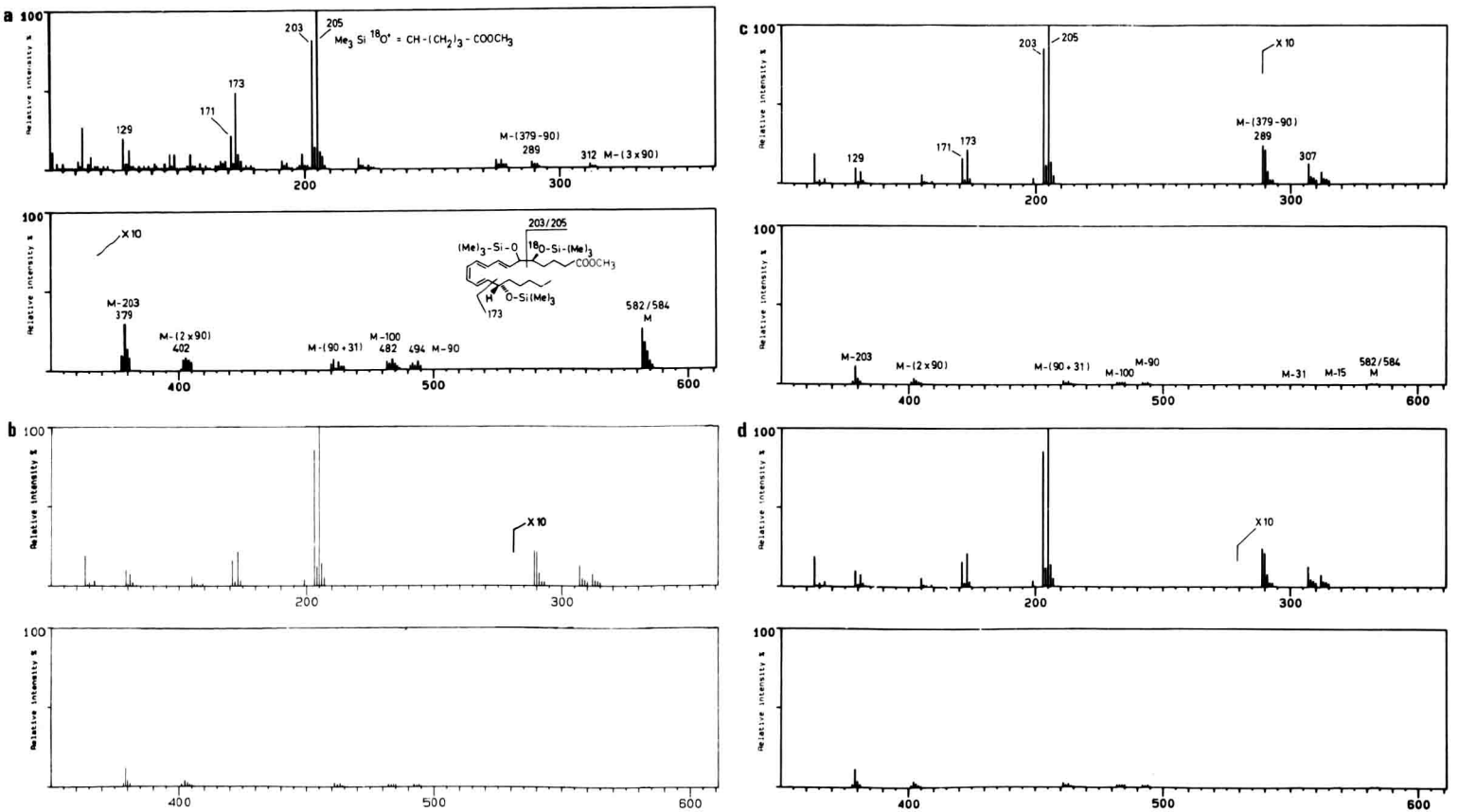

Fig. 5. Mass spectra or the Me_3Si derivatives of LXA_4 and its isomers obtained from human leukocyctes maintained under an atmosphere rich in $^{18}O_2$ and incubated with 15-HETE (50 μM) and A23187 (2.5 μM). ^{18}O-labeled Me_3Si derivative of the methyl ester of A) LXA_4, B) 11-trans LXA_4, C) 6s-11-trans-LXA_4 and D) 6s-LXA_4. The prominent ions and their positions are indicated. Again, ^{18}O was incorporated into the C-5 position of LXA_4 and each isomer[10,12].

that the oxygen atoms at either carbon-6 of LXA_4 or carbon-14 of LXB_4 as well as their isomers were not exclusively derived from $^{18}O_2$. Results obtained with either 15-HPETE or 15-HETE were virtually identical. These findings and results of alcohol trapping studies suggested the involvement of unstable epoxide intermediates in the formation of both LXA_4 and LXB_4[11-14].

Although it is clear that several distinct biosynthetic routes can be involved in the formation of tetraene containing eicosanoids[6,7], the finding that 15-HETE is also transformed by activated leukocytes to these products provided us with a model for studying a more limited biosynthetic path operative in their formation. Moreover, this finding provides a basis for exploring cell-cell interactions in the formation of lipoxin A_4 and lipoxin B_4 (e.g. transcellular metabolism of 15-HETE)[11,12]. In this route, schematically summarized in Figure 2, 15-HETE is converted to 5(S)-hydroperoxy-15(S)-hydroxy-6,13-trans-8,11-cis-eicosatetraenoic acid by activated cells which is further transformed to a 5(6)-epoxide tetraene. One proposed intermediate is 15(S)-hydroxy-5,6-epoxy-7,9,13-trans-11-cis-eicosatetraenoic acid[10-12]. Such an epoxide or its equivalent could be enzymatically transformed to either lipoxin A_4 (by the action of an epoxide hydrolase) or lipoxin B_4 (by attack of the C-14 position with the generation of an 8-cis double bond) (Figs. 2 and 3). Other isomers may be generated by non-enzymatic hydrolysis of the 5(6)-epoxytetraene or by isomerizations of LXA_4 or LXB_4 from conditions encountered upon isolation or by interactions of these compounds with metal-containing proteins[11,12].

This scheme of events is supported by several lines of evidence:

(1) 15-HETE serves as a precursor for formation of both LXA_4 and LXB_4 in activated leukocytes (which excludes the involvement of an epoxide intermediate at the 14(15) position);
(2) the pattern of isotopic oxygen incorporation in 5,15-DHETE, LXA_4, LXB_4, and their isomers (Figs. 4 and 5);
(3) the absolute stereochemistry of LXA_4 and LXB_4;
(4) time course of formation of these compounds by leukocytes (Fig. 6); and
(5) identification of alcohol trapping products (i.e. 15-HETE derived 5,15-dihydroxy-14-O-alkyleicosatetraenoic acids) originating from a 5(6)-epoxide tetraene (13,14).

Further evidence for the role of a 5(6)-epoxytetraene intermediate in the biosynthesis of these compounds was obtained by preparing 15(S)-hydroxy-5(6)-oxido-7,9,13-trans-11-cis-eicosatetraenoic acid by total chemical synthesis[13,14]. When added to purified human liver cytosolic epoxide hydrolase, the synthetic epoxide was rapidly (less than 5 seconds) and quantitatively converted into lipoxin A_4[14]. This system provides a clear model for evaluating the enzymatic formation of lipoxin A_4 (Fig. 7). It remains to be determined whether a similar enzyme is solely responsible for the formation of LXA_4 by human leukocytes. Others have also postulated the role of epoxide tetraenes in the formation of lipoxins and related compounds[15-20] and have isolated lipoxins of the 5 series which are formed from eicosapentaenoic acid[21]. In accordance with this observation we recently proposed that tetraene-containing compounds derived from arachidonic acid be denoted as lipoxins (LX) of the four series (i.e. lipoxin A_4 or LXA_4 and lipoxin B_4 or LXB_4) and those derived from eicosapentaenoic acid be termed lipoxins of the five series (i.e. lipoxin A_5 or LXA_5 and lipoxin B_5 or LXB_5) rather than lipoxenes[22].

In addition to the above-mentioned route of lipoxin formation, we have recently found that eosinophil rich granulocyte suspensions obtained from the peripheral blood of eosinophilic donors can generate LXA_4 from endogenous sources of arachidonate when exposed (in vitro) to ionophore A23187 (Fig. 8). In this study, neither lipoxin B_4 nor 6S-LXA_4 were consistently detected in extracts from these incubations. These eosinophil

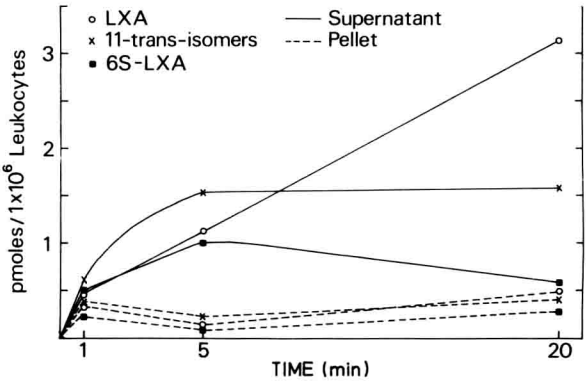

Fig. 6. Time course of LXA_4 generation by human leukocytes. Cells were incubated with 15-HETE (50 μM) and A23187 (2.5 μM) at 37°C. At the indicated times, the incubation mixtures were rapidly centrifuged and resulting pellets and supernatants were isolated and extracted (n=3). The relative amounts of LXA_4, 6S-LXA_4 and the 11-trans-isomers are shown. At 60 seconds, approximately equal amounts of LXA_4 are associated with cell pellets (- - -) as with supernatant (———). At times greater than 5 min LXA_4 is accumulated in the supernatant.

rich granulocyte suspensions were obtained from three donor groups which included eosinophilia due to an allergic disorder, reactions to drugs, and hypereosinophilic syndrome. In each case, eosinophils from these patient categories generated leukotriene C_4 in amounts 20-50 times greater than LXA_4 from endogenous sources of arachidonic acid when exposed to the ionophore A23187[23]. The values obtained for LXA_4 are expressed per 30 x 10^6 leukocytes per incubation, since this was the mean value of cells (or total