TOPICS IN LIPID RESEARCH

FROM STRUCTURAL ELUCIDATION TO BIOLOGICAL FUNCTION

EDITED BY
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From structural elucidation to biological function

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PREFACE

Over the last decade many lipid chemists and biochemists have made a conscious effort to move from purely structural investigations to those which throw more light on the biological function of lipid molecules. Lipid molecules of rather simple structure have potent biological activities in their own right and are not just the convenient adjuncts of the more exciting membrane proteins - a view not uncommon in the late sixties and earlier seventies!

For many years lipids were considered to be molecules containing just C, H and O, related to fatty acids and hydrocarbons, with high oil-water partition coefficients; more recently, however, it has been realized that the more complex structures such as glycolipids and lipoproteins, and indeed glycolipoproteins, are fascinating both as structural challenges and as functional elements of the cell.

The contributions to this book grew out of a meeting, held in Cambridge in April 1986 by the Royal Society of Chemistry, concerned with the inter-relationships between structure and function for lipid molecules, and their macromolecular ordered systems.

Our choice of individual topics was undoubtedly personal; we felt, however, that the areas chosen - platelet activating factor, eicosanoids, glycolipids, membrane structure and function and environmental adaptation - are some of those areas in which there has been a very successful coming together of physical and chemical work at the molecular level with investigations at supra-molecular level involving biophysicists, biochemists, physiologists and pharmacologists.

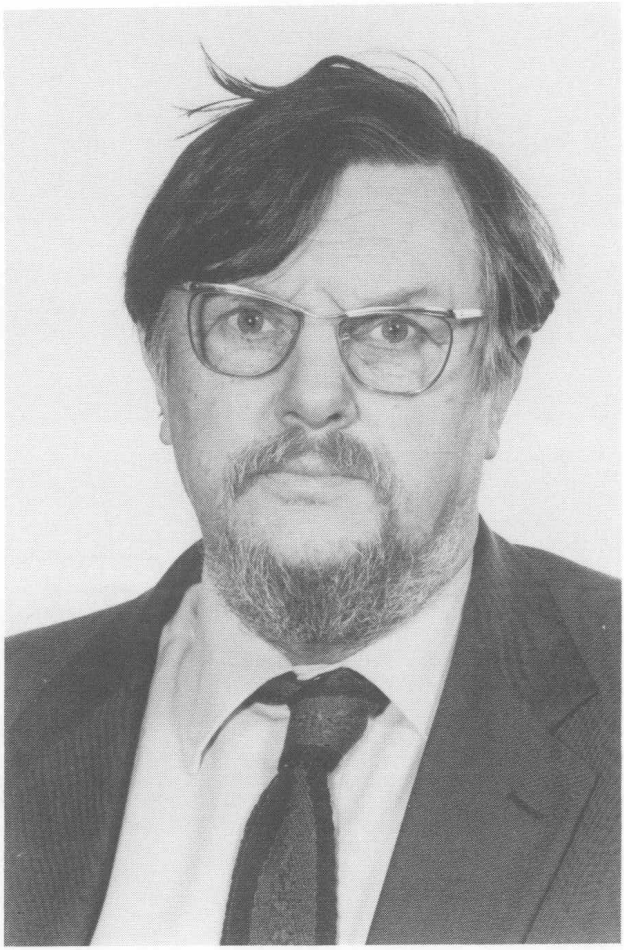
In each of the sections of the book we have contributions some of which are review papers and some of which are more detailed research papers. We have tried to present a leavened mixture of papers ranging from those with a chemical and structural bias to those with a more functional and biological basis.

Our overall intention was to present in this book a synopsis of current thinking in those areas of lipid research which we felt to be most interesting. This is especially true of the round table discussion on aspects of membrane probes and anaesthetics representing as it does the edited, and at that only slightly edited, verbatim recording of an unscripted verbal duel between the various participants.

We would like to express our thanks to all those who took part in the Round Table for being both coherent and incisive in what they had to say. Our thanks go also to all the other contributors to the book whose manuscripts were of a high standard thus making our editorial task that much easier.

We should like to dedicate this book to Alec Bangham on the occasion of his sixty-fifth birthday on 10th November 1986, in recognition of his contribution towards understanding the relationships between structure and function for membrane lipids.

Cambridge, August 1986 Roger Klein and Brigitte Schmitz



Alec Bangham, MD, FRS

CONTENTS

Section 1

PLATELET ACTIVATING FACTOR

CHEMISTRY OF PLATELET ACTIVATING FACTOR (PAF, PAF ACETHER, AGEPC) – 1-O-ALKYL-2-ACETYL-sn-GLYCERO-3-PHOSPHOCHOLINES – AND SOME PAF ANTAGONISTS

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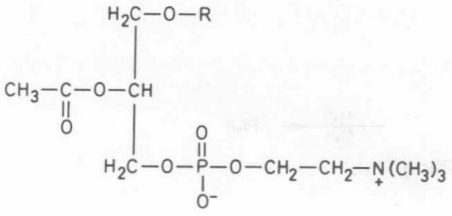
INTRODUCTION

The platelet activating factor (PAF) was first isolated from rabbit blood cells (Benveniste et al., 1972) and later identified as a mixture of 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholines having predominantly saturated alkyl moieties with 16 and 18 carbon atoms (Benveniste et al., 1979; Demopoulos et al., 1979); unsaturated alkyl moieties were found recently (Mueller et al., 1984; Weintraub et al., 1985). Numerous biological effects of this potent lipid mediator and the structural requirements for such actions are described in several review articles (e.g., Benveniste and Vargaftig, 1983; Braquet et al., 1986; Pinckard et al., 1982; Snyder, 1985; Weber, 1986), the proceedings of two symposia (Benveniste and Arnoux, 1983; Winslow and Lee, 1986), and a monograph (Snyder, 1986).



PAF

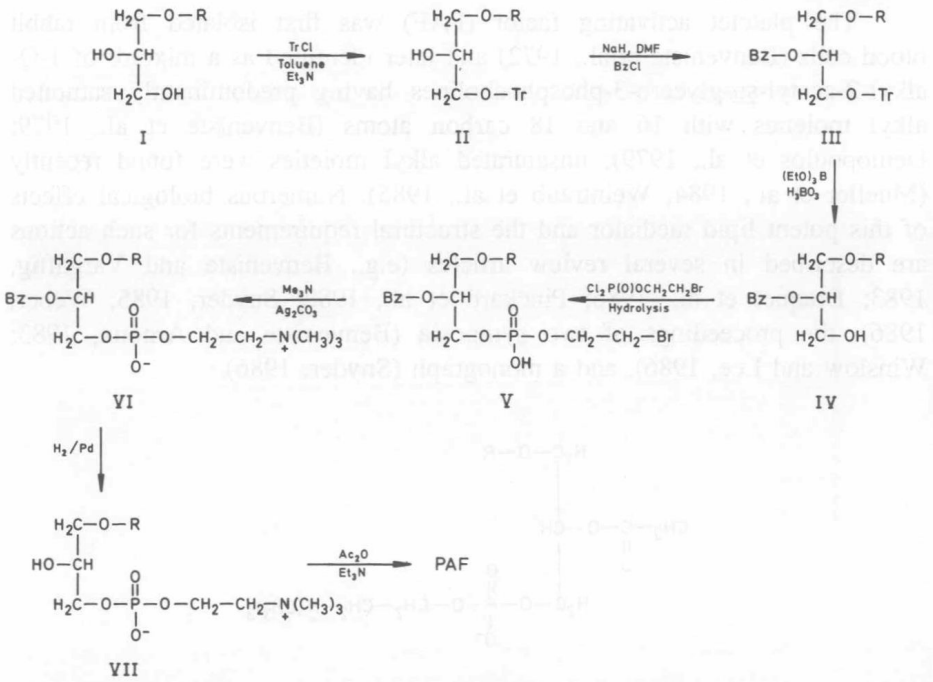
As an extension of an earlier survey (Mangold, 1983), the present article gives an account of work aimed at the synthesis and semi-synthesis

of PAF including radioactively labelled preparations as well as structurally related compounds tagged with a photoreactive group. In addition, this review provides information on PAF antagonists that were synthesized or isolated from natural sources.

SYNTHESIS OF 1-O-ALKYL-2-ACETYL-sn-GLYCERO-3-PHOSPHOCHOLINES

Efficient procedures for the synthesis of 1-O-alkyl-2-acylglycerophospholipids with a satisfactory degree of positional and chiral purity are well worked out (Eibl, 1981, 1984; Eibl and Woolley, 1986; Paltauf, 1983). As a rule, the synthesis of 1-O-alkyl-2-acetyl-sn-glycero-3-phosphocholines

Synthesis of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines from 1-*O*-alkyl-*sn*-glycerols



starts out from a 1-O-alkyl-sn-glycerol which is prepared by alkylation of 1,2-isopropylidene-sn-glycerol with an alkyl methanesulfonate followed by

hydrolytic removal of the protecting group (Baumann and Mangold, 1964). In the latter reaction and in all following steps, experimental conditions must be employed that minimize racemization and avoid migration of substituents in positions 2 and 3 as far as possible. The consecutive introduction of the acetyl group in position 2 and of the phosphorylcholine moiety in position 3 of the glycerol backbone leads to mixtures of positional isomers due to acyl migration (Godfroid et al., 1980; Hirth et al., 1983). In order to avoid this isomerization reaction, it is advisable to introduce the phosphorylcholine moiety in position 3 before the most labile bond is formed by acetylation of the hydroxy group in position 2. This strategy is applied in the route of synthesis shown above (Heymans et al., 1981). The primary hydroxy group of a 1-O-alkyl-sn-glycerol, I, is protected by tritylation to yield II, and the secondary hydroxy group is protected by introduction of a benzyl group; detritylation of compound III affords IV.

1-O-Alkyl-2-O-benzyl-sn-glycerol, IV, the key intermediate in this synthesis, can be obtained by several other routes as well (Eibl, 1984; Fujita et al., 1982; Ohno et al., 1985; Tsuri and Kamata, 1985). The phosphorylation of IV with bromoethylphosphoric acid dichloride is well worked out. Nevertheless, it may be of advantage to use dimethylphosphoryl chloride for phosphorylation followed by removal of the protecting methyl groups and hydrolysis (Bittman et al., 1984). The yield of VI may be improved by preparing first the corresponding ethanolamine phospholipid - via a phospholane intermediate - followed by quaternization (H. Eibl, private communication, 1986). The catalytic hydrogenolysis of the protecting benzyl group in position 2 of VI does not lead to migration of the phosphorylcholine moiety and, therefore, acetylation of VII with acetic anhydride, preferably in the presence of dimethylaminopyridine, affords PAF of high chemical purity and high biological activity (Hirth and Barner, 1982; Muramatsu et al., 1981; Surles et al., 1985).

The removal of the protecting benzyl group in VI by catalytic hydrogenolysis excludes the preparation of PAF having unsaturated alkyl moieties by this route of synthesis. The synthesis of unsaturated PAF is possible by protecting the primary hydroxy function of an unsaturated 1-O-alkyl-sn-glycerol with a p-methoxytrityl group and the secondary hydroxy function with a benzoyl group. The p-methoxytrityl group is removed by acid hydrolysis under relatively mild conditions and thus a 1-O-alkyl-2-benzoyl-sn-glycerol becomes available as the key intermediate instead of IV. Following phosphorylation and amination, the protecting benzoyl group is removed by alkaline hydrolysis and unsaturated PAF is obtained by acetylation of the lyso compound thus formed (Hirth et al., 1983). Another route of synthesis makes use of the methoxyethoxymethyl group for

protecting the secondary hydroxy function in an unsaturated rac-1-O-alkylglycerol. After introduction of the phosphorylcholine moiety, the protecting group is removed by reaction with zinc bromide in methylene chloride and the resulting lyso compound is acetylated to afford rac-1-O-alkyl-2-acetylglycerophosphocholine. The latter substance is treated with phospholipase A_2 (Wells and Hanahan, 1969) and the resulting 1-O-alkyl-sn-glycero-3-phosphocholine is separated from the unaffected 'unnatural' 3-O-alkyl-2-acetyl-sn-glycero-1-phosphocholine and reacetylated to yield the desired PAF (Surles et al., 1985).

Semi-synthesis of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines

Preparation of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines
from the choline plasmalogens of beef heart

1-*O*-(1'-Alkenyl)-2-acyl-*sn*-glycero-3-phosphocholines (Choline plasmalogens) —Hydrogenation→

1-*O*-Alkyl-2-acyl-*sn*-glycero-3-phosphocholines —Methanolysis→

1-*O*-Alkyl-*sn*-glycero-3-phosphocholines —Acetylation→

1-*O*-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholines

Preparation of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines
from the neutral ether lipids of shark liver oils

1-*O*-Alkyl-2,3-diacyl-*sn*-glycerols —Enzymatic hydrolysis→

1-*O*-Alkyl-2-acyl-*sn*-glycerols —1. Hydrogenation, 2. Phosphorylation, 3. Amination→

1-*O*-Alkyl-2-acyl-*sn*-glycero-3-phosphocholines —1. Partial hydrolysis, 2. Acetylation→

1-*O*-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholines

Preparation of 1-*O*-alkyl-2-acetyl-*sn*-glycero-3-phosphocholines
using cell suspension cultures of rape (*Brassica napus*)

1-*O*-Alkylglycerol —Rape cells→

1-*O*-Alkyl-2-acyl-*sn*-glycero-3-phosphocholines —1. Partial hydrolysis, 2. Acetylation→

1-*O*-Alkyl-2-acetyl-*sn*-glycero-3-phosphocholine

SEMI-SYNTHESIS OF 1-O-ALKYL-2-ACETYL-sn-GLYCERO-3-PHOSPHOCHOLINES

Naturally occurring ether lipids such as the ethanolamine plasmalogens of bovine brain (Benveniste et al., 1979) or the choline plasmalogens of bovine heart (Demopoulos et al., 1979), the neutral ether lipids of shark liver oils (Muramatsu et al., 1981), and the 1-O-alkyl-2-acyl-sn-glycero-3-phosphoethanolamines of bovine erythrocytes (Kumar et al., 1984) offer themselves as starting materials for the preparation of 1-O-alkyl-2-acyl-sn-glycero-3-phosphocholines from which PAF can be prepared easily. The latter intermediates can also be obtained by utilizing the capability of cell suspension cultures of rape to acylate, phosphorylate, and aminate exogenous 1-O-alkyl-sn-glycerols (Weber et al., 1984; Weber and Mangold, 1985). Three procedures for the preparation of PAF are outlined on the previous page.

LABELLED COMPOUNDS

1-O-Alkyl-2-acetyl-sn-glycero-3-phosphocholines labelled with ^{3}H, ^{14}C or another radio-isotope are needed in numerous biochemical and biomedical studies. ^{3}H-Labelled preparations that are obtained by catalytic tritiation of the 1-alkenyl moieties of choline plasmalogens (Demopoulos et al., 1979) or the alkenyl moieties of 'unsaturated PAF' derived from shark liver oils (Muramatsu et al., 1981) are available from NEN, Boston, Mass., U.S.A. and Amersham International, Amersham, Bucks., U.K., respectively. ^{3}H-Labelled compounds prepared by tritiation of synthetic PAF containing (Z)-9-alkenyl moieties (Surles et al., 1985; Wyrick et al., 1985) are commercially available as well. Unsaturated PAF could, of course, also be labelled with ^{125}I or ^{131}I. [^{14}C]PAF labelled in the choline moiety can be obtained by reacting 1-O-alkyl-2-acetyl-sn-glycero-3-phosphoethanolamines with [^{14}C]methyl iodide in the presence of a crown ether (Kumar et al., 1984). PAF containing a ^{14}C-labelled alkyl moiety can be prepared by incubating a cell suspension culture of rape with a tagged 1-O-alkyl-sn-glycerol (Weber and Mangold, 1985). Plant cell cultures could, of course, also be used for the facile preparation of PAF labelled with ^{31}P. In addition to radioactively labelled compounds, those marked with a stable isotope, such as deuterium, may be of interest in certain areas of research (Wyrick et al., 1985).

Also of interest in this connection are two analogs of the platelet activating factor that are labelled with an azido group in position 2 of the glycerol backbone, VIII, (Ponpipom and Bugianesi, 1984) and with both a photoreactive azido group and radioactive iodine, IX, (Bette-Bobillo et al.,