32384

A PROGRESS REPORT

to

The Atomic Energy Commission
Washington, D. C. 20545

SPIN-LABELED MEMBRANES

submitted by

Alec D. Keith

Period covered: September, 1973 to the present

NOTICE

This report was prepared as an account of work sponsored by the United States Government. Neither the United States nor the United States Atomic Energy Commission, nor any of their employees, nor any of their contractors, subcontractors, or their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness or usefulness of any information, apparatus, product or process disclosed, or represents that its use would not infringe privately owned rights.

MASTER

Biophysics Department
The Pennsylvania State University
University Park, PA 16802

July, 1974

DISTRIBUTION OF THIS DOCUMENT IS UNLIMITED

DISCLAIMER

This report was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor any agency Thereof, nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial product, process, or service by trade name, trademark, manufacturer, or otherwise does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or any agency thereof. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government or any agency thereof.

DISCLAIMER

Portions of this document may be illegible in electronic image products. Images are produced from the best available original document.

ABSTRACT OF PROPOSED WORK

The effects of membrane structural perturbation induced by a number of semispherical molecules will be investigated. These investigations will concentrate on physiological processes which have a coincident detectable effect on the motion of spin labels. The interrelationship between membrane physical state and cytoplasmic viscosity will be investigated through the technique of using nickel as a paramagnetic line-broadening agent to subtract ESR signals outside membrane-bounded zones. The use and synthesis of ^{15}N spin labels will be further developed and the general treatment of electron spin exchange and magnetic dipole interactions continue to be developed for the purpose of making nickel more useful and better understood as a line-broadening agent.

PROGRESS REPORT

I. Membrane structural perturbation:

Eletr et al. (ref. 42) turned out to be a central observation for a considerable amount of research. They showed that Adamantane and BHT are capable of disordering hydrocarbon domains in membrane phospholipids. These perturb the temperature dependence of spin label motion, cell growth and oxygen uptake of whole cells. This general type of membrane effect will be further described in proposed work.

ADAMANTANE

BHT

II. Cytoplasmic viscosity:

Keith and Snipes (ref.40) also turned out to be an important research approach. As an immediate follow-up to determine the average restriction to spin label motion imposed by the aqueous cytoplasm we carried out research on sarcoplasmic reticular vesicles using spin labels that could be localized in and on the vesicle membranes (53). Appropriate electron microscopy was carried out which verified that NiCl_2 used in the experiments did not deform the vesicles. The general approach of ref. 40 was that NiCl_2 broadened away nitroxide signals within about 15 Å spacing and that nickel is membrane impermeable, therefore that nickel would subtract away nitroxide signals outside membrane-bounded regions but not inside. Membrane-permeable spin labels are then free to probe the bounded space without an interfering signal from outside the bounded region. Some cells and cellular preparations are deformed by the NiCl_2 treatment and we have not yet learned how to prevent deformation to these preparations. Red blood cells are such an example and, of course, we

will not use such samples that deform until we have developed a means of preventing deformation.

Considerable research, using this general capability of probing the viscosity of local zones is presently being undertaken and will be treated in proposed research.

III. Phase transitions or temperature-dependence of physical state:

In the past we have used spin-label motion as a parameter to correlate with biological function to help make assignments of inferred phase transitions to physical state changes in the membrane lipids. References 44, 45, 51 and 55 were of this general type where earlier observations were extended to other systems. Ref. 55, however, shows an important novel aspect in addition. As squirrels of the San Bernardino Mountains of Southern California undergo hibernation and drop their body temperature to around 1°C there is a simultaneous production of about 8 mole percent of lysophosphatides in liver and heart tissue. Membrane preparations or phospholipid extracts from these tissues show no spin label-detectable phase transition whereas those from active squirrels do. The addition of lysophosphatidyl choline to the active phospholipid vesicles removes the transition in the same manner as that of the preparations from the hibernating animals. Since the tissue preparations from hibernating animals have much more cold tolerance than do those from active animals these findings are probably highly significant.

IV. ^{15}N spin labels (47)

We embarked on research to develop ^{15}N spin labels (ref. 47). We believe that ^{15}N spin labels have a good future; however, the development of suitable chemistry to synthesize these spin labels in good yield from available precursors is difficult. We can now synthesize piperidines in about 20% yield and pyrrolidines in about 10% yield. We are currently developing

the required chemistry to synthesize oxazolidines from available precursors. Several (3-4) schemes exist in the literature for synthesizing 2-amino-2-methylpropanol, the immediate precursor of oxazolidines by the Keana synthesis; however, when scaled down these procedures are not feasible, have very low yield or do not work at all. We are currently developing the scheme shown below and are trying to maximize yield on each step.

We believe we can get to step V with 50% yield and then to step VI with 4% yield. This would yield 0.8 g of spin labeled 2N12 for about \$1000 initial cost. Some companies sell ^{14}N spin labels for \$1/mg; therefore, based on their sale price, the synthetic cost of ^{15}N oxazolidines is in line.

V. Other efforts

Some of the other references shown are carried out in collaboration with Professor W. Snipes primarily in his laboratory, are primarily supported by other sources, or are review articles requiring no primary research (38,39,41,44,46,48,49,50 and 52).

PUBLICATIONS:

33. Keith, A. D., B. J. Wisniewski, S. A. Henry and J. C. Williams. Membranes of yeasts, in Lipids and Biomembranes of Eucaryotic Microorganisms (ed. J. Erwin) Academic Press, 1973. pp. 259-321.
34. Keith, A. D., R. J. Mehlhorn, K. Freeman and A. V. Nichols. Spin labeled lipid probes in serum lipoproteins. *Chem. Phys. Lipids*. 10: 223-236 (1973).
35. Williams, M. A., D. W. Taylor, J. Tinoco, M. A. Ojakian and A. D. Keith. Utilization of C₂₀-polyunsaturated fatty acid desaturase mutant. *Biochem. Biophys. Res. Comm.* 54:1560-1566 (1973).
36. Mehlhorn, R. J., W. Snipes and A. D. Keith. Spin label motion in fatty acids. *Biophys. J.* 13:1223-1231 (1973).
37. Keith, A. D., M. Sharnoff and G. E. Cohn. A summary and evaluation of spin labels used for probes for biological membrane structure. *BBA (Biomemb. Rev.)* 300:379-419 (1973).
38. Torres-Pereria, J., R. Mehlhorn, A. D. Keith and L. Packer. Changes in membrane lipid structure of illuminated chloroplasts - Studies with spin-labeled and freeze-fractured membranes. *Arch. Biochem. Biophys.* 160:90-99 (1974).
39. Cohn, G., A. D. Keith and W. Snipes. Spin-label translational diffusion in solid tristearin. *Biophys. J.* 14:178-188 (1974).
40. Keith, A. D. and W. Snipes. Viscosity of cellular protoplasm. *Science* 183:666-667 (1974).
41. Snipes, W., J. Cupp, J. Sands, A. Keith and A. Davis. Calcium requirement for assembly of the lipid-containing bacteriophage PM2. *BBA (Biomemb.)* 339:311-322 (1974).
42. Eletr, S., M. A. Williams, T. Watkins and A. D. Keith. Perturbations of the dynamics of lipid alkyl chains in membrane systems: Effect on the activity of membrane-bound enzymes. *BBA* 339:190-201 (1974).
43. Snipes, W., J. Cupp, G. Cohn and A. Keith. ESR analysis of the nitroxide spin label tempone in single crystals of the reduced tempone matrix. *Biophys. J.* 14:20-32 (1974).
44. Linden, C. D., A. D. Keith and C. F. Fox. Correlations between fatty acid distribution in phospholipids and the temperature dependence of membrane physical state. *J. Supramol. Biol.* 1:523-534 (1973).
45. Wade, N. L., R. W. Breidenbach, A. D. Keith and J. M. Lyons. Phase changes in castor bean mitochondria. *J. Plant Physiol.*, in press.
46. Keith, A. D. and M. A. Williams. Yeast lipid mutants: Isolation, maintenance and spin label studies, in Methods in Enzymology, Academic Press, N.Y., in press.
47. Keith, A. D., D. Horvat and W. Snipes. Spectral characterization of ¹⁵N spin labels. *Chem. Phys. Lipids*, in press.
48. Snipes, W., A. Keith and P. Wanda. Active transport of choline by a marine pseudomonad. *J. Bacteriol.*, in press.
49. Keith, A. D. Chemistry of fatty acids. *Enciclopedia della Chimica*, Firenze, Italy. In press.
50. Keith, A. D., D. Deamer and J. K. Raison. Functional aspects of the interrelationships between the lipid and protein components of biological membranes. *Science*, in press.
51. Lyons, J. M., A. D. Keith and I. J. Thomason. Temperature induced phase transitions in nematode lipids and their influence on respiration. In manuscript.

52. Snipes, W., J. Douthwright, J. Sands and A. D. Keith. Control of phospholipid synthesis and viral assembly by bacteriophage PM2. In manuscript.
53. Morse, P. D. II, M. Ruhlrig, W. Snipes and A. D. Keith. Viscosity profile of sarcoplasmic reticular vesicles. In manuscript.
54. Boltz, R. C., R. K. Riley, H. Schraer and A. D. Keith. Membrane structural changes induced by Ca^{++} : Evidence from spin labeling and electron microscopy. In manuscript.
55. Aloia, R., J. Lyons, W. Snipes and A. D. Keith. Spin label evidence for the role of lysophosphatides in hibernation. In manuscript.

TABLE OF CONTENTS

- COO-2223-12 Eletr, Williams, Watkins and Keith. Perturbations of the dynamics of lipid alkyl chains in membrane systems.
- Coo-2223-10 Snipes, Cupp, Cohn and Keith. Electron spin resonance analysis of the nitroxide spin label 2,2,6,6-tetramethylpiperidone-N-oxyl (TEMPONE) in single crystals of the reduced tempone matrix.
- COO-2223-16 Snipes, Cupp, Sands, Keith and Davis. Calcium requirement for assembly of the lipid-containing bacteriophage PM2.
- COO-2223-5 Mehlhorn, Snipes and Keith. Spin label motion in fatty acids.
- COO-2223-8 Keith, Mehlhorn, Freeman and Nichols. Spin labeled lipid probes in serum lipoproteins.
- COO-2223-6 Cohn, Keith and Snipes. Spin label translational diffusion in solid tristearin.
- COO-2223-17 Snipes and Keith. Viscosity of cellular protoplasm.
- COO-2223-13 Keith, Sharnoff and Cohn. A summary and evaluation of spin labels used as probes for biological membrane structures.
- COO-2223-18 Preprint