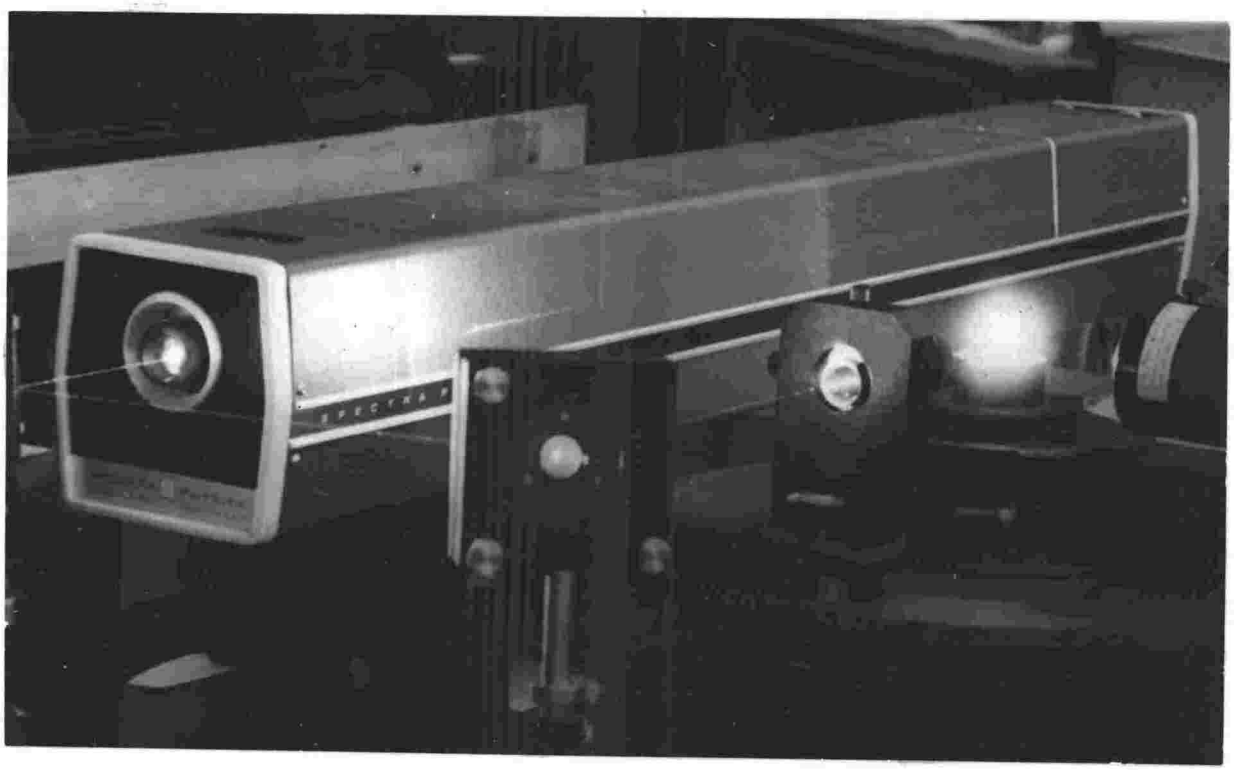
BIOPHYSICAL STUDIES OF MACROMOLECULES



P.R.WILLS

FOREWORD

This thesis is presented in fulfilment of the requirements for the degree of Doctor of Philosophy in Biochemistry at the University of Auckland by Peter Rowland Wills.

A thesis is very rarely born of a single person's labour and this one is no exception. I would therefore like to express my gratitude to the many people who have helped me at various stages along the way, and trust that the final result does justice to their hopes. In particular, I would like to acknowledge the wise guidance I have received from my supervisors, Dr R. Geddes (Department of Biochemistry) and Dr J.D. Harvey (Department of Physics). Their encouragement and understanding in changing circumstances has been of great assistance to me and will always be remembered. Several other friends who I would like to mention have helped in specific ways: Martin Upsdell gave me advice about computing and taught me Algol; Greg Stratton and Nan Pin Chee prepared samples of glycogen; Warren Davison managed the photography; Helen Parkin provided all sorts of assistance in the day to day routine of the laboratory; Crispen Gardiner arranged working space for me at the University of Waikato; John and Judi Winslade gave me the key to their home in Mt Eden.

Thanks are also due to Professor A.G. Renwick in whose department I have had the benefit of working for the last four years.

The thesis was typed by Ms L. Bailey and Mrs S. Zimmerman. Their patience and expertise are evident on every page and the fact that the task was completed at considerable personal cost to them is appreciated.

My list of acknowledgements would not be complete without a special note to my wife Lynette who has had to live through the four years during which this thesis has been in preparation. She has helped me to do all sorts of things, ranging from getting started to getting finished, but mostly it has been enough just for her to be there.

This thesis began as a brief excursion into the field of laser light scattering. At that stage it was to be completed within a year or so and submitted for the degree of Master of Science. However, the opportunity to extend the work into a Ph.D. offered itself and I accepted, knowing there would be plenty to interest and challenge me. Glycogen was the obvious choice of substances to examine since it was regularly prepared with high purity in our laboratory. It soon became evident that other techniques had to be used in conjunction with intensity fluctuation spectroscopy if the maximum benefit was to be gained from this new biophysical method. We therefore decided to attempt to give a complete hydrodynamic description of glycogen making use of a minimum of data (Chapter 3). This led to an examination of the theories which treat macromolecules as hydrodynamic particles (Section 1.2). It was recently found that glycogen can be modified using disulphide-bond breaking reagents, so a whole new series of measurements were made on the new material. The results of this study have led to a reappraisal of our knowledge of the molecular structure of glycogen.

We were also interested in the possibility of using our laser light scattering equipment to make measurements on protein solutions (Chapter 4). We began with bovine serum albumin and gathered information on its interaction with salicylate which correlated well with the previous work of W.N. Vant in our laboratory. What can be accomplished with difficulty is usually

more interesting that what can be accomplished with ease, and so we decided to examine the properties of lysozyme using intensity fluctuation spectroscopy. This substance is right at the lower limit of the size range of molecules which can be examined using our equipment, but we met with some degree of success and learnt many valuable lessons.

No-one who believes science is a philosophical adventure can write a thesis without his prejudices becoming evident at some stage. I have therefore started with a brief consideration of some of the deeper questions facing the type of research in which I have participated. The thoughts in Section 1.1 correspond to many evenings and lunchtimes spent in spirited conversation and debate with a number of close friends, one of whom is no longer here. Some of the broader aspects of scientific philosophy were discussed in papers which I gave at consecutive annual Religious Studies Colloquia at the University of Auckland. The published versions of these papers are included in Appendix IV because they form an integral part of the intellectual background from which this thesis has originated. I acknowledge the profound effect which my teachers (Professor John Morton, Dr Robert Mann, Mr David Williams), fellow students (Bill Vant, Bill Wilson, Mike Bevan) and friends (Fletcher Cole, Greg Judkins) have had on the development of the ideas expressed in these philosophical essays.

Each section of this thesis has been written so that it can be read and understood without knowledge of all the preceding material. This has necessitated the rigorous use of a cross-referencing system allowing definitive material not covered in one section to be found elsewhere.

Peter R. Wills

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ABSTRACT

Hydrodynamic theories of macromolecular structure have been critically examined and used in the experimental study of the conformation of various biological molecules. This work has been carried out giving careful consideration to ancient and modern wisdom.

The fundamental molecular structure of liver glycogen has been investigated using a variety of biophysical techniques, including intensity fluctuation spectroscopy. It has been found that above a certain minimum size, molecules of this material are hydrodynamically equivalent to one another, and behave as if comprised of ideal spherical subunits. Smaller molecules do not have a smooth hydrodynamic surface and display a much higher degree of frictional interaction with the aqueous solvent. It has also been shown that when treated with disulphide-bond breaking reagents, large glycogen molecules are disrupted, but the structure of the subunits is undisturbed. The role of protein in glycogen structure has been confirmed by these studies.

Intensity fluctuation spectroscopy has also been applied to the study of protein conformation. The frictional coefficients of bovine serum albumin monomers and dimers have been measured, and an apparent conformational change in the monomer detected upon the binding of salicylate. The unfolding and subsequent aggregation of lysozyme when it is thermally denatured have been observed and the hydrodynamic radii of the native, folded state and the expanded, unfolded state of protein have been measured. There is a well defined transition temperature for the denaturation process.

CHAPTER ONE PROLEGOMENA

CHAPTER TWO EXPERIMENTAL PROCEDURES

CHAPTER THREE THE MOLECULAR SIZE AND SHAPE OF LIVER GLYCOGEN

CHAPTER FOUR LASER LIGHT SCATTERING STUDIES OF PROTEINS

GLOSSARY OF SYSTEMATIC NAMES

DNA	Deoxyribonucleic acid
Iothalamic acid	5 - Acetamido - 2,4,6 - triiodo - N - methyl - isophthalamic acid
PPO	2,5 - Diphenyloxazole
POPOP	1,4 - Di - 2 - (5 - phenyloxazolyl) - benzene
salicylic acid	2 - hydroxy benzoic acid