THE AUSTRALIAN NATIONAL UNIVERSITY

MACROMOLECULAR ANALYSIS SERVICE GROUP

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STAFF

Senior Fellow:

Ernest Howard Creaser, M.A., Ph.D. (Cantab.)

Research Fellow:

Keith William Bentley, M.Sc. (Well.) Ph.D. (ANU)

Research Student:

James Alexander Lindsay, B.Sc. (ANU)

Visiting Research Worker:

John Langridge, M.Sc. (N.Z.), Ph.D. (Adel.)

Senior Technical Officer:

Kathleen Anne Britt, A.A.I.M.L.T., B.Sc., (ANU)

Ultracentrifuge:

Michael Perri

Amino Acid Analyser Operator:

Ian David Davies

The group was established in May 1974 with two areas of responsibility. Firstly, to carry on research on protein evolution and newer methods of protein degradation and synthesis which were no longer appropriate to the Department of Genetics upon its reorganisation. Secondly, to operate the Amino Acid Analyser and Analytical Ultracentrifuge Services of the School and to provide advice and assistance on problems of protein and amino acid analysis. The group is presently housed in Block 'M'.

Service Operations

(a) Ultracentrifugal Analysis (M. Perri)

Two machines are operated as a service for the School; these are a Spinco Model E with multiplex automatic scanner and an M.S.E. 75 Centriscan. Due to the fact that the machines are geographically separated, an Academic Councillor was appointed by Faculty Board to act as an interface between the operator and users in the main Research School of Biological Sciences building - ne is currently Dr. Paul Scotti, of Developmental Biology. With the operation of the two machines it is possible to keep a continuing service even though one machine may be out of action for repairs at any one time. In the six months from May, some 150 analytical runs have been performed. Of these, 57 have been sedimentation velocity and 94 equilibrium runs. In general the sedimentation

runs were done to determine the purity and size of proteins and the equilibrium runs were done to provide information on density of DNA samples. Analyses by Departments of the users were; Developmental 64, Genetics 68, Population 10 and Environmental 4.

(b) Amino Acid Analysis (I. Davies)

The group has two analysers in operation; a Beckman 120C machine operated with the lithium system, and a much modified 120B with automatic multiple-sample-analysis facility. The machines required extensive servicing and re-setting up after the move into 'M' Block, but are now almost in full operation. Since the move we have done some 176 analytical runs. These were made up of 52 analyses of Legume seed proteins, 18 runs of RUDP carboxylase, 16 virus protein analyses, 17 thermophilic bacterial proteins, 45 cobalt methodology analyses and 26 analyses of chemically synthesised peptides.

RESEARCH

The research operations of the group are continuing projects which carry on from when the members of the unit were in the Genetics Department and for this year there will be some overlap in reporting. Four lines of research are currently being pursued. Firstly, a study of the evolution of the enzyme histidinol dehydrogenase; secondly, investigation of the evolution of Australian animals, as shown by the sequences of their cytochrome c molecules; a study of the value of cobalt chelates in the degradation and synthesis of polypeptides and, lastly, a programme of synthesis of small peptides with enzyme activities.

1. *Histidinol dehydrogenase evolution* (E. Creaser, J. Lindsay, K. Britt, M. Torronen)

This is a continuing programme to study the evolution of this enzyme as deduced from its amino acid sequence and to elucidate the various control mechanisms concerned in its biosynthesis in prokaryotes and lower eukaryotes. Details of the present states of the investigation are in the Genetics section.

2. *Cytochrome c in Australian animals* (E. Creaser, K. Bentley)

Analysis of the structure of cytochrome c continues from work started in Genetics with Dr. John Baldwin. Determination of the sequence of cytochrome c has been a very useful indicator of the evolution of the organisms containing the molecules, especially where other evidence, such as the fossil record, is not available or is incomplete. We have analysed cytochrome c from Echidna and Platypus by amino acid analysis and peptide mapping. Preliminary results show that the closest cytochromes c are those from frogs and rattlesnakes, which differ from those of Echidna and Platypus by approximately 13 amino acid residues, whereas mammals and

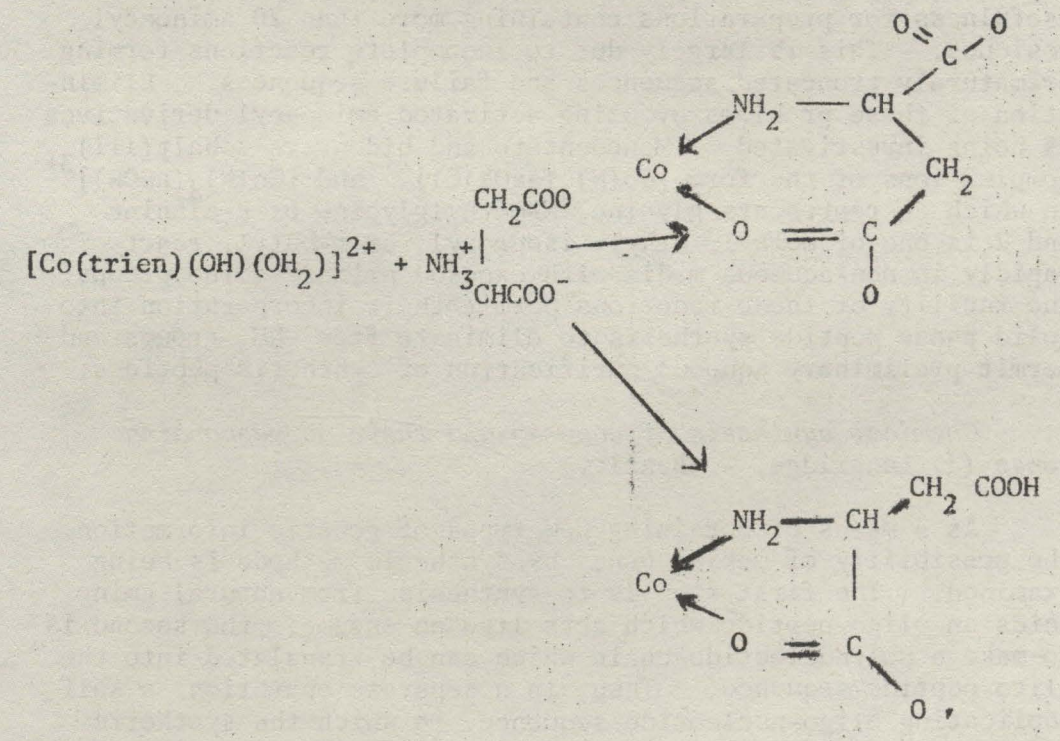
marsupials differ by some 18 amino acids. Peptide maps are consistent with this degree of change and it is hoped the complete sequences will be available this year. Other sequences, such as Murray Cod and Tuatara are proposed for investigation.

3. *Use of cobalt chelates in synthesis and degradation of Peptides* (K. Bentley, E. Creaser)

(a) Cobalt(III) chelates in protein sequence determination

Investigations continue on the use of cobalt chelates in peptide degradation. *cis*-Hydroxyaquo(tetramine)cobalt(III) ions at pH 8.0 and 25-60° have been shown to be effective in promoting the hydrolysis of amino acid esters, dipeptide esters, peptides and proteins.

For small peptides both the condensation reaction and subsequent peptide bond hydrolysis have been shown to be stoichiometric and specific for the N-terminal residue. The amino acid complexes β-$[Co(trien)(NH_2CHR'COO)](ClO_4)_2$ have been prepared and their properties examined. The hydrolysis of peptides containing aspartic acid and cysteine are more complex owing to the presence of alternative mechanisms (aspartic acid) and precipitation of the reagent (cysteine). These have been investigated further. For aspartic acid residues the pathways



in which both the 5 and 6 membered chelated species arise in the products has been shown to be temperature dependent (e.g. 40° (5:6) 85:75; 80° (5:6) = 27 , 73). Cysteinyl peptides can be modified by oxidation to cysteic acid or converted to S-CH_3 or S-benzyl. Amino ethylation of the sulphydryl groups prior to cobalt attack cannot be used, as chelation occurs at all three possible positions, the proportion of each product being temperature and pH dependent.

(b) Synthesis of modified amino acids (K. Bentley, M. Cosgrove)

Bidentate or tetradentate (e.g. ethylenediamine triethylene-tetramine) (hydroxyaquo)cobalt(III) complexes in which the (OH) and (OH_2) sites are available for substitution by α-amino and α or β carboxyl functions of amino acids to form either 5- or 6-membered chelate rings, have been found useful in the synthesis of modified amino acids. The intermediates are temperature stable at pH 1-11. They can be chemically modified in high yield by reaction with known amino acid derivatizing reagents, e.g. Fluram, FDNB, DNS, $POCl_3$ etc. before dechelation and product purification.

(c) Peptide synthesis (K. Bentley)

Methods for the synthesis and modification of clinically important peptides has, with few exceptions, little economic usefulness for preparations containing more than 20 aminoacyl residues. This is largely due to incomplete reactions forming prematurely truncated sequences and failure sequences. Elimination of these problems by using activated aminoacyl derivatives is being investigated. Monodentate and bidentate cobalt(III) complex ions of the form $[Co(N)_4(aaOR)Cl]^{2+}$ and $[Co(N)_4(aaOR)]^{3+}$ in which aa represents glycine, N-methylglycine or β-alanine and R is one of methyl, ethyl, isopropyl, or t-butyl, react rapidly in non-aqueous media with exposed primary amino groups. The facility of these reactions permit their incorporation into solid phase peptide synthesis to eliminate free -NH_2 groups and permit preliminary aqueous purification of synthetic peptides.

4. *Chemical synthesis of enzymes and their corresponding genes* (J. Langridge, K. Bentley)

As a means of obtaining new types of genetic information the possibility of making genes by synthetic methods is being examined. The first step is to synthesise from natural amino acids an oligo-peptide which acts like an enzyme; the second is to make a polynucleotide chain which can be translated into the oligo-peptide sequence. Then, in a separate operation, a self replicating oligo-nucleotide sequence, to which the synthetic gene can be attached, is selected from randomly polymerized nucleotides by the action of DNA-dependent RNA polymerase. To achieve the first step, amino acids are coupled by solid-phase peptide synthesis to design a substrate-binding site around the esterolytic activity of free histidine. A series of oligo-peptides has been made and is being tested for ability to bind substrate molecules before rearranging their chemical bonds.