GORDON RESEARCH CONFERENCE ON LIPID METABOLISM - 1985

Final Report

December 1985
(For the period 1 January 1985 - 31 December 1985)

Richard E. Pagano, Ph.D.

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DD FORM 1473, 84 VA
The Gordon Research Conference on Lipid Metabolism was held June 17-21, 1985 at Kimball Union Academy, Meriden, N.H. This conference covered the following major topics: physical chemistry of lipids, enzymological aspects of lipid metabolism, metabolism of inositol lipids, lipid transfer between membranes, lipid translocation in cells, ether lipids, liposomes and anti-lipid antibodies, and glycolipids/sphingolipids. These topics were covered during nine three-hour sessions held during mornings and early evenings, leaving time for informal discussions in the afternoons, late evenings and at mealtimes. The chairperson of each session provided an overview, and integrated the discussion period. In addition to the formal oral presentations, there were more than 50 poster presentations over a four day period, which complemented the lectures. Discussion and interchange of ideas throughout the conference was excellent.

A brief summary of the conference sessions appears below along with a copy of the program.

Session I: "Physical Chemistry of Lipids"

The opening session was chaired by A. Kleinfeld, known for his work on fluorescence techniques in the study of lipids and membranes. D. Small discussed phase transitions and physical properties of lipids, indicating the way in which having two acyl chains linked to a polar head group can profoundly affect the behavior and domain formation of lipid systems. D. Wolf then talked about the use of diI molecules as probes of mobility within membranes. He showed that these molecules can monitor heterogenous lipid phases in the same membrane, and gave examples of such behavior both in model and in natural membrane systems. He also touched on some of the pitfalls of measuring lateral diffusion in membranes. D. Schachter discussed his studies on monitoring the fluidity of the inner and outer leaflets of the erythrocyte membrane, showing that the outer leaflet appears to be more fluid than the inner one. He also showed that treatments such as cholesterol enrichment and benzyl alcohol could have a preferential effect on one leaflet or the other. Finally, D. Friend discussed the use of filipin as a probe to detect sterol domains in cellular membranes. This molecule forms a complex with sterols in membranes and gives rise to easily detected features when treated cells are viewed in the electron microscope. Some of the micrographs shown were quite striking since they showed continuously connected membranes containing domains of significantly different sterol concentrations, with no apparent physical barrier.

Session II: "Lipid Metabolism/Enzymology, Session I"

This session concerned the study of the proteins involved in the synthesis and organization of lipids into membranes. The chairman, D. Vance, briefly summarized aspects of the regulation of lipid
biosynthetic enzymes. P. Choy discussed the regulation of phosphatidyl choline (PC) in mammalian heart by CDP-choline transferase, which is found in both cytosolic and membrane bound forms. The cytosolic forms are found in high and low molecular weight forms; the high molecular weight form is an aggregate of the low molecular weight one and has a much higher synthetic activity. Next, an exciting and controversial talk was presented by R. Bishop who described a possible "flippase" that could generate lipid asymmetry. A water soluble analog of PC was synthesized and used to assay transport across rat liver microsomes. It was found that this transport was saturable, inhibitable, and protease-sensitive, indicating that a protein carrier molecule could be involved. G. Carman presented some of his recent studies on phospholipid synthesis in S. cerevisiae, concentrating on the links between phosphatidyl serine (PS) and phosphatidylinositol (PI) synthesis. Each is formed by enzymes utilizing CDP-diglyceride (CDP-DG) as a precursor. Using CDP-DG bound to a sepharose matrix, an affinity column was made that proved useful in the isolation and purification of PS and PI synths.

Session III: "Lipid Metabolism/Enzymology, Session II"

This session was chaired by M. Kates. C. Raetz focused on CDP-DG synthesis and degradation in E. Coli, using mutant isolation and recombinant DNA technology. Both CDP-DG synthase and CDP-DG hydrolase genes have been cloned in this way. The roles of these enzymes in maintaining the CDP-DG pool size was discussed as well as the possible mechanism of CDP-DG hydrolase activity. J. Cronan then discussed lipid-protein interactions of E. Coli pyruvate oxidase. Addition of lipids to the isolated enzyme results in a 20-50 fold increase in activity, although there are mutant forms of the enzyme that do not show this activation. Comparison of the DNA sequences for the mutant and wild type genes revealed elements in the carboxyl terminus region of the protein that can contribute to lipid binding. W. Schneider then described his work in the laboratory of Brown and Goldstein on the LDL-receptor. Recombinant DNA technology was employed to characterize both the LDL binding site and the cytoplasmic domain of the receptor. In particular, LDL-receptors obtained from patients deficient in LDL internalization had markedly different cytoplasmic domains from control patients. K. Hostetler discussed the use of drugs which act to specifically inhibit lysosomal phospholipases, as well as the finding of a new class of inhibitor proteins which may play a role in modulating the activity of lysosomal phospholipases.

Session IV: "Inositol Lipids"

This session was chaired by M. Hokin-Neaverson who was responsible for initially describing the phosphatidylinositol (PI) cycle and thus gave a very enlightening historical perspective to this important field. B. Agranoff discussed polyphosphoinositides in the nervous system, and his recent findings on the potential importance of the cyclic polyphosphoinositides in signal transduction. P. Downes related the theme of PI breakdown to calcium mobilization using the parotid gland as an experimental system. The GTP-dependent binding
protein was mentioned as a possible link between receptor binding and the PI specific phospholipase C activation. R. Bell presented a new model for diacylglycerol activation of protein kinase C, demonstrating that optimal activation of protein kinase C required the participation of 4 phosphatidylserine molecules and one molecule of diacylglycerol. This presentation stimulated much discussion and was the highlight of the evening. L. Cantley discussed the possibility that oncogene products may be involved in the phosphorylation of PI, and that this might be involved cell transformation.

Session V: "Lipid Translocation, Session I"

This session was the first of two sessions dealing with lipid movements between membranes. It was chaired by H. Pownall who gave a brief overview of the field. K. Wirtz presented data on two lipid transfer proteins, one specific (for PC) and one non-specific, and speculated on their mode of action. In the case of the PC-specific protein, a series of structural analogs of phosphatidylcholine were used, giving some insights into the molecular details of the hydrophobic pocket in which the lipid sits during transfer between membranes. J.W. Nichols gave a detailed kinetic analysis of lipid transfer between membranes, and suggested that the non-specific transfer protein functions by increasing the "off-rate" of lipids from membranes. M. Phillips discussed his studies on cholesterol efﬂux from cells and presented a detailed kinetic model for this process. An exciting finding of his studies was the difference in cholesterol efﬂux from normal and tumor cells and the possible role of other lipids in modulating this process. The final talk of this session was given by R. Sleight in which the use of fluorescent lipid analogs to study lipid movements in living cells was presented. Possible molecular mechanisms for lipid movement were discussed and different models were presented for each of a series of lipids in cultured cells.

Session VI: "Lipid Translocation, Session II"

This session, detailing the molecular mechanisms by which lipid molecules move about within cells, was chaired by T.E. Thompson, who summarized the formal mechanisms by which lipid translocation is expected to occur. R. Simoni discussed his recent study on the intracellular transport of cholesterol from the endoplasmic reticulum to the plasma membrane of CHO cells, made possible by the development of a new technique for rapid isolation of plasma membranes from cells, after "pulsing" with radiolabeled precursors for various lipids. A striking finding was that newly synthesized cholesterol and phosphatidylcholine appear to move to the plasma membrane by different molecular mechanisms (vesicular movement vs molecular transfer). E.A. Dawidowicz presented his studies on lipid translocation in Acanthamoeba. His major finding was that newly synthesized phosphatidylcholine appears at the plasma membrane by a process of vesicular movement. He also presented some preliminary evidence for the existence of an ER flipase protein in rat liver microsomes. G. van Meer discussed his recent findings on the movement of fluorescent lipids in epithelial cells. Fluorescent lipids were first incorporated
into liposomes containing the glycolipid GDla and these were then “fused” with the surface membrane of MDCK cells which had been infected with influenza virus. The fate of the fluorescent lipids in the treated cells could then be followed. The main focus of these studies was to determine whether certain fluorescent lipids could pass the tight junction in epithelial cells. Although this question was left unanswered, there was much enthusiasm about the approach used, and optimism that it will soon be possible, through the use of quenching agents, to determine whether transmembrane movement of a plasma membrane lipid is a prerequisite for the lateral diffusion of the lipid past the tight junction.

Session VII: "Ether Lipids"

F. Snyder, the session chairman, gave an interesting and informative historical overview of the ether lipid field. D. Hanahan then discussed platelet activating factor (PAF), an ether lipid, and its interaction with cells. The number of high affinity binding sites on most cell types is only several hundred, demonstrating the enormous potency of PAF. The results of studies using various structural analogs of PAF, some of which are competitive inhibitors, were also given. R. Wyke discussed the relationship between PAF metabolism and arachidonate metabolism in neutrophils, presenting data showing a synergistic effect between products of arachidonate metabolism and PAF function. For example, in the presence of 5-HETE, approximately 100-fold less PAF is required to produce degranulation in neutrophils. A. Hajra discussed the metabolism of ether lipids, and showed that two of the enzymes in ether lipid metabolism, alkyl DHAP synthase and DHAP acyl transferase, are located in the peroxisomes. Both Hajra, and the subsequent speaker, H. van den Bosch, presented some data on Zellweger's syndrome, a disease in which no peroxisomes are present, and in which ether lipid metabolism is deficient. van den Bosch's talk included a discussion of recent attempts at prenatal diagnosis of this disease by analysis of ether lipid metabolites.

Session VIII: "Liposomes"

This session dealt with anti-lipid antibodies, and with liposome targeting. J. Weinstein was session chairman. C. Alving gave an overview of his studies where it has been demonstrated that it is possible to make a variety of both polyclonal and monoclonal anti-lipid antibodies. Moreover, he indicated that many of these antibodies are naturally occurring. A. Janoff presented evidence that the antibodies from patients with Lupus actually recognize lipid molecules in the hexagonal phase. This finding was the basis for a new liposome-based assay for Lupus-positive sera being developed by Janoff and co-workers. D. Papahadjopoulos summarized his recent efforts to effect antibody-directed targeting of liposomes to specific cells, and to induce liposome fusion with endocytic compartments using a new kind of "pH sensitive" liposome. The talks in this session were extremely well received, and much of the material presented was quite new to the audience.
Session IX: "Glycolipids/Sphingolipids"

The session chairman was S. Gatt, who presented a brief overview of sphingolipid metabolism, indicating the various disease states which have been identified with defects in sphingolipid metabolism/degradation. V. Ginsburg discussed glycolipid differentiation antigens detected by an ingenious technique employing monoclonal antibodies and thin-layer chromatography. Specific staining patterns for one glycolipid found in synaptic layers of the retina were also shown. S. Spiegel presented recent studies on the use of fluorescent gangliosides to study the dynamics of gangliosides in cells, and their interactions with the extracellular matrix. K. Sandhoff discussed the lysosomal degradation of sphingolipids, and emphasized the role of particular activator proteins which appear to be required for lipid degradation. Data was presented showing that the activator proteins can also bind and stimulate the transfer of sphingolipids between liposomes. He also presented some data on the insertion of exogenous gangliosides (fluorescent and spin-labeled) into cells, and indicated an enzymatic method by which integration of the "foreign" molecules could be distinguished from simple binding to the cell surface.

FINAL PROGRAM FOR 1985 LIPID METABOLISM GORDON CONFERENCE

Monday A.M. "Physical Chemistry of Lipids"
Chairman: Alan Kleinfeld

1. Donald Small
   "The physical properties of lipids--From alkanes to phospholipids"

2. David Wolf
   "Probing the lateral organization of membrane lipids"

3. David Schachter
   "Lipid fluidity of the individual hemileaflets of human erythrocyte membranes"

4. Daniel Friend
   "Cholesterol (polyene-sterol) localization in natural membranes"

Monday, P.M. "Lipid Metabolism/Enzymology, Session I"
Chairman: Dennis Vance

1. Patrick Choy
   "Regulation of phosphatidylcholine biosynthesis in mammalian hearts"

2. Robert Bishop
   "Transmembrane lipid transport by an ER Flipase"
3. George Carman "Phospholipid biosynthesis in Saccharomyces cerevisiae"

Tuesday, A.M.
Chairman: Morris Kates
1. Chris Raetz "Genetic analysis of CDP-DG metabolism in E. coli"
2. John Cronan "Metabolic function of a protein-lipid interaction"
3. Wolfgang Schneider "The LDL receptor--Structural insights"
4. Karl Hostetler "Regulation of lysosomal phospholipid catabolism"

Tuesday, P.M.
Chairperson: Mabel Hokin-Neaverson
1. B.W. Agranoff "Polyphosphoinositides in the nervous system"
2. C. Peter Downes "Inositol lipids and signal transduction"
3. Robert Bell "Diacylglycerols function as intracellular regulators of protein kinase C"
4. Lewis Cantley "Oncogenes and polyphosphoinositides"

Wednesday, A.M.
Chairman: Henry Pownall
1. K.W.A. Wirtz "Mode of action of the phosphatidylcholine transfer protein"
2. J.W. Nichols "Molecular mechanisms of lipid transfer"
3. Mike Phillips "Cholesterol efflux from cells"

Wednesday, P.M.
Chairman: T.E. Thompson
1. Robert Simoni "Intracellular transport of cholesterol from the endoplasmic reticulum to the plasma membrane"
2. E.A. Dawidowicz  "Intracellular transport of lipids in Acanthamoeba"

3. Gerrit van Meer  "Lipid movement after implantation into the plasma membrane of living cells"

Thursday, A.M.
Chairman: Fred Snyder
1. Donald Hanahan  "Biochemical nature of platelet activating factor interaction with cells"
2. Robert Wykle  "Interrelationship of arachadonate factor"
3. Amiya K. Hajra  "Ether lipid metabolism in peroxisomes"
4. H. van den Bosch  "The Cerebro-Hepato-Renal (Zellweger) Syndrome: An inborn error of ether lipid biosynthesis"

Thursday, P.M.
Chairman: John Weinstein
1. Carl Alving  "Antibodies to phospholipids, lipid bilayers, and liposomes"
2. Andrew Janoff  "Structural specificity of anti-lipid antibodies"
3. Demetrios Papahadjopoulos  "Ligand-directed targeting of liposomes: New strategies for enhancing cytoplasmic delivery of liposome contents"

Friday, A.M.
Chairman: Shimon Gatt
1. Sarah Spiegel  "Probing the dynamics and functions of gangliosides using fluorescent gangliosides"
2. Victor Ginsburg  "Glycolipid differentiation antigens detected by monoclonal antibodies"
3. Konrad Sandhoff  "Role of activator proteins in sphingolipid metabolism"