STUDIES ON LACTOFERRIN,
A METAL BINDING PROTEIN IN HUMAN MILK

A Thesis presented in partial fulfilment
of the requirements for the degree of
Doctor of Philosophy
in Chemistry
at Massey University

Jeffrey Ernest Plowman

1979

"Its a bit late in the day to introduce the idea now, but almost any mammal's milk would be easier to modify than cow's milk. Pig's milk is actually nearest to human milk. Camel milk and mare's milk have a better balance for humans. Sheep's milk is OK and so is goat's. Reindeer milk would be a bit fat, dog's milk a bit thin. Now, otter's milk could be just right. Perhaps we should look into it."

M. Bateman ("Sunday Times" - London, 16 March 1975)

ABSTRACT

Lactoferrin (Lf), isolated from human colostrum, has been complexed with a variety of transition metal ions. In addition to binding two iron(III) or copper(II) ions it strongly and specifically binds two cobalt(III), chromium(III) and manganese(III) ions, in the presence of bicarbonate. Such strong, specific binding of copper(II) to lactoferrin will not occur in the absence of bicarbonate, instead only a weak interaction is observed. Lactoferrin has also been shown to weakly bind manganese(II), cobalt(II), nickel(II), zinc(II), cadmium(II), lead(II) and mercury(II), though manganese(II) will undergo aerial oxidation in the presence of the protein and bind as manganese(III). These metal ion protein complexes have been examined using the techniques of fluorescence, electronic, e.s.r. and Resonance Raman spectroscopy. The close similarity between the spectra of the complexes Fe_2Lf, Cu_2Lf, Co_2Lf, Mn_2Lf and Cr_2Lf with those of transferrin and ovotransferrin reinforce the postulate that the metal binding sites in all three proteins are similar. The sites in all three proteins are essentially rhombic in character and contain 2-3 tyrosyl residues and at least one histidyl residue. A distinct heterogeneity is evident from the e.s.r. spectrum of Cr_2Lf and metal ion replacement studies indicate that chromium in one site is more labile than the other. In addition to the specific sites lactoferrin appears to have a number of non-specific sites on the outside of the protein capable of weakly coordinating metal ions such as chromium(III) and copper(II). Differences evident in the non-specific sites available to copper when manganese(III) is bound to lactoferrin, to those available when iron(III), cobalt(III) and chromium(III) are bound, suggest that the stronger binding of manganese(III) results from it inducing a different conformational change in the protein to those other metals and one that favours the higher stability of its metal-protein complex.

A series of small molecular weight complexes of iron(III) with bi-, tri-, tetra- and hexadentate ligands, containing phenolate groups were prepared and examined by electronic,

e.s.r. and Mössbauer spectroscopy. Complexes of copper(II) with nitrogen bases and chloro- and bromo-substituted phenolates were prepared and examined by electronic and e.s.r. spectroscopy. Results from these studies would favour the involvement of three tyrosyl residues and two cis histidyl residues in the metal-binding sites of lactoferrin and from studies on the copper complexes it seems likely that one histidyl and one tyrosyl are axially coordinated to the metal. Evidence from a single crystal x-ray structure is presented which would favour the interaction of bicarbonate ion with iron(III) via a solvent (water) molecule.

ACKNOWLEDGEMENTS

I am most grateful to:

Dr E. W. Ainscough and Dr A. M. Brodie for their invaluable assistance, advice and encouragement through all stages of this project.

Dr W. C. Tennant, Dr D. G. McGavin and Dr A. R. Gainsford of Chemistry Division, D.S.I.R., Petone, for their assistance in running and analysing e.s.r. and Mössbauer spectra and for the use of their Mössbauer spectrometer.

Dr K. L. Brown of Chemistry Division, D.S.I.R., Petone, for the single crystal x-ray structural analysis of one of my compounds.

Professor N. F. Curtis of the Chemistry Department, Victoria University, Wellington, for the use of his Faraday Balance.

Professor A. D. Campbell of the Chemistry Department, Otago University, Dunedin, for microanalyses.
Professor T. M. Loehr of Oregon Graduate Centre, Beaverton, Oregon, for running and analysing spectra of some metal-lactoferrin solutions.

Dr A. W. Addison of the Chemistry Department, Drexel University, Philadelphia for supplying two of the ligands used in Chapter 2.

Dr S. V. Rumball, Dr E. N. Baker, Dr G. G. Midwinter, Mr A. Trow, Mr S. J. Bloor and others of the Chemistry/Biochemistry/Biophysics Department, Massey University, for their assistance and advice.

Mrs L. Reid for typing this thesis and Mrs J. Trow for preparation of some of the diagrams.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES