# The Leukotrienes

## CHEMISTRY AND BIOLOGY

Edited by
Lawrence W. Chakrin
and
Denis M. Bailey

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*Chemistry and Biology*

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## *Chemistry and Biology*

*Edited by*

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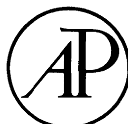


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# PREFACE

Leukotrienes are formed from the unsaturated fatty acid, arachidonic acid, through an unstable epoxide intermediate, leukotriene $A_4$. Enzymatic conversion yields leukotriene $B_4$ by hydration and leukotriene $C_4$ by addition of glutathione. Subsequently, leukotriene $C_4$ is metabolized to leukotriene $D_4$ and leukotriene $E_4$ respectively through elimination of a $\gamma$-glutamyl, and later a glycine, residue.

The extraordinary tale of the more than 40 years of scientific progress from the discovery of one of the mediators of anaphylaxis, slow-reacting substance (SRS), through the chemical identification of SRS-A as a mixture of leukotrienes $C_4$, $D_4$, and $E_4$ to a comprehensive understanding of these fascinating molecules is told in *The Leukotrienes*. In particular, the last decade has witnessed an enormous increase in research on the chemistry and biology of these and related substances, especially as they relate to the etiology of allergic and inflammatory diseases. This research interest has been fueled by the identification of lipoxygenase products from human platelets in 1974 and the recognition in 1977 of the relationship of the SRSs to the arachidonate lipoxygenase enzyme system. These and other evolutionary events have been put into perspective in the various chapters of *The Leukotrienes,* all of which are authored by major contributors to the field.

Readers interested in the role that the leukotrienes may play as mediators of immediate hypersensitivity reactions and inflammation will find this volume thoughtful and provocative, as will scientists specifically interested in exploiting arachidonic acid metabolism for the development of therapeutic agents specifically designed to either inhibit the synthesis or antagonize the effects of the leukotrienes.

Lawrence W. Chakrin
Denis M. Bailey

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# 1

## *Historical and Continuing Perspectives on the Biology of the Leukotrienes*

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## I. INTRODUCTION

At a time when the biology, chemistry, and pharmacology of the leukotriene constituents of slow-reacting substance of anaphylaxis ($LTC_4$, $LTD_4$, and $LTE_4$) and of the chemotactic leukotriene ($LTB_4$) have come of age, there may be some merit in reevaluating some of the contributions to this field over a period of several years, during which the technologies available did

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not permit the structural definition of the sulfidopeptide leukotrienes. The concept of a membrane-derived mediator that was generated via the oxidative metabolism of arachidonic acid and was composed of a lipid backbone and a sulfidopeptide adduct was not conceivable at a time when the recognized chemical mediators were basic amines or polypeptides. The lack of chemical definition of the moiety prompted the suffix "anaphylaxis," to emphasize that the product was derived from an immediate-type hypersensitivity reaction and to require that this mechanism of generation be part of the final definition (Brocklehurst, 1953). Thus, a substantial portion of the history of the field relates to the isolation and characterization of structurally unique moieties, the sulfidopeptide leukotrienes, as immunologic reaction products with profiles of pharmacologic activity distinct from those of other known chemical mediators. Indeed, the cumbersome definition of slow-reacting substance of anaphylaxis (SRS-A) may account for the fact that some of the critical observations were made in parallel by several investigators without a full appreciation of the relationship of their reports.

## II. ISOLATION AND CHARACTERIZATION OF SRS-A

### A. Discovery and Initial Characterization

It is generally held that SRS-A was initially described by Kellaway and Trethewie (1940) as a product of an immediate-type hypersensitivity reaction, recovered from the isolated perfused guinea pig lung, which constricted smooth muscle tissue more slowly than did histamine. In considering the literature, however, it is quite possible that Harkavy (1930) recognized a similar substance in the sputum of asthmatic humans, that is, a contractile material that he believed to be distinct in nature from histamine. It was not possible to address seriously the isolation of SRS-A until Brocklehurst (1953) utilized an antihistamine (now known to be of the $H_1$ class) to block the *in vitro* contractile response of smooth muscle to histamine in the perfusate or diffusate of an immediate-type hypersensitivity tissue reaction and fully uncover that to SRS-A. Utilizing this bioassay, both Brocklehurst, and Strandberg and Üvnas addressed the problem of isolation. Both laboratories were able to demonstrate that SRS-A was a unique "polar lipid," on the basis of its physical properties (Brocklehurst, 1962; Strandberg and Üvnas, 1971). Strandberg and Üvnas (1971) were also able to conclude that SRS-A was distinct from the family of lipid moieties identified as prostaglandins. The capacity of SRS-A to bind to various support media as well as to proteins and phospholipids represented an additional complication. Thus, those interested in the isolation and characterization of SRS-A were limited to

systems that generated relatively small amounts of material—that is, the immunologic release of SRS-A from guinea pig lung tissue or the release by 48/80 from the cat's paw (Änggård *et al.,* 1963)—and were further handicapped by very substantial losses during isolation attempts.

In the late 1960s, Austen and his colleagues recognized that SRS-A was released into the peritoneal fluid by an intraperitoneal antigen–antibody reaction utilizing either hyperimmune rabbit or rat serum (Orange *et al.,* 1967, 1968a, 1969). These observations not only provided a system for generating larger amounts of SRS-A than had previously been available, but also revealed for the first time that the generation of SRS-A did not require a conventional immediate-type hypersensitivity reaction. Indeed, it was demonstrated that the generation of SRS-A in the peritoneal cavity of the rat was dependent on the presence of rat neutrophilic polymorphonuclear leukocytes and of an intact complement system, and did not require the presence of histamine-containing mast cells within that cavity and its adjacent tissues (Orange *et al.,* 1968a). That SRS-A could be generated in the peritoneal cavity by a mast cell-dependent reaction involving rat IgE-type immunoglobulin (Orange *et al.,* 1970) indicated for the first time that in a single species the chemical mediator could be provided by cellular interactions involving different antibody classes and apparently different cells of origin. As had been done by earlier workers, Orange and co-workers separated SRS-A from proteins by precipitation of the latter with ethanol, and SRS-A was then isolated by conventional chromatographic methods depending on hydrophobicity, charge, and molecular size. Base hydrolysis was also utilized to eliminate the phospholipids, and the apparent molecular weight of this moiety was indicated to be approximately 500 (Orange *et al.,* 1973). Various methods were employed for volatilizing this material, but all were unsuccessful as preparations for mass spectral analysis, suggesting that SRS-A might be highly involatile (Orange *et al.,* 1973). Accordingly, an elemental analysis by spark source spectroscopy revealed that the SRS-A, derived from the rat peritoneal cavity by the interaction of antigen with hyperimmune serum and then partially purified, was enriched for sulfur (Orange *et al.,* 1973). This finding was compatible with the apparent involatility of the material. Nonetheless, because it was difficult to define a "control substance" that would prove that the SRS-A was truly enriched for sulfur, it was necessary to confirm this conclusion by an independent approach.

### B. Methods for Inactivation

That confirmation, based on the inactivation of SRS-A by arylsulfatases (AS) of many species, was, in retrospect, fortuitous but not correct. Three

lines of evidence were used to substantiate the capacity of arylsulfatases to inactivate SRS-A: the inactivation was achieved by partially purified arylsulfatases from a variety of different sources including limpet, snail, and human eosinophils (ASB), human lung tissue (ASA and ASB), and rat basophil leukemia cells (ASA and ASB) (Orange *et al.,* 1974; Wasserman *et al.,* 1975a; Wasserman and Austen, 1976, 1977); human esinophils from hypereosinophilic donors, which are enriched for ASB, preferentially inactivated SRS-A, as compared to human neutrophils, which are essentially devoid of the enzyme (Wasserman *et al.,* 1975b); and partially purified SRS-A prevented arylsulfatase from cleaving the synthetic substrate, *p*-nitrocatechol sulfate (Wasserman *et al.,* 1975a; Wasserman and Austen, 1976, 1977).

It is now evident that the loss of biologic activity in SRS-A on treatment with the various arylsulfatases must have been due to their contamination by dipeptidases, which converted $LTD_4$ to the less spasmogenic $LTE_4$, as measured on the guinea pig ileum. The capacity of eosinophils from hypereosinophilic donors to inactivate SRS-A is now attributed to the fact that such cells generate hypochlorous acid through the interaction of a peroxidase, hydrogen peroxide, and chlorine, because they are in an "activated state," as compared to neutrophils from normal donors (Lee *et al.,* 1982, 1983; Henderson *et al.,* 1982; Weller *et al.,* 1983). Neutrophils have the same capacity for sulfidopeptide inactivation, but only when they have been activated, as would occur with phorbol myristate acetate (Lee *et al.,* 1982, 1983). Interestingly, it has been confirmed that the synthetic sulfidopeptide leukotriene constituents of SRS-A are remarkably active inhibitors of purified human eosinophil ASB on its synthetic substrate (Weller *et al.,* 1981). Presumably, the enzyme interacts with the sulfidopeptide leukotrienes as if they possess sulfur-bearing aromatic rings, and molar concentrations 3–4 logs less than that of *p*-nitrocatechol sulfate are sufficient to interfere significantly with cleavage of that substrate (Weller *et al.,* 1981).

## III. RECOGNITION OF THE COMPONENTS OF SRS-A, THE LEUKOTRIENES

### A. Calcium Ionophore Activation

In the early 1970s it was possible to demonstrate that an almost pure population of basophil leukemia cells of human origin would respond to activation with the calcium ionophore, so as to release a material indistinguishable by chromatographic and pharmacologic characteristics from the immunologically generated SRS-A of rat origin (Lewis *et al.,* 1975). This observation made it possible for others in the field to utilize the ionophore as an activating principle for generating SRS-A from cell types without de-