STUDIES OF MACROMOLECULAR INTERACTIONS

by

DONALD JOHN WINZOR B.Sc. Ph.D. (Adel)

Department of Biochemistry

University of Queensland

A compilation of published papers presented for the Degree

of

Doctor of Science

in

The University of Adelaide

1975

PREFACE

This thesis contains published accounts of original research conducted in the period 1961-1974, during which time my interest has centred on the quantitative study of rapidly and reversibly associating macromolecular systems. Within the same time period two reviews on this subject have also been compiled, the first at the instigation of Professor S.J. Leach for inclusion in a volume of the Molecular Biology series published by Academic Press: the second, written in collaboration with Professor L.W. Nichol, was requested by Dr. A.R. Peacocke for the Clarendon Press series of Monographs in Physical Biochemistry. These two reviews are listed (by title only) as an Addendum.

Since the biological implications of rapid, reversible interactions in relation to metabolic control have been considered extensively, it is surprising that relatively little attention has been paid to the development of methods for the experimental detection and quantitative study of such equilibria. The initial stimulus for the present research endeavours stemmed from the observation of positive concentration dependence for the sedimentation coefficient of gluten proteins (paper III-1). At that time (1962) experimental studies of associating systems were in their infancy despite the fact that Gilbert [Discussions Faraday Soc. 20, 68 (1955)] had provided the mathematical solution to their migration behaviour some seven years earlier. Chapter I contains a collection of papers concerned with the development of methods for the quantitative study of associating

systems. Papers dealing with the consequences and study of preferential binding of ligands to macromolecules are collected in Chapter II. Chapter III contains studies of interacting systems by methods developed in the previous two Chapters, and the final Chapter presents a miscellany of manuscripts on non-interacting systems: papers dealing with the fractionation of wheat proteins are included in this section. Manuscripts within a Chapter are presented chronologically.

A major section of the research contained in this thesis is the development of methods for the study of reversibly interacting systems, a project that has been done largely in collaboration with Professors L.W. Nichol and A.G. Ogston, the present and former Heads of the Department of Physical Biochemistry at the Australian National University. My transfer in 1968 from the CSIRO Wheat Research Unit to the Biochemistry Department at Queensland University has heralded the commencement of a further fruitful research liaison - with Dr. C.J. Masters, who has introduced me to areas of biochemistry into which I would not otherwise have ventured. To all of my research colleagues, and to those three in particular, I am extremely grateful for their friendship, advice and assistance.

October, 1975 D.J.W.

CONTENTS

CHAPTER I. STUDIES OF INTERACTING SYSTEMS: METHODOLOGICAL ASPECTS

I-1 Studies of Chemically Reacting Systems on Sephadex. 1. Chromatographic Demonstration of the Gilbert Theory,
D.J. Winzor and H.A. Scheraga
Biochemistry 2, 1263 (1963)

I-2 Studies of Chemically Reacting Systems on Sephadex. 2. Molecular Weights of Monomers in Rapid Association Equilibrium,
D.J. Winzor and H.A. Scheraga
J. Phys. Chem. 68, 338 (1964)

I-3 The Determination of Equilibrium Constants from Transport Data on Rapidly Reacting Systems of the Type A + B $\rightleftarrows$ C,
L.W. Nichol and D.J. Winzor
J. Phys. Chem. 68, 2455 (1964)

I-4 Migration of Rapidly Equilibrating Systems Comprising Two Reactants with Equal Velocities,
L.W. Nichol and D.J. Winzor
Biochim. Biophys. Acta 94, 591 (1965)

I-5 Effects of Concentration Dependence in Gel Filtration,
D.J. Winzor and L.W. Nichol
Biochim. Biophys. Acta 104, 1 (1965)

I-6 Reconciliation of Zonal and Frontal Studies of Concentration Dependent Migration in Gel Filtration,
D.J. Winzor
Biochem. J. 101, 30C (1966)

I-7 Detection of Interaction in Gluten Extracts by Gel Filtration,
D.J. Winzor
Arch. Biochem. Biophys. 113, 421 (1966)

I-8 Interpretation of Zonal Asymmetry in Gel Filtration,
M.V. Tracey and D.J. Winzor
Arch. Biochem. Biophys. 117, 184 (1966)

I-9 Reaction Boundaries and Elution Profiles in Column Chromatography,
L.W. Nichol, A.G. Ogston and D.J. Winzor
J. Phys. Chem. 71, 726 (1967)

I-10 Evaluation of Gel Filtration Data on Systems Interacting Chemically and Physicallly,
L.W. Nichol, A.G. Ogston and D.J. Winzor
Arch. Biochem. Biophys. 121, 517 (1967)

I-11 Quantitative Studies of Rapid Association Equilibria by Gel Filtration. The z-Average Elution Volume of a Reaction Boundary,
D.J. Winzor, J.P. Loke and L.W. Nichol
J. Phys. Chem. 71, 4492 (1967)

I-12 Enantiography of Elution Profiles in Frontal Gel Filtration,
J.A. Ronalds and D.J. Winzor
Arch. Biochem. Biophys. 129, 94 (1969)

I-13 Gibbs-Donnan Effects in Gel Chromatography,
L.W. Nichol, W.H. Sawyer and D.J. Winzor
Biochem. J. 112, 259 (1969)

I-14 A Differential Chromatographic Study of Macromolecular Changes Governed by Environmental Factors,
P.A. Baghurst, L.W. Nichol, R.J. Richards and D.J. Winzor
Nature (London) 234, 299 (1971)

I-15 Theoretical Gel Chromatographic Behaviour of Irreversibly Isomerizing Systems,
S.M.A. Meggitt, L.W. Nichol and D.J. Winzor
J. Phys. Chem. 77, 352 (1973)

I-16 The Origins and Consequences of Concentration Dependence in Gel Chromatography,
L.W. Nichol, M. Janado and D.J. Winzor
Biochem. J. 133, 15 (1973)

I-17 The Use of Gel Chromatography to Study Rapid Equilibria of the Type A + B $\rightleftharpoons$ C + D,
S.J. Lovell and D.J. Winzor
Arch. Biochem. Biophys. 156, 640 (1973)

I-18 Interpretation of Migration Patterns for Interacting Mixtures of Reactants that Travel in Opposite Directions,
L.W. Nichol, G.D. Smith and D.J. Winzor
J. Phys. Chem. 77, 2912 (1973)

I-19 Difference Gel Chromatography of Kinetically Controlled Systems: Irreversible Polymerization,
L.W. Nichol and D.J. Winzor
J. Phys. Chem. 78, 460 (1974)

I-20 Determination of a First-Order Rate Constant by Difference Gel Chromatography: Dissociation of Insulin by Alkali,
S.J. Lovell, L.W. Nichol and D.J. Winzor
FEBS Letters 40, 233 (1974)

I-21 A Gel Chromatographic Procedure that Corrects for Donnan Effects in Studies of Ligand Binding: Its Application to the Interaction of Copper Ions with Bovine Serum Albumin and Deoxyribonucleic Acid,
D.O. Jordan, S.J. Lovell, D.R. Phillips and D.J. Winzor
Biochemistry 13, 1832 (1974)

I-22 Quantitative Interpretation of Concentration-Dependent Migration in Gel Chromatography of Reversibly Polymerizing Solutes,
P.A. Baghurst, L.W. Nichol, A.G. Ogston and D.J. Winzor
Biochem. J. 147, 575 (1975)

I-23 The Treatment of Thermodynamic Non-Ideality in Equilibrium Studies on Associating Solutes,
A.G. Ogston and D.J. Winzor
J. Phys. Chem. (in press)

CHAPTER II. PREFERENTIAL BINDING OF LIGANDS: CONSEQUENCES AND METHODS OF STUDY

II-1 A Theoretical Study of the Binding of Small Molecules to a Polymerizing Protein System. A Model for Allosteric Effects,
L.W. Nichol, W.J.H. Jackson and D.J. Winzor
Biochemistry 6, 2449 (1967)

II-2 Consideration of Kinetic Data on α-Chymotrypsin in Terms of Oligomer Activities,
D.J. Winzor, J.P. Loke and L.W. Nichol
Biochem. J. 108, 159 (1968)

II-3 Curve-Fitting of Allosteric Binding Data,
L.W. Nichol, G.D. Smith and D.J. Winzor
Nature (London) 222, 174 (1969)

II-4 Reversible Adsorption of Enzymes as a Possible Allosteric Control Mechanism,
C.J. Masters, R.J. Sheedy, D.J. Winzor and L.W. Nichol
Biochem. J. 112, 806 (1969)

II-5 The Differential Adsorption of Aldolase Isoenzymes in Rat Brain,
F.M. Clarke, C.J. Masters and D.J. Winzor
Biochem. J. 118, 325 (1970)

II-6 Preferential Binding of Competitive Inhibitors to the Monomeric Form of α-Chymotrypsin,
L.W. Nichol, W.J.H. Jackson and D.J. Winzor
Biochemistry 11, 585 (1972)

II-7 Use of Affinity Chromatography for the Quantitative Study of Acceptor-Ligand Interactions: The Lactose Synthetase System,
P. Andrews, B.J. Kitchen and D.J. Winzor
Biochem. J. 135, 897 (1973)

II-8 Evaluation of Equilibrium Constants by Affinity Chromatography,
L.W. Nichol, A.G. Ogston, D.J. Winzor and W.H. Sawyer
Biochem. J. 143, 435 (1974)

II-9 Cooperative Binding of Oxytocin to Bovine Neurophysin,
D.B. Hope, M. Wälti and D.J. Winzor
Biochem. J. 147, 377 (1975)

II-10 Evaluation of Equilibrium Constants for the Interaction of Lactate Dehydrogenase Isoenzymes with NADH by Affinity Chromatography,
R.I. Brinkworth, C.J. Masters and D.J. Winzor
Biochem. J. (in press)

II-11 Thermodynamic Requirements for the Cross-Linking Theory of Lymphocyte Activation : The Interpretation of Dose Response Curves,
W.H. Sawyer and D.J. Winzor
Immunochemistry (in press)

CHAPTER III. INTERACTING SYSTEMS: APPLICATION OF METHODS TO OTHER EXPERIMENTAL SYSTEMS

III-1 Association of Gluten Proteins in Solution,
D.J. Winzor
Biochim. Biophys. Acta 74, 144 (1963)

III-2 Titration Behaviour of Bovine Thrombin,
D.J. Winzor and H.A. Scheraga
Arch. Biochem. Biophys. 104, 202 (1964)

III-3 Partial Characterization of Interactions in Aqueous Gluten Extracts,
J.A. Ronalds and D.J. Winzor
Arch. Biochem. Biophys. 129, 456 (1969)

III-4 Dissociation of Alkaline Phosphatase from *Escherichia coli* Under Conditions of Enzymic Assay,
D.J. Winzor
Biochim. Biophys. Acta 200, 423 (1970)

III-5 The Molecular Size of Enzymically Active Aldolase A,
C.J. Masters and D.J. Winzor
Biochem. J. 121, 735 (1971)

III-6 Interaction of Aldolase with the Troponin-Tropomyosin Complex of Beef Muscle,
F.M. Clarke, C.J. Masters and D.J. Winzor,
Biochem. J. 139, 785 (1974)

III-7 Effects of Phosphate on the Dissociation and Enzymic Stability of Rabbit Muscle Lactate Dehydrogenase,
S.J. Lovell and D.J. Winzor
Biochemistry 13, 3527 (1974)

III-8 Carboxylesterases (EC 3.1.1). The Molecular Sizes of Chicken and Pig Liver Carboxylesterases,
P.A. Inkerman, D.J. Winzor and B. Zerner
Can. J. Biochem. 53, 547 (1975)

CHAPTER IV. MISCELLANEOUS PUBLICATIONS

IV-1 Chromatography of the Proteins from Wheat Flour Soluble in Acetic Acid,
D.H. Simmonds and D.J. Winzor
Nature (London) 189, 306 (1961)

IV-2 Chromatographic Fractionation of the Acetic Acid Soluble Proteins of Wheat Flour on Carboxymethyl-Cellulose,
D.H. Simmonds and D.J. Winzor
Aust. J. Biol. Sci. 14, 690 (1961)

IV-3 Properties of Gluten Fractions Prepared by Ion-Exchange Chromatography on Carboxymethyl-Cellulose,
J.W. Lee, M.V. Tracey, D.J. Winzor, C.W. Wrigley and H. Zentner
Aust. J. Biol. Sci. 16, 717 (1963)

IV-4 Estimation of Molecular Weights from Zonal Ultracentrifugal Data,
D.J. Winzor
Biochem. Biophys. Acta 133, 171 (1967)

IV-5 An Experimental and Theoretical Investigation of Boundary Spreading in Gel Chromatography,
G.E. Hibberd, A.G. Ogston and D.J. Winzor
J. Chromatog. 48, 393 (1970)

IV-6 Estimation of Molecular Weight Distributions by Gel Chromatography,
N.W.H. Cheetham and D.J. Winzor
J. Chromatog. 48, 400 (1970)

IV-7 Polysaccharides of Tropical Grass Species. 2. Electrophoretic Analysis and Fractionation of *Setaria sphacelata* Hemicellulose,
N.W.H. Cheetham, R.J. McIlroy and D.J. Winzor
Carbohyd. Res. 21, 479 (1972)

IV-8 Polysaccharides of Tropical Grass Species. 3. Solution Properties of Hemicellulose B from *Setaria sphacelata*,
N.W.H. Cheetham, R.J.McIlroy and D.J. Winzor
Carbohyd. Res. 23, 57 (1972)

IV-9 Effects of Lipid Removal on the Molecular Size and Kinetic Properties of Bovine Plasma Arylesterase,
B.J. Kitchen, C.J. Masters and D.J. Winzor
Biochem. J. 135, 93 (1973)

IV-10 Further Evidence for the Concept of Bovine Plasma Arylesterase as a Lipoprotein,
M.M. Don, C.J. Masters and D.J. Winzor
Biochem. J. (in press)

ADDENDUM. REVIEW ARTICLES (by title only)

Analytical Gel Filtration,
D.J. Winzor
Physical Principles and Techniques of Protein Chemistry (S.J. Leach, ed.), Vol. A, p. 451. Academic Press, New York, 1969

Migration of Interacting Systems,
L.W. Nichol and D.J. Winzor
Clarendon Press, Oxford, 1972

STUDIES OF MACROMOLECULAR INTERACTIONS

Summary of Thesis presented by D.J. Winzor for the Degree of D.Sc. at the University of Adelaide

The thesis comprises fifty two accounts of original research performed in the period 1961-1974. Chapter I contains twenty three manuscripts dealing with methodological aspects of the study of associating systems that are either rapidly equilibrating or subject to kinetic control. Included in this Chapter are papers on the development of frontal gel chromatography as a method for detecting and studying quantitatively rapid interactions of the type $nA \rightleftarrows C$, $A + B \rightleftarrows C$ and $A + B \rightleftarrows C + D$. Osmotic and Donnan effects are shown to operate in gel chromatography, and quantitative expressions developed for their descriptions. An experimental gel chromatographic method of obtaining ligand binding data that are corrected for Donnan effects is presented. The Chapter concludes with a manuscript on the validity of current procedures of allowing for thermodynamic non-ideality in studies of reversibly polymerizing solutes.

Chapter II considers specifically the preferential binding of ligands to macromolecular systems, with major emphasis on the consequences of such binding and on methods for quantitative study of the pertinent equilibria. A model for allosteric behaviour based on protein polymerization is presented, and

methods described for distinction between this model and those based on protein isomerization. The feasibility of obtaining a complete quantitative characterization of a preferential binding system is illustrated with the α-chymotrypsin - phenylpropiolate system, in which the association constant for the binding of ligand to dimer is shown to be four-fifths of that for the ligand-monomer interaction. Also included in this Chapter are three manuscripts on the use of affinity chromatography for the quantitative study of the interactions responsible for the specific ligand effect. The final paper on ligand binding outlines the thermodynamic requirements of the cross-linking theory of lymphocyte activation by mitogens, a theory that has antagonists as well as supporters within the ranks of immunochemists.

Chapter III encompasses seven papers on protein systems that have proved to be associating systems under the conditions of study: gluten, thrombin, alkaline phosphatase (*E. coli*), aldolase, rabbit muscle lactate dehydrogenase and pig liver carboxylesterase. In addition there is a brief report of a velocity sedimentation study of the interaction between aldolase and the troponin-tropomyosin complex of beef muscle.

Chapter IV presents a miscellany of manuscripts on non-interacting systems, including three on the purification of gluten fractions. Also included is an experimental study of boundary spreading in gel chromatography that led to the formulation of an alternative random walk theory of solute migration. Three papers on the physicochemical characterization of polysaccharides are also presented. The Chapter concludes

with two manuscripts on bovine plasma arylesterase, which has been investigated to determine whether the arylesterase is an enzymic polypeptide in association equilibrium with lipid and/or lipoprotein fractions of plasma, or whether the enzyme should be regarded as a lipoprotein in its own right: the latter alternative is favoured.

To the author's knowledge this thesis contains no material published or written by any other person, except when due reference is made in the text. No part of this work has been submitted previously by the author for a degree at the University of Adelaide or at any other University.

Of the joint publications included in this thesis the author considers that he has been a major contributor to manuscripts I-1, I-2, I-5, I-11, I-12, I-14, I-17, I-21, II-2, II-10, III-3, III-7, III-8, IV-6, IV-7 and IV-8; a minor contributor to manuscripts I-19 and II-1; and an equal contributor to the remainder.

LIBRARY NOTE:

The following articles have been removed due to copyright.