Inspired by Lipids: The Morton Lecture Award Presentation

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Abstract

Lipids are key molecules for membranes, energy storage and signalling. I have been privileged to have worked in such a diverse field and in organisms from microbes to humans. Here I will describe some of those contrasting areas which range from environmental impacts to food production and on to human health. It has been a fascinating journey which still continues to excite me.

Introduction

I was inspired to take a career as a biochemist by a lecture I received in my final year as an undergraduate at the University of Birmingham. At that time I was considering several options for my future but then I had a lecture from J. N. (Tim) Hawthorne about phospholipids. With the use of radiolabelling, it became clear that mammalian membrane phospholipids turned over at significant rates (typical $T1/2$'s being 24-48 hrs). But the really exciting thing was that some inositol lipids had a $T1/2$ of a few minutes! The mystery of why they had this characteristic really intrigued me and I was lucky enough to gain a place as a PhD student in Tim's lab. It was a great time to be a student and even more so in a field that was just opening up.

Tim had a useful habit of putting new students to work on a subject that had already been explored but needed some completion. Thus, I was able to have my name on a paper within a few months. My research followed up R. H. (Bob) Michell's work and showed that PtdIns kinase was located in the plasma membrane – giving a clue to its function [1]. Both Tim Hawthorne and Bob Michell were awardees of the Morton Lecture Award – I had excellent teachers!

I completed my PhD early and was able to spend six months as a "post-doctoral" (on a student stipend) in Tim's lab. This gave an opportunity to explore a couple of 'ideas' I had, both of which turned out to be valid. Tim was very encouraging and allowed me to explore fully these topics – I am not sure that all the form-filling and 'accountability' would have allowed this nowadays.

In the late 1960's, it was customary to spend a post-doctoral abroad – usually in the U.S.A. So I trawled through possibilities and ended up with two offers from the University of California. I elected to go to Paul Stumpf's lab at Davis, on the basis of a personal recommendation that he was 'a great guy to work for'. It was a splendid choice although it was in the alien area of plants (having taken Medical Biochemistry and Pharmacology at University, I had not even had a lecture on photosynthesis!)

Like most things I have found in life, once one gets to grips with details, most subjects are fascinating. So it was with plants. To brush up on my background, I attended Stumpf's lectures and learnt a lot more about the diversity of plant lipids. My research was to look at fatty acid synthesis in germinating peas. The work went well and it wasn't long before I had my first paper in the area [2]. We also characterised features of the synthesis of very long chain (>18C) fatty acids, work which shed light on the action of some important herbicides [3].

While doing experiments with radiolabelling, I had a great example of John Schloss's maxim that it is better to be lucky than smart (Table 1). I wanted to find out how the supply of energy (particularly ATP) would influence fatty acid biosynthesis during germination. I chose a selection of inhibitors, one of which was arsenite – which acted on the lipoic acid component of pyruvate and alpha-ketoglutarate dehydrogenases. Amazingly, arsenite completely inhibited stearate radiolabelling from acetate while allowing palmitate synthesis unchecked [4]. Thus, we accidentally discovered the condensing enzyme KAS II (Beta-ketoacyl-ACP synthase II) which clearly had the appropriate structural characteristics of vicinal sulphydryl groups to allow arsenite to bind. Since that time a third condensing enzyme, KAS III, has been characterised in plants. We made a contribution to this area also [5].

Lung Disease and Pulmonary Surfactant

My first academic appointment was in Cardiff. To the north of the city lie the "Valleys" where heavy industry (coal mining, quarrying, steel making) used to exist. So lung diseases such as silicosis were prevalent. In conjunction with other scientists in the Cardiff area, I worked on silicosis but was even more fascinated by pulmonary surfactant. This specialised lipo-protein mixture (Table 2) prevents lung collapse at end respiration and allows breathing to continue normally. We isolated surfactant from a number of mammals and showed how three characteristics were common:

1 – Surfactant was 90% lipid with only small amounts of (specialised) proteins [6, 7].

2 – The main lipid was phosphatidylcholine with a high level of its dipalmitoyl-molecular species.

3 – Phosphatidylglycerol (a rare mammalian component) was the second most abundant phospholipid.

We were contacted by Alec Bangham who had noted our Biochem J paper [6]. After discussions, we were all involved in the production of an artificial lung surfactant. This could be used for replacement therapy for premature babies suffering from respiratory distress of the newborn where their own surfactant has not yet been produced. We chose an artificial

PtdCho/PtdGro mixture because we were concerned about allergic reactions to foreign proteins in surfactants isolated from animals. As it turned out, usually only one treatment is needed so allergic side-effects are minimal. Thus, animal-derived surfactants are used routinely nowadays although all preparations are effective [8] when instilled.

So successful has replacement therapy proven that breathing problems due to a lack of surfactant are essentially unknown. So from a situation where thousands of babies were dying each year, acute respiratory disease of the newborn has been virtually eliminated – a most rewarding outcome.

Alpha Linolenic Acid – The World's Most Abundant Fatty Acid

Around 65% of the total fatty acids of vegetation is alpha-linolenic acid, which is also an essential fatty acid for our good health. Clearly this is the most prevalent fatty acid on earth. But how is it made?

Early experiments by Tony James's group at Unilever had shown that it was formed by three sequential desaturations of stearic acid. The same laboratory also provided early evidence that complex lipids could act as substrates for some desaturations [9]. Thus, when we began work on the problem of exactly how alpha-linolenic acid was made, there were tantalising pieces of evidence but not a whole picture.

Radiolabelling experiments revealed two important facts. First, at early times, the radiolabelled alpha-linolenate was confined exclusively to monogalactosyldiacylglycerol (MGDG), and second, specific radioactivities of oleate and linoleate were essentially the same in both ER and plastid lipids whereas other fatty acids were very varied [10]. The former observation implied that MGDG could be a substrate for the conversion of linoleate to linolenate and we showed this directly soon after [11]. The explanation of specific radioactivities for oleate and linoleate implied free exchange between different lipids as now currently accepted generally in plants [12].

At the time when we showed that MGDG could be a substrate for linoleate desaturation, there were other experiments suggesting that PtdCho was the substrate [13]. We now know that both experiments were correct with PtdCho acting in the E.R. of oil seeds while MGDG was the substrate in plastids of leaf tissue. Appropriate and distinct desaturases are present in these two subcellular locations [14].

Poikilotherms at the Mercy of the Environment

Unlike mammals, most organisms cannot change their internal temperature. They are therefore, often subject to environmental stress. One such organism is the common soil protozoon *Acanthamoeba castellanii*. This organism is vital for maintaining soil bacteria at relatively constant levels [15]. To do this it must be able to phagocytose and lower environmental temperatures may impede this process by causing membrane lipids to be below their transition temperature. This ruins the characteristic 'fluid mosaic' arrangement of membranes. To correct this problem, the most common remedy is for organisms to increase unsaturation of membrane lipids. Unusually for an 'animal', *A.castellanii* can form the essential fatty acid linoleate and it is this acid that is formed from oleate during adaptation to growth at low temperature [16]. The enzyme responsible uses PtdCho as a substrate and is induced by low environmental temperatures [17]. So a sequence exists where the environmental temperature is reduced, desaturase activity induced, unsaturation of membrane lipids increased and phagocytosis recommences (Table 3) [18].

Omega-3 (n-3) Polyunsaturated Fatty Acids (PUFA's) and Good Health

Plentiful reports in the literature of the benefits of fish oil (rich in n-3 PUFA's) persuaded me to work with Bruce Caterson (an expert in arthritis – http://www.cardiff.ac.uk/people/view/81135-caterson-bruce) on the molecular mechanisms by which dietary n-3 PUFAs could be of benefit in osteoarthritis. In the latter disease, two characteristic conditions are prevalent. First, there is chronic inflammation and second, activation of proteinases breaks down the cartilage of the joint. So, using a model system of connective tissue explants, we probed the effect of exogenous PUFA's. To our surprise, n-3 PUFAs such as eicosapentaenoic acid (EPA), not only reduced characteristic proteins of inflammation (e.g. interleukin-1) and the expression of cyclooxygenase ('inflammatory' COX-2 but not 'housekeeping' COX-1) but also had beneficial effects on proteinases, such as ADAMTS-4 and -5 [19]. n-6 PUFA had no beneficial effect and there was a difference in the efficacy of different n-3 PUFA [20].

Our data attracted the attention of a pet food manufacturer, Hill's Pet Foods, because certain breeds of dogs are prone to arthritis. When n-3 PUFAs were included in the diet of arthritic beagles there was a spectacular improvement (U.S. Patent application 11/0577 was filed on February 14th 2005 "a method for decreasing cartilage damage in dogs") in only a short time. It is now common for pet food manufacturers to include n-3-PUFA in their formulae.

As we have discussed [21], the ability of n-3 PUFA to give rise to anti-inflammatory signalling molecules [12] is relevant to a host of illnesses with chronic inflammation as a primary confounder. One such complaint is Alzheimer's disease, the major dementia. Alzheimer's disease affects more than 35 million people globally and is predicted to impact double that number by 2030 [http://www.alz.co.uk/research/worldalzheimerreport2015.pdf]. Many groups have worked in this important area but there is often controversy about the benefits of dietary n-3 PUFA.

We have made a modest contribution to this debate by utilising a model mouse, the Tg2576 which suffers cognitive impairment following production of beta-amyloid plaques [22]. Increasing dietary n-3 PUFA (in this case, docosahexaenoic acid, DHA) benefitted cognitive performance when fed from an early age [23]. However, it was noticeable that lipid metabolism following feeding was much more complex than might have been expected. Thus, the extra DHA in the diet was incorporated selectively into certain brain phospholipids. PtdSer and PtdEtn were the two phospholipids with the highest levels of DHA but only PtdEtn (both diacyl and ether forms) was modified significantly by diet [24]. This was interesting since ethanolamine phospholipids have been implicated in Alzheimer's disease [e.g. 25].

The distinct acyl compositions of different membrane lipid classes were noticeable and were very similar for the cortex and hippocampus areas but somewhat different from the cerebellum [24]. These data pointed to the sophisticated regulation of lipid metabolism. Amongst the unanswered questions of brain biochemistry are to unravel the details of the control of metabolism as well as what role the lipid classes and their acyl compositions play in brain function.

As mentioned above there has been (and still is) some controversy about how beneficial n-3 PUFAs are for brain function. Critics often point to the fact that giving increased amounts of DHA in the diet seldom helps patients suffering cognitive impairment. However, it may then be too late because nerves are already damaged/reduced in number. Certainly, there is persuasive evidence from animal experiments and for young infants that n-3 PUFAs are vital for appropriate nerve function and cognition [e.g. 26-28]. Recently there has been increasing interest in how n-3 PUFAs can alter mood such as reducing aggression or anxiety [29]. There is still much to discover!

Supplies of Food Lipids are Critical

Over the last 50 years, consumption of oils and fats has increased at about 5% year on year. With limited agricultural land and elevated demands for vegetable oils as renewable chemicals and food, it is obvious that we have a serious issue [30]. One way to improve

supplies is to increase productivity but to do this we must understand how oil accumulation is controlled. We have applied flux control analysis [31] to this problem.

There are two basic methods to examine flux control – top-down and bottom-up (Fig. 1). In bottom-up control analysis (BUCA) a specific way of altering the activity of a single enzyme step (using inhibitors or gene manipulation) is needed. By examining each enzyme step in turn, a complete picture of control can be accumulated. In top-down control analysis (TDCA) the metabolic pathway is divided into two with a chosen intermediate connecting the two parts. Thus, multiple inhibitors are not needed, and indeed, the manipulations of Block A or of Block B (Fig 1) could be via several steps. TDCA provides an immediate view over the pathway and by further dividing up reactions within a given Block one eventually gains the same information as BUCA.

We have applied TDCA to a number of important oil crops – oil palm [32, 33], olive [32], soybean [34] and oilseed rape [35]. Usually more control was present in fatty acid synthesis (Block A) than in lipid assembly (Block B). Oilseed rape (*Brassica napus*) cv. Westar was an exception [35]. Accordingly we focussed on lipid assembly in this plant. From previous experiments we believed that the final enzyme in oil biosynthesis, diacylglycerolacyltransferase (DGAT) was important [36]. So we overexpressed the major isoform (DGAT1), and, sure enough, this manipulation increased oil accumulation as well as shifting control characteristics [37]. To follow up this experimental observation, transgenic lines were trialled in fields and in two successive seasons (with variable weather conditions) gave 8% increased yields [38]. This 'modest' increase is worth about 2 billion Canadian dollars, so is not insignificant!

Conclusion

In this short essay, I hope I have given enough detail of some of the exciting areas of lipid biochemistry that I have worked in. Although we have sometimes reached significant conclusions, it always seems to me that there are many interesting facts for an experimental biochemist still to uncover. Perhaps I could conclude by wondering if, as Robert Louis Stevenson once said "to travel hopefully is a better thing than to arrive and the true success is to labour"

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Table 1 – Schloss's Maxim

SCHLOSS'S MAXIM

Given the choice between being lucky or smart

-   It is always better to be lucky.

The worst possible situation is to be very smart

but extremely unlucky

Because then you know just how

badly off you are!

Table 2 – Composition of pulmonary surfactant

Lipids (90%) - 87% polar lipids - 75% PtdCho (50% dipalmitoyl species)
- 10% PtdGro
- 13% non-polar lipids – cholesterol and triacylglycerol

Proteins (10%) - SP-A, SP-B, SP-C, SP-D - (SP-B and SP-C are exceptionally hydrophobic)

Table 3 – Adaptation of *A.castellanii* to low temperatures

1. Low environmental temperature (shock)
2. Δ12-desaturase induced
3. Increased conversion 18:1 → 18:2 etc.
4. Decreased membrane order (Increased 'fluidity')
5. Phagocytosis recommences

Figure 1 – Legend

In the pathway S→P, the Bottom-up approach to control analysis (BUCA) manipulates the activity of individual reaction steps. For a linear pathway addition of all the control values equals 1.

In the Top-down approach to control analysis (TDCA) the pathway is divided into two with a chosen intermediate (D). By manipulating each block independently, one determines which block (A or B) exerts the most control. Thus, an overall view of the pathway is gained. By further division of the reactions in each Block, eventually TDCA provides the same information as BUCA.