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CONTINUING SERIES: BIOCHEMISTRY RESEARCH AT PREDOMINANTLY UNDERGRADUATE INSTITUTIONS

Rodney F. Boyer, Series Editor

LIPIDS AND LIPASES: A HISTORY OF BIOCHEMISTRY RESEARCH AT ST. OLAF COLLEGE

H. Stewart Hendrickson
Department of Chemistry
St. Olaf College
Northfield, Minnesota 55057

As I begin my twenty-fifth year at St. Olaf College, it does not seem that I have been here that long. I have been quite fortunate to have had continuous research support during that time, good facilities, and most important, good students. Our work now ranges from organic synthesis to enzymology and recombinant DNA. It all began with an interest in lipids as a graduate student with Professor Herbert E. Carter at the University of Illinois.

Getting Started

I came to St. Olaf College in 1968, after a B.A. at Pomona College, a Ph.D. at the University of Illinois, a postdoctoral year at U.C.-Berkeley, two years as a research chemist at the U.S. Dept. of Agriculture Western Regional Lab in Albany, California, and three years as Assistant Professor of Biochemistry and Physiology at the University of Texas Southwestern Medical School at Dallas. Although a biochemistry course had been introduced the previous year by a part-time instructor from the University of Minnesota, I was the first full-time biochemist in the Chemistry Department. I already had my first two-year NSF research grant at Texas, and was able to bring a second NSF grant to St. Olaf. This was a propitious time to come to St. Olaf; the Chemistry Department was moving into a new Science Center and the new biochemistry teaching program was supported by a Research Corporation grant. With equipment I brought with me from Texas and also provided through the Research Corporation grant, I had a well-equipped lab for research. As I look back at this beginning, I value my postdoctoral experience and the fact that I had already begun my own research career. This enabled me to begin research soon after my arrival at St. Olaf.

Phosphoinositides

Prior to coming to St. Olaf, my research had centered around phosphoinositides, phospholipids thought to have an important, yet then unidentified, role in biological membranes. I was the first to isolate intact phosphatidylinositol mono- and bis-phosphate from brain during my postdoctoral research with Professor Clinton Ballou at U.C.-Berkeley. I subsequently studied their interactions with metal ions and proteins. During the new-faculty orientation beginning my first year at St. Olaf, I presented an invited paper on the physical properties and interactions of phosphoinositides at a New York Academy of Sciences meeting in New York.[1]

In the fall of 1968, my first student (Jim Reinertsen '69; M.D.) approached me with a desire to do research during the January interim. Jim turned out to be by far my best student. He is now a rheumatologist and President of the Park-Nicollet Medical Center in the Minneapolis area. We worked out a project where Jim synthesized trans-cyclohexane-1,2-diol bisphosphate as a model for phosphatidylinositol bisphosphate. He then determined binding constants with a series of metal ions which we compared to binding constants for deacylated phosphatidylinositol bisphosphate. He finished this project during the spring and we published the results in

Biochemistry.[2] Jim worked in my lab the following summer and produced results for a second paper. This was not a bad beginning for my research at St. Olaf.

For the next few years I continued to have summer undergraduate research students and we continued work on phosphoinositides. In 1970 I received a NIH grant to support a postdoc, and in 1972 when my NSF grant was not renewed, I switched to NIH as the main support of my research. NIH support continued until 1989 when NSF again came to my aid. With my first postdoc, Ed Scattergood, we shifted our research to phospholipid enzymology. With the aid of several undergraduates, we developed an apparatus to study planar, "black film", bilayer membranes, and studied the effects of phospholipases A2 and C on these membranes.[3]

First Sabbatical - PhospholipaseA2Activity on Phospholipid Monolayers

In anticipation of my first sabbatical leave in 1974-75 with Professor Laurens van Deenen at the University of Utrecht in The Netherlands, I began work on phospholipase A2 (PLA2). We set up a homemade monolayer trough and balance to study the effect of phospholipases on phospholipid monolayers. This was modeled after work in the Utrecht laboratories. John Schaus ('75; Ph.D.) began this work which we continued into the early 1980s.

As I planned my first sabbatical, I submitted a competitive renewal proposal to NIH to support my leave and the following year. I finalized arrangements for my sabbatical before receiving final word on my new grant. This was an act of faith; if I had not done this we would not have gone. It was not until my family and I had been in Holland for a month that I received a written announcement that my grant was funded. If not funded, we could barely afford to live in Holland, much less travel back to the States! As it turned out, this was an excellent place to begin my phospholipase A2 work (Utrecht is an international center for phospholipase research) and a great experience in international living for my family.

At Utrecht, I began work on "black-film" bilayer membranes in van Deenen's lab. This was very touchy and did not seem to be producing useful results, so I then decided to pursue a project of my own using the monolayer trough designed for enzyme kinetic studies in Professor de Haas's lab. A former student of de Haas had reported on the inhibition of phospholipase A2 by local anesthetics. I planned to determine the penetration of these amphiphiles into phospholipid monolayers and correlate their presence with enzyme inhibition. It turned out that this correlation was not good since the anesthetics also interacted with the enzyme in solution and prevented its penetration into the interface. I published this work in two papers after my return.[4] It was a successful sabbatical.

More Monolayers

After my return to St. Olaf, I constructed a second-generation monolayer trough modeled after the one in Utrecht. We also purified several-hundred mg of porcine pancreatic PLA2, starting with a bucketful of pancreas from the Hormel processing plant in Austin, Minnesota. Cheryl Willman ('77; M.D.) did some very fine research on the interfacial mechanism of local anesthetic inhibition of PLA2 using our new monolayer apparatus.[5] Later, a very clever student (John Eklund 78; M.D./Ph.D.) with computer interfacing experience approached me about doing a project. During the spring and summer John succeeded in interfacing the monolayer apparatus to a micro processor and computer. He knew much more about this than I, and I let him proceed on his own. At the end of summer it was complete and worked. This apparatus supported several more students and resulted in further publications.[6]

Beginning with my first research grant, I have always had a part-time lab technician. In 1978 I was looking for a new technician without much luck. My wife, Betty, an organic chemist who had worked the previous year as a half-time instructor in chemistry, was interested in taking the job since our children were then in high school. That began a very fruitful collaboration which continues to the present.

Phospholipid Synthesis - The Sulfur Phase or Life in the Skunk Works

In 1979 Peter Dedon ('79; M.D./Ph.D.) began our entry into phospholipid synthesis by making alkyl phosphoryl cholines, which we needed to form mixed micelles for kinetic studies of PLA2. We then gained enough confidence to attempt the chiral synthesis of a bisthiolester analog of phosphatidylcholine (thio-PC)[7] to be used for the continuous spectrophotometric assay of PLA2 (the thiol product, thio-Iysophosphatidylcholine, reacts in a coupled reaction with a thiol reagent, such as DTNB, to produce a chromophore). This began a long line of thio-phospholipid analog syntheses[8] and, from time to time, a smelly lab (which does not endear us to other faculty and students, but did result in a more efficient hood!).

In 1982-83, with our chiral thio-PC in hand, I did my second sabbatical with Professor Ed Dennis at U.C.-San Diego. The Dennis lab is a center for PLA2 research in the U.S. At the same time my wife synthesized phospholipids for Scripps Research Institute in La Jolla. I used our thio-PC to do a detailed kinetic study of cobra venom PLA2.[9] This was another very productive and successful sabbatical.

Phospholipid Synthesis - The Fluorescent Phase (Enlightenment)

In 1980 Phil Rauk ('81; M.D.) synthesized our first fluorescent phospholipid, 1,2-bis-pyrenebutanoyl-PC. This proved useful for a continuous fluorescence-based assay of PLA2; the substrate exhibits excimer fluorescence while the products emit as monomers.[10] We have continued to synthesize many other fluorescent-labeled phospholipids. Andy Batchelder ('85; M.D.) began work on a novel fluorescent phospholipid, Prodan-PC[11]. After some frustration, he finally succeeded in an enzymatic synthesis of this analog. He was so excited that he burst into my class as I was beginning a lecture to tell me of his success. It was a memorable moment for both of us, although he was a little embarrassed later.

Return to Phosphoinositides

In 1988 I prepared for my third sabbatical (1989-90) with Professor Hays Griffith at the University of Oregon. My NIH grant was up for competitive renewal in 1989, and I wrote proposals to both NIH and NSF with a change of direction. I proposed to study a bacterial (*B cereus*) phosphatidylinositol-specific phospholipase C (PI-PLC) which the Griffith lab had begun to research several years previous (this represented a full-circle return to the phosphoinositide research of my early career). I had hoped to bring a thiophosphate analog (C-S-P bond) of PI with me to facilitate a continuous spectrophotometric assay similar to the one we had developed for PLA2.

This was a bit too ambitious, and we did not yet have the analog. After some frustration in starting a research project in Eugene, I decided to switch direction and asked a young German postdoc (Andreas Kuppe) in the Griffith lab to teach me recombinant DNA techniques. He was very happy to do so, and I helped work on an expression plasmid for the PI-PLC gene which he had earlier cloned. This was stimulating experience which introduced me to recombinant DNA. My support for that year and the following turned out to come from NSF. Although I received the best study section report ever from NIH, I did not make the low funding cut, but NSF took pity on me. Again, it was not until I started work in Eugene that I received written funding approval of my grant - another act of faith!

Entrepreneurial Venture

While I was working at the University of Oregon, my wife got a full time job as the chief phospholipid synthetic organic chemist for Molecular Probes, Inc., a small specialty chemical company also in Eugene. Through this we developed a good relationship with Dr. Richard Haugland, the President, founder and research director. After our return to St. Olaf, we began an entrepreneurial venture in which we synthesize and market our fluorescent- and thiophospholipid

analogs through Molecular Probes. They now have over a dozen of our analogs, the sales of which provide extra funds for our research - an example of creative funding.

First Thiophosphate Analogs of Phosphatidylinositol

By 1991 we succeeded in synthesizing the first thiophosphate substrate analog of PI (thio-PI) and also a fluorescent analog.[12] This thio-PI allows the first continuous assay of PI-PLC and will enable us to do detailed kinetic studies. Jeannette Johnson ('92) began some very fme kinetic studies wIth these two substrates. My wife continues to work with students on the complicated syntheses of phosphoinositide analysis. We also have an efficient *E coli* expression system which provides all the pure PI-PLC. we need. We thus take pride that we "do It all,'' from organic synthesis to enzymology and recombinant DNA. In addition we collaborate with other university research groups outside of St. Olaf.

Pharmaceutical Collaboration

Another collaborative research project began in 1987 with Hoffman-La Roche (Nutley, NJ). We evaluate potential anti-inflammatory drugs, which they synthesize, as inhibitors of PLA2. This project funds undergraduate students to work part-time during the academic year and full-time In the summer. This is a good way for sophomore students to get experience in our lab prior to their summer research. Generous funding by Hoffman-La Roche also provided us with capital for our venture with Molecular Probes, Inc.

Pro- and Retrospective

Looking back on my years at St. Olaf, I have seen a change in undergraduate research. In a recent *CUR Newsletter* I responded to an earlier editorial by Mike Doyle on research at undergraduate institutions.[13] I have seen a recent decline in undergraduate research by faculty, and students have to be more actively recruited and lack long-term commitment. Continuous grant support and a part-time technician have been very important in my research. The latter insures that research continues through the academic year when I am more involved in teaching (I have always had. a full teaching load). Collaboration with my wife has also played a major role. My wife now works two-thirds-time. This year I have a visiting scientist, Professor Anatoly Bushnev, from Moscow. I support students part-time during the academic year and they often work two summers. Good students can pick up the necessary techniques and learn the relevant biochemistry (most of our students do not take the biochemistry course until their senior year).

We look forward in the future to synthesizing more sophisticated PI analogs and learning more about the bacterial PI-PLC and the more interesting mammalian PI-PLC which is involved in the phosphoinositide cascade of signal transduction (we have done some very preliminary work with the eukaryotic enzyme). I hope to entice more students into biochemical research. I regret that space prevents me from acknowledging all of the students who have worked in my lab - they total close to fifty. Many have been coauthors on my publications and have presented papers at national and regional meetings. I consider research to be an integral part of teaching, and the students have made it all worthwhile.

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