A Life in Science

A Journey started in 1959 and (hopefully) still not ended

1962-66 PhD project Exeter University, UK

The original aim of my PhD project was to synthesize polymers containing different sequences of the optical isomeric residues of glutamic acid (L- and D-glutamic acid). The scheme was to synthesize the sequences L-D, L-L-D, L-L-L-D and L-L-D-D dipeptide, tripeptide and tetrapeptides and then polymerize the respective peptides to produce the sequences: $[LDLD]_n$ $[LLDLLD]_n$ $[LLLDLLLD]_n$ and $[LLDDLLDD]_n$. Model building had shown that the resultant polypeptides would fold into helices with different configurations to the standard α-helix structure e.g. a possible 3_{10} helix. If all this seems a bit naïve in the cold light of day do not forget that I started this project only just 11 years after Pauling had published the original paper on the α-helical structure of polypeptides and proteins.

However, the peptide synthesis methods available at that time (viz: coupling via activated *p*-nitrophenyl ester linkages or via carbodiimide condensation reactions) although adequate for single amino acid addition, produced some degree of racemisation when linking peptide sequences. This is a known problem with this approach because of planar azlactone formation in the intermediate stage of the reaction which can then open to the opposite optical isomer. Hence the optical rotatory dispersion spectroscopy used to analyse the conformations was confounded by the unknown levels of racemates in the polymer sequences. Newer techniques since this time have resulted in avoidance of this problem and later extension of the work succeeded in producing the relevant polypeptides.

As an aside for those of you less familiar with optical stereoisomers, the nomenclature of assigning L- or D- configurations has largely been superseded in stereochemistry by the Cahn-Ingold-Prelog convention where the geometry is assigned by the distribution of groups around the central asymmetric carbon atom according to the atomic number of the adjacent elements. However, in peptide chemistry the L- and D- assignments have been maintained. I always thought this was due to the innate conservatism of biochemists but in refreshing my knowledge of the field for this talk I found out that there is a problem with amino acid geometry. Clearly the amino acids assigned to the L-configuration have identical distribution around the asymmetric centre and are S-configuration in the Cahn-Ingold-Prelog system except for L-cysteine. Here the sulphur atom because it is in the second row of the periodic table has a higher atomic number than any of the

other substituents and the configuration is designated R-. To avoid this anomaly it is preferable to retain the old L- and D- configurations.

As is almost universal with PhD projects (in chemistry anyway) the project went off in a tangent to investigate the conformations by nuclear magnetic resonance spectroscopy, mainly because we had just obtained a 60mHz NMR spectrometer in the laboratory. This technique was in its early days and the resultant paper was the first time it had been used to track unfolding and refolding of polypeptide structures – it was my first publication and actually had more than 100 citations in the literature (almost a citation classic). My career obviously peaked at this step since my citation rate decreased for my subsequent publications.

The optical rotatory dispersion spectra for ordered helical structure and unfolded structure are shown here:

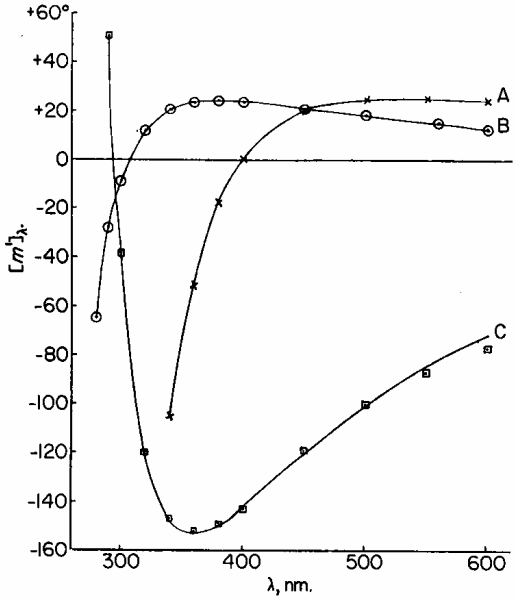


Figure 1. Optical rotatory dispersion of solutions in chloroform at 33°: A, poly(γ-benzyl L-glutamate) (IV); B, poly(γ-benzyl-L-glutamyl-γ-benzyl-D-glutamate) (II); C, poly(γ-benzyl-D-glutamyl-γ-benzyl-L-glutamate) (III).

This transition can be followed via the change in the spectra and we correlated it in this work with the gradual change in the NMR spectra demonstrating the gradual shift in the resonance characteristics around the side-chains of the polypeptide and the peptide bond.

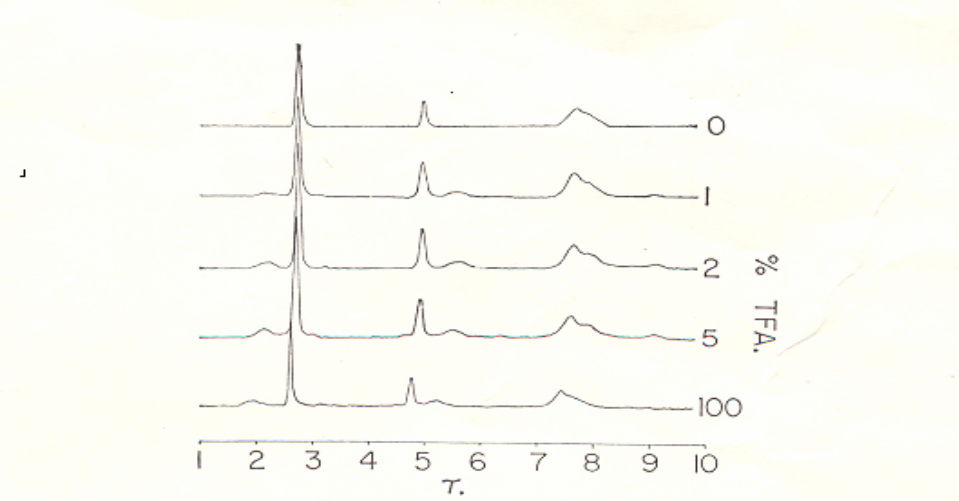


Figure 3. ^{1}H nmr spectra (60 MHz) of poly(γ-benzyl-L-glutamyl-γ-benzyl-D-glutamate) (II) in $CDCl_3$–TFA at 33°.

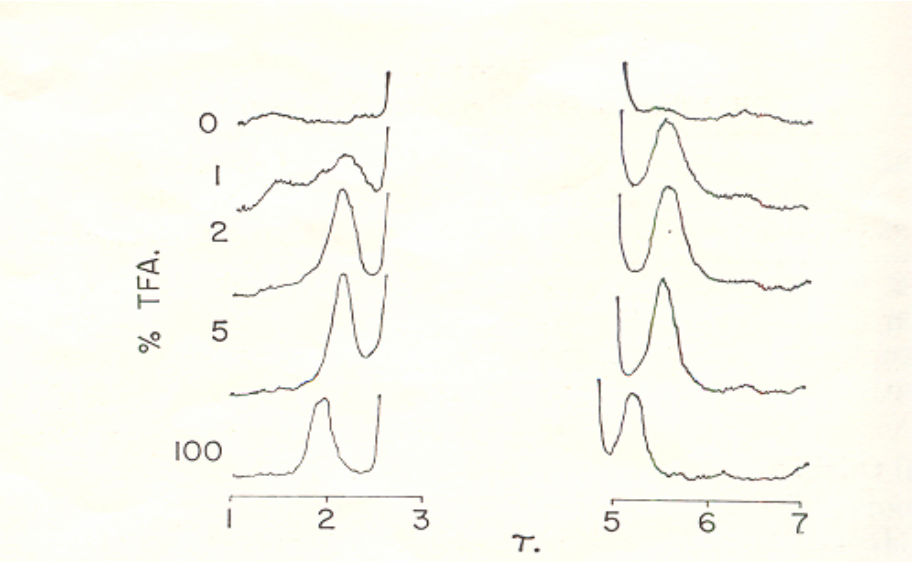


Figure 4. ^{1}H nmr spectra (60 MHz) of poly(γ-benzyl-L-glutamyl γ-benzyl-D-glutamate) (II) in $CDCl_3$–TFA at 33°; NH and α-CH regions; 150 accumulations.

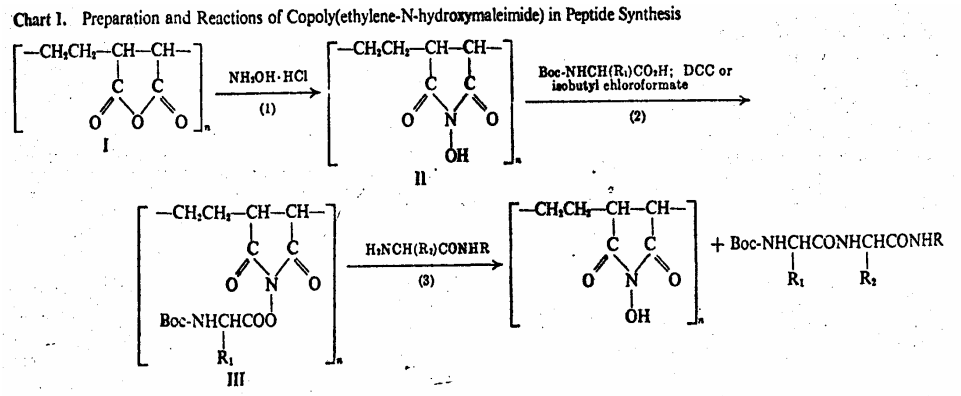
I had originally interpreted the chemical shifts in terms of differences in the relaxation times due to the ordered structure. However, later work by Walter Gratzer at King's College London showed that the chemical resonance peaks were actually significantly shifted by the shielding effect of the ordered structure and the apparent appearance in the spectra was a result of the chemical shift changing.

At the time I was finishing my PhD I had applied for post-doctoral positions at Harvard Medical School and The Weizmann Institute in Rehovoth Israel. I was quite keen on the Weizmann position but the post was for a year later

than the finish of my PhD and I accepted the Harvard position. As it happened I had a period of illness before finishing the PhD and I had to delay by about 8 or 9 months so in fact I could have gone to Israel. However, I would actually have been there right in the middle of the six-day war so as it was things worked out for the best!

1966-68 Harvard University Medical School, USA

The work carried out in Professor Blout's laboratory was an extension of the approach to peptide synthesis pioneered by Merrifield – solid-state peptide synthesis on an insoluble resin platform. Rather than building up the growing peptide chain on the resin base, as in the Merrifield approach, the route in Blout's laboratory was to link the amino acid residue to an insoluble polymer resin base but via an activated ester link which exchanged from the resin in the presence of unprotected amines. Hence any unreacted amino acid residue remained out of solution and could be removed from the growing polypeptide simply by filtration. This avoided the problems of missing and truncated sequences that arise in the Merrifield procedure and also maintained a fast rate of reaction. I found that this could be induced to go even faster when carrying out the reaction in a blender.



Using this method we synthesized the Ribonuclease S (twenty residue) peptide. However, it became apparent that as the peptide chain increased in length, especially when the peptide started to adopt secondary structure in solution, the kinetics of the reactions became prohibitively slow.

Although Harvard Medical School is the world's premier institution in the biomedical field and the social and cultural time I had there was one of the best times I had in my work career the actual work was relatively unsatisfactory. I felt the project ended up in somewhat of a dead end and there was another project in the lab' that I was actually more interested in and I felt in addition they were going about it the wrong way!

1968-69 **Imperial College, London, UK**

On my return to England I was involved in a study of the spinach ferredoxin protein by electron paramagnetic resonance (EPR) and magnetic optical rotatory dispersion spectroscopy carried out in the laboratory at Imperial College as part of a program for determining the structure around the iron-sulphide chromophore that was the active centre of the molecule responsible for its electron transport function. These atoms were thought to form a cage with the electronic configuration delocalized in the reduced form and a good model for electron paramagnetic resonance spectroscopy analysis (due to the unpairing of the electrons). My main interest was in matching the magnetic optical rotatory dispersion (MORD) spectra with the EPR profiles and showing the influence of the structure on the spectra in the presence or absence of a magnetic field. However I did carry out EPR studies on the spinach ferredoxin. We purchased sacks of spinach from Covent Garden market which we then proceeded to extract with litres of acetone! O,H and S committee these days would be horrified at our technique. This is also the one and only time that I used liquid helium. The main drawback with this material was the open vacuum container kept on blocking up with solid oxygen that we would evaporate by use of a metal rod! With the EPR spectrometer (with radio wave guides partly made up with war-time surplus material from Malvern Radar Establishment) the instrument could be operated in two modes with different magnetic field powers. We had to screw on extra magnetic faces for the high-power level but had to remember to tighten the screws. One of the researchers set up the magnet but forgot to put in the holding screws and when the electric power was turned on the two faces crashed together destroying the caviton sample chamber! This cost £300 which was not small beer in those days. The PhD student responsible kept a very low profile for a time after this incident.

The work I published on the MORD work emphasized the relationship to the classification by Shashoau for such spectra and the relationship to the electronic properties surrounding the iron-sulphur chromophore. I was not particularly enthused with the paper I published but at a later meeting no less a person than Carl Djerassi (the inventor among other achievements of the contraceptive pill) praised the paper as showing evidence for the theories surrounding the cage structure of the chromophore!

1970-73 **MRE, Porton Down UK**

Extending my expertise in optical rotatory dispersion(ORD)/circular dichroism(CD) to investigate protein structure was valuable in obtaining a Medical Research Council Fellowship at The Microbiological Research Establishment (MRE) at Porton Down (aka The Germ Warfare Establishment).

MRE was processing bacterial proteins via continuous bacterial culture and producing large quantities of medically useful agents. One of the first products from this procedure was L-asparaginase which had been shown earlier to be an effective therapy for acute lymphoblastic leukaemia. MRE was keen to develop the enzyme as a therapeutic reagent as an initial step to downplay its germ warfare role and improve its public relations profile. A few years after I left it was closed down as a defence establishment and became a Public Health Services Laboratory. The aim of the project with the asparaginase enzyme was to gain more knowledge of its physicochemical properties and determine whether such knowledge could be used to raise the therapeutic efficacy.

The L-asparaginase enzyme from *Erwinia caratovora* (now reclassified as *Erwinia Chrysanthemi*) has a molecular weight of 135,000 and consists of four subunit chains of approximately 35,000 mol.wt. Ultracentrifugation studies showed that the enzyme had a very compact conformation in solution (a frictional ratio close to 1). The ORD and CD spectra showed the profiles typical of ordered proteins with significant helical conformation. When taken up in 8M urea the spectra undergo transitions typical of complete unfolding and denaturation of the protein as long as the pH of the solutions was well away from the pI of 7.5 to 8. However, when taking the protein into a surfactant such as sodium dodecyl sulphate (SDS) the spectra indicate a more ordered structure and ultracentrifugation data indicated dimeric rather than monomeric subunits.

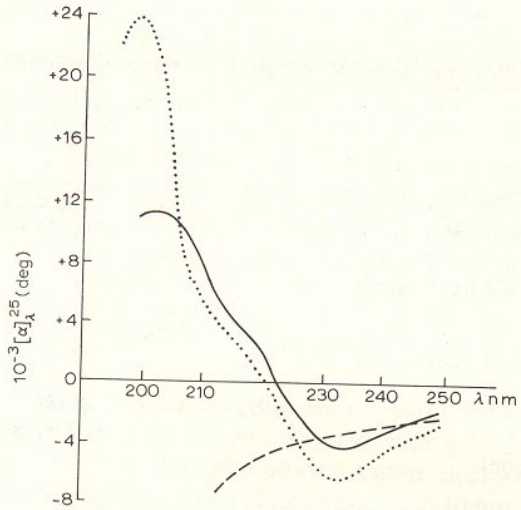


Fig. 1. Optical rotatory dispersion spectra of L-asparaginase isolated from *Erwinia carotovora*. Solution prepared in pH 7.0 buffer, 0.1 I sodium phosphate and 0.1 I sodium chloride; protein concentration 0.024–0.030 mg/ml. Experiments at 25 °C, initially in pH 7.0 buffer (———); and then after the addition of 8.0 M urea and incubation at 37 °C for 2 h (– – –); and also after treatment with 0.1% sodium dodecylsulphate and incubation at 37 °C for 24 h (· · · · · ·).

Reconstitution of the urea-denatured subunits results in a return to the native enzyme when carried out at pH 6-7 with 70% recovery of activity but when carried out at pH >10 gives only 10% recovery and the CD profile typical of the SDS-treated material.

More detailed investigations of the kinetics of the denaturation/refolding via optical rotation showed that the unfolding of the native structure and dissociation into subunits occurred over a number of hours but pretreatment with SDS followed by urea almost instantaneously gave disordered conformation. The conclusion was that the dissociation of the tetramer to subunits was the rate-determining step and the subsequent unfolding of the secondary structure was quite rapid.

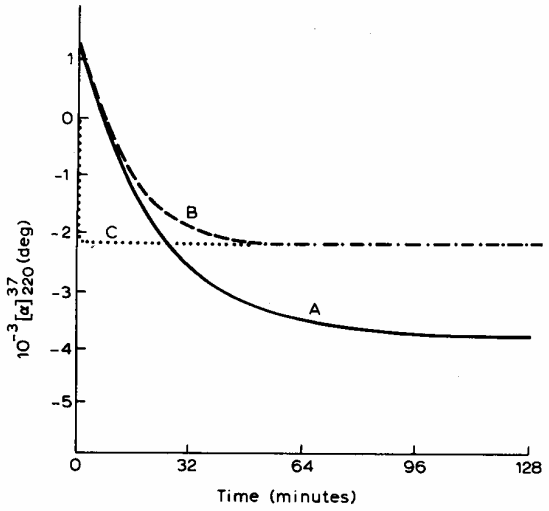


Fig. 7. Kinetics as based on optical rotation of the dissociation and unfolding of *Erwinia carotovora* L-asparaginase. Optical rotation at 220 nm measured as a function of time at 37 °C in pH 7.0 buffer, 0.1 I sodium phosphate and 0.1 I sodium chloride, (A) with 7.5 M urea; (B) with 7.5.M urea and 0.1% sodium dodecylsulphate; (C) after the pre-dissociation of enzyme subunits in 0.1% sodium dodecylsulphate at 37 °C for 24 h, followed by treatment with 7.5 M urea.

This work suggested a possible mechanism of increasing the therapeutic efficacy of the enzyme although I think the work went along other lines. This material is still one of the main components in a multi-drug treatment for lymphoblastic leukaemia and was only recently reapproved in the EU listing of therapies.

In my time at Porton Down I also carried out conformational studies on the ion-gating peptide alamethicin. This peptide is unusual in that it contains the non-standard amino acid residue 2-aminoisobutyric acid. Later work showed that this is residue strongly induces helical conformations in peptides. At the time I worked on the peptide it was thought to be a cyclic peptide joined by cross-linked glutamyl-glutamine residues through the γ-chain. However, work carried out in later years indicated that the peptide was in fact linear

but forming trimers. The CD studies I carried out showed a strongly ordered structure typical of α-helix formation although the wavelength of the trough in the spectra was blue-shifted by 3 nm from the typical α-helix pattern. However, the model that we built up suggested that it could easily form into a helix structure.

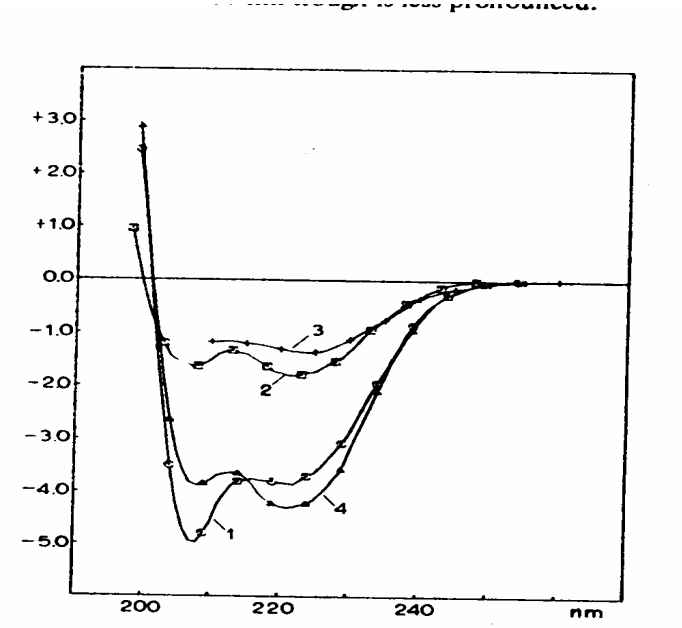


Fig. 1. Circular dichroism of alamethicin: 0.18 mg/ml, 1.00 mm path, 25°, mean residue MW 93; solvents 1, 100% ethanol; 2, 10% ethanol in water; 3, 6.0 M guanidine hydrochloride; 4, 1% sodium dodecyl sulphate (computer-drawn curves).

Proposed helical structure of alamethicin molecule:

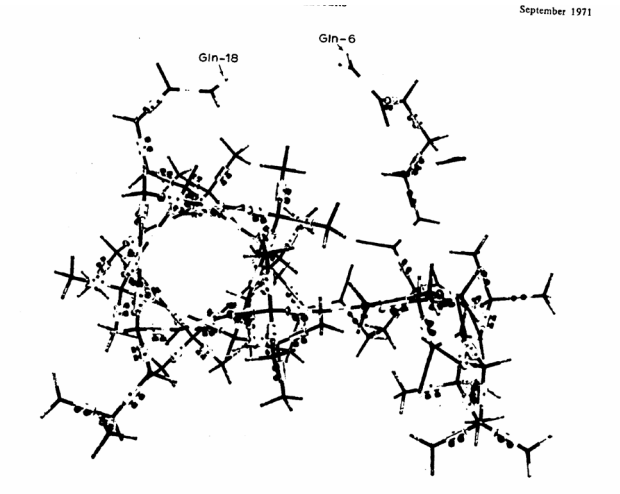


Fig. 2. CRE (Cambridge) molecular model of alamethicin illustrating the potential for α-helical formation by up to a maximum of 60% of the residues; being those 11 residues located between the two prolines including Gln-6.

Acknowledgement

The other side-project that I pursued was measuring the ORD/CD spectra of poly-γ-D-glutamic acid, almost a reprise of my PhD project. The interest in this molecule is that forms the protecting coat of the anthrax bacillus and hence there were large quantities available at Porton Down! The interesting feature of the spectra was that the ionized form molecule (sodium salt) apparently did not completely unfold as is common with other polypeptides but adopted an ORD spectrum compatible with a β-pleated sheet conformation.

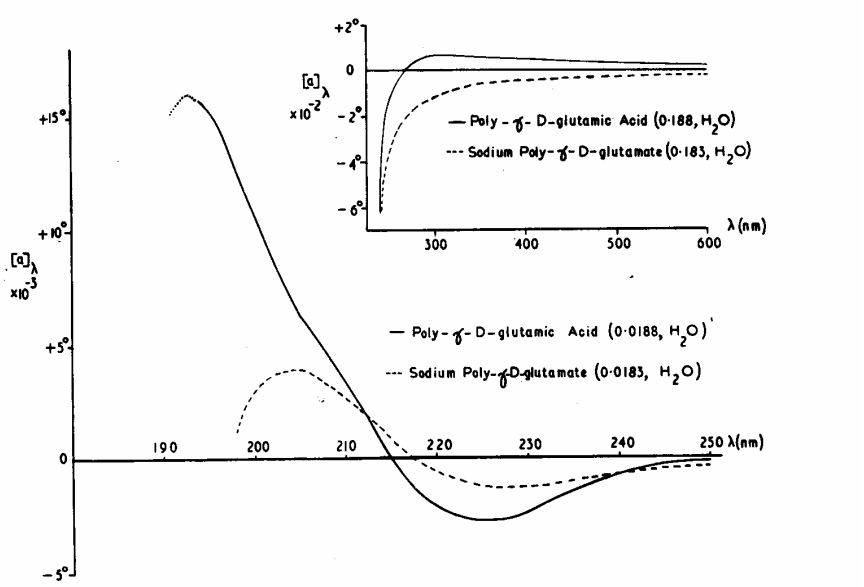


Fig. 1. Optical rotatory dispersion spectra of poly-γ-D-glutamic acid (——) dissolved in water and sodium poly-γ-D-glutamate (- - - -) dissolved in water. The far-u.v. maximum for the poly-γ-D-glutamic acid is subject to some uncertainty due to noise and has been shown dotted.

When I published the paper on this work I did flirt with the idea of including Watson & Crick's phrase from their first DNA paper – "it has not escaped our attention that this structure offers a simple mechanism for genetic replication", i.e. "it has not escaped my attention that this structure offers a reason for the unusual stability of the anthrax organism". However, (A) I am not as arrogant as Watson & Crick and (B) my career was not on such solid grounds as theirs!

1973-76 Papanicolaou Cancer Institute, Miami USA

The department that I worked in at the Pap Cancer Institute had nothing to do with cancer but was involved with the chemistry and physiology of the angiotensin-bradykinin vasoactive system. The Institute maintained us under sufferance mainly because the Department head, James Ryan, actually brought in most of the funding to the Institute. My contribution mainly involved again ORD/CD studies of peptide analogs of bradykinin. In

another throwback to earlier work, bradykinin was the first physiologically active peptide synthesized by the Merrifield peptide synthesis method. The Merrifield methodology was carried out in the laboratory to produce the analog peptides although I was only peripherally involved. By this time the procedure had evolved to a computer-controlled assembly where you had all the relevant components lined up in flasks and pressed a button to start the actual chemical process.

While we were at Miami, Valerie and I met the Brazilian scientist Rocha e Silva who had discovered bradykinin. He was quite an eccentric individual and as I will be soon semi-retired at the time. He had isolated bradykinin from a dog that had been bitten by the South American snake *Bothrops Jarararca*. This snake not only injects kininase that releases the blood-pressure lowering agent bradykinin into the bloodstream but also injects what are called bradykinin-potentiating peptides which block the action of angiotensin-converting enzyme (ACE) to produce angiotensin II that maintains blood pressure with the result that in a matter of minutes the blood pressure drops to zero. The work I was involved in that point was related to the investigations by Squibb pharmaceutical that led to the current ACE-inhibitor drugs for treatment of high blood pressure.

We studied peptides with dehydroproline substitutions in the sequence (for native proline residues) and the subsequent changes in conformation revealed by the CD spectra. The concept behind the study was the restriction of the number of possible conformers with the dehydroproline residues even as compared to the restrictive prolyl residues. This work was extended to the bradykinin potentiating peptide where the influence of gradually shortening the chain to see where the secondary structure became important. The work demonstrated that the peptides started adopting some sort of ordered structure in solution when five amino acid residues formed the chain.

1976-81 John Curtin School for Medical Research ANU Canberra

Transferring from the Pap Cancer Research Institute/University of Miami to ANU in Canberra I returned to the study of protein secondary and tertiary structure. I was involved in studies of the oxygen-transport protein hemocyanin. This protein had a more complex tertiary structure than asparaginase.

The native hemocyanin is a globular protein with two distinguishable fractions (17S and 25S) of different sedimentation coefficients. We demonstrated that the two components were both hexamers with at least 6 different subunits. However, since at least two of the subunits were actually dimers the 25S fraction is a virtual dodecamer.

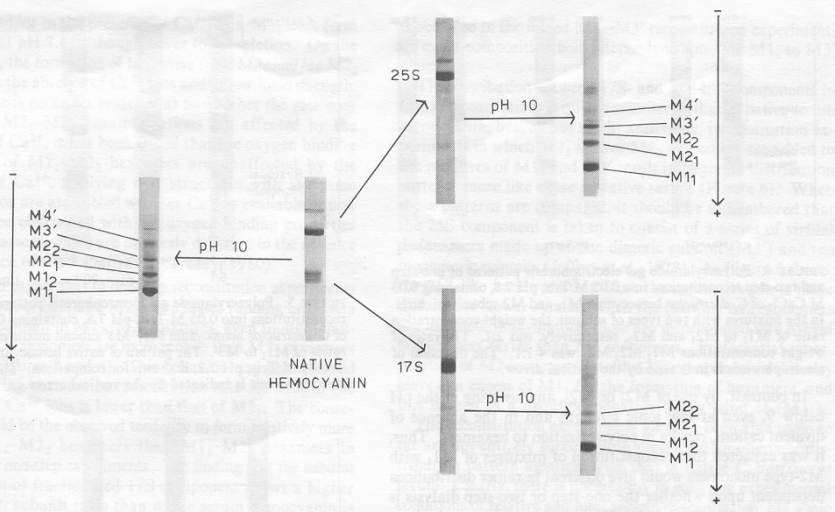


FIGURE 1: Polyacrylamide gel electrophoresis patterns of native *C. destructor* hemocyanin and the individually separated 17S and 25S components run in 0.05 M Tris, pH 7.8. The gel patterns of the respective alkaline dissociation products of hemocyanin serum, 17S components and 25S components run in 0.05 M glycine, pH 10, containing 0.1 mM EGTA are also shown. The individual subunits $M1_1$, $M1_2$, $M2_1$, $M2_2$, M3′, and M4′ that have been identified are marked. The direction of electrophoresis is indicated by the vertical arrows.

As with the asparaginase enzyme the denaturation in urea, followed by absorbance spectroscopy, demonstrated a time-dependent unfolding. Because of the complex nature of the subunit distribution it was difficult to differentiate the contributions of polymeric dissociation from disruption of the secondary structure of the subunits.

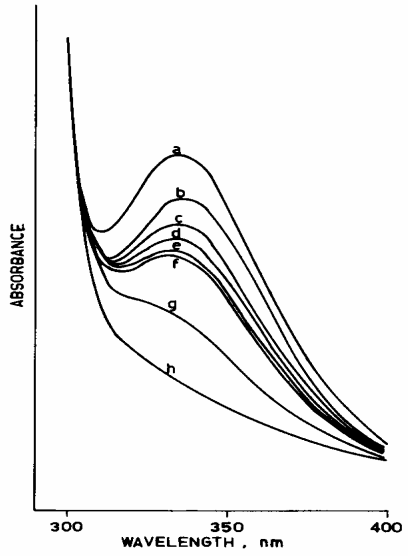


FIG. 2. Change in the absorbance of a solution of *Cherax destructor* hemocyanin, in the wavelength region 400 to 300 nm, over a period of time after dilution into 6 M urea. Curve (a) native hemocyanin, curves (b) to (f) taken at 5-min intervals after dilution into 6 M urea, curve (g) 2 h after dilution, and curve (h) 24 h after dilution.

We were pursuing models of the dissociation kinetics with French collaborators at the Francois Rabelais University in Tours when unfortunately my Fellowship at ANU came to a close.

Two other projects were studied while at ANU. I carried out some CD studies on sulfatase A enzyme. Earlier work had suggested that in the course of catalysis involving the sulfatase A, incorporation of sulfur from the substrate, resulted in loss of activity and unfolding of the secondary structure. Our studies did not replicate this unfolding – the substrate-modified sulfatase retained the CD profile characteristic of ordered structure and we concluded that some other artifact was leading to loss of activity in the earlier studies.

I also pursued some theoretical analysis of peptide sequences based on a speculation by Leslie Orgel at the Salk Institute in San Diego that prebiotic nucleotide sequences adopted alternating pyrimidine and purine bases due to steric restrictions. Using one of the early tables of hydrophobicity of the naturally-occurring amino acid residues it was clear that there is a strong correlation between amino acid properties and the central base of the respective codon. Virtually all codons with a central purine base (adenine or guanine) code for a hydrophilic residue while all codons with a central pyrimidine base (cytosine or uracil) code for a hydrophobic residue.

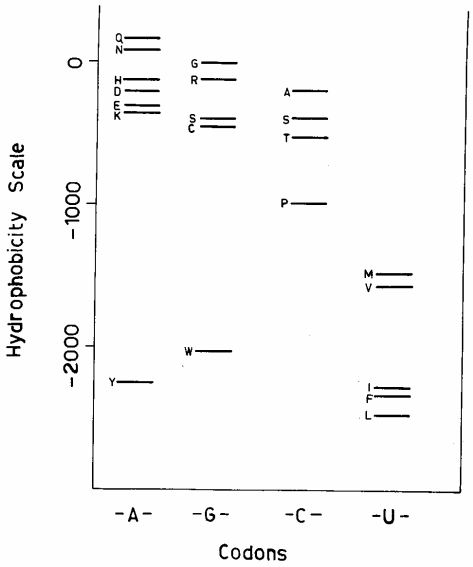
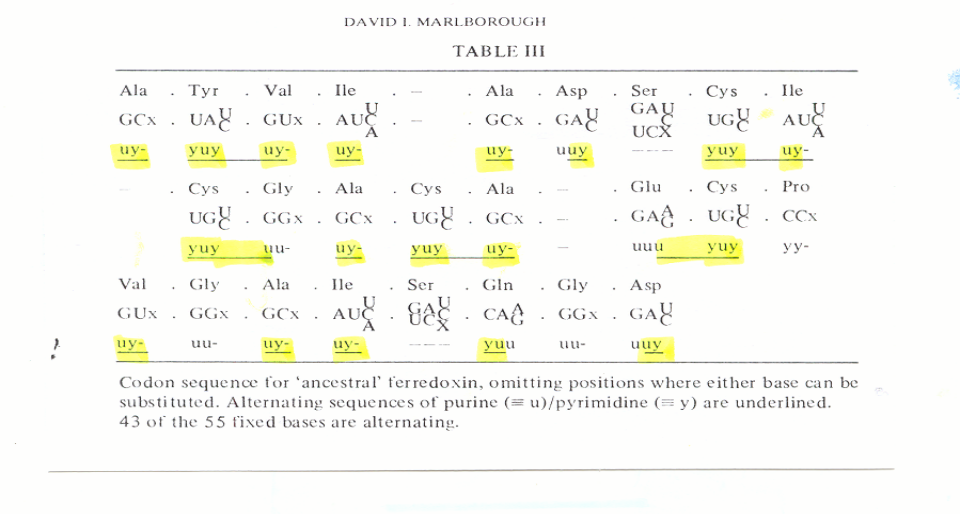


Fig. 1. The hydrophobicity values of the 20 common amino acids published by Bull and Breese (1974) arranged according to magnitude and in columns corresponding to the central base of their coding triplets.

Assuming the early proteins or peptides in a pre-biotic environment would involve electron-transport features as a significant prerequisite for a functioning self-assembly then 'redox' would predominate. A number of authors had argued that iron-sulphur proteins such as ferredoxin would be

possible candidates for the earliest type of protein. A so-called "ancestral" ferredoxin sequence proposed by Dayhoff does indeed contain residues preponderantly containing alternating purines/pyrimidines in the codons with 3 to 4 times the expected frequency in the sequence.
A-Y-V-I-…-A-D-S-C-I-…-C-G-A-C-A-…-E-C-P-V-G-A-I-S-Q-G-D



DAVID I. MARLBOROUGH

TABLE III

Ala	.	Tyr	.	Val	.	Ile	.	–	.	Ala	.	Asp	.	Ser	.	Cys	.	Ile	
GCx	.	UA U/C	.	GUx	.	AU U/C/A	.	–	.	GCx	.	GA U/C	.	GAU/C UCX	.	UG U/C	.	AU U/C/A	
uy-		yuy		uy-		uy-				uy-		uuy		----		yuy		uy-	
–	.	Cys	.	Gly	.	Ala	.	Cys	.	Ala	.	–	.	Glu	.	Cys	.	Pro	
		UG U/C	.	GGx	.	GCx	.	UG U/C	.	GCx	.	–	.	GA A/G	.	UG U/C	.	CCx	
		yuy		uu-		uy-		yuy		uy-		–		uuu		yuy		yy-	
Val	.	Gly	.	Ala	.	Ile	.	Ser	.	Gln	.	Gly	.	Asp					
GUx	.	GGx	.	GCx	.	AU U/C/A	.	GAU/C UCX	.	CA A/G	.	GGx	.	GA U/C					
uy-		uu-		uy-		uy-		----		yuu		uu-		uuy					

Codon sequence for 'ancestral' ferredoxin, omitting positions where either base can be substituted. Alternating sequences of purine (≡ u)/pyrimidine (≡ y) are underlined. 43 of the 55 fixed bases are alternating.

Such a restriction on the nucleotide sequence (limiting the sequence to alternating purine and pyrimidine bases) will have a significant effect on the nature of the transfer-RNA molecule with the typical ladder structure where there is internal bonding between the bases in the sequence containing the anti-codon loop. Typically the loop has seven nucleotides including the anti-codon sequence before joining on to the internally-bonded chain. If any mutation occurs near the loop sequence the whole linkage would have to frame-shift by two bases to accommodate the alternating structure thus introducing an extra two nucleotides into the loop. Lo and behold there are two known mitochondrial sequences that have the mutated loop of nine nucleotides and they code for tyrosine and tryptophan – the two residues that do not line up with the hydrophobic sequence given in the earlier chart! I did try and get something published on this but the initial submission was rejected and by that time my Fellowship at ANU had finished and I was in the tribulations of trying to get another job so didn't follow it up.

As I was running out of options of continually getting University Fellowships (at age 43!) I had to get a real job for a change. I spent two years working for Johnson & Johnson in their drug regulatory affairs department but was not involved in any laboratory work so it was of very little interest until the chance came up to join Alex Pucci's group at Monoclonal Australia Pty Ltd developing immuno-based diagnostic tests

1984-94 Monoclonal Australia, Sydney and Melbourne

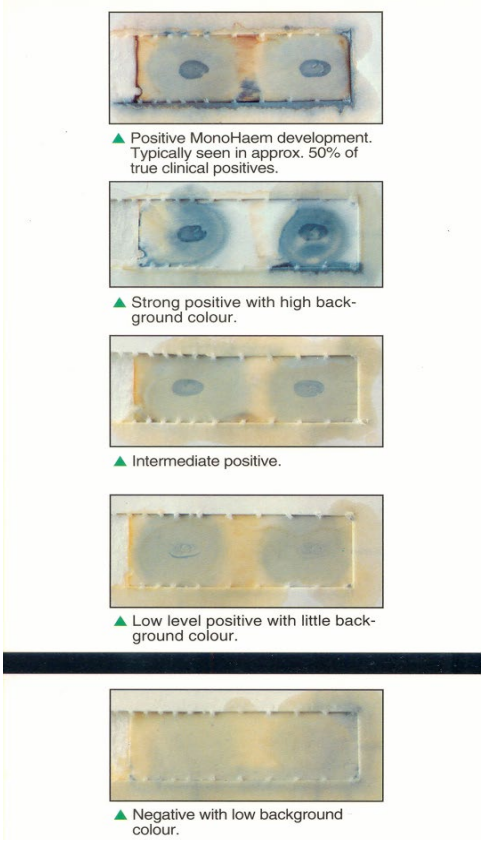
When the position at Monoclonal Australia became available we were actually living two blocks away from the laboratory so although she had advertised in Scientific journals in London and New York I actually replied from around the corner!

The change of emphasis to a more commercial application involved a move to immunochemistry and an understanding of how the immunoreagents could be adapted to rapid point-of-care diagnostic tests. The main emphasis of the project that I was responsible for was to work up a rapid test for detection of bleeding from the large bowel. This had been shown since the early 60s to be strongly indicative of the presence of colorectal cancer which is the most wide-spread cancer across both sexes in the Western world.

The main test for diagnosis of bleeding from the large bowel was one produced by SmithKline Diagnostics – the Hemoccult slide test. A faecal smear was placed on a support filter impregnated with gum guaiac. Native haemoglobin has a low level of pseudoperoxidase activity and will oxidise suitable substrates in the presence of hydrogen peroxide to give a chromophoric end-product in exactly the same way that ELISAs work. Hence if any intact blood is present in the faecal smear and the slide is developed with hydrogen peroxide the alpha-guaiacol (the principal in gum guaiac) is converted to an insoluble blue end-product of oxidation.

The main problem with this methodology is that any haemoglobin will give a positive peroxidase reaction and hence the test must be carried out after the subject under test goes on a diet free of any other peroxidase or pseudoperoxidase-containing components (such as lean meat or vegetables with high oxidase levels).

At Monoclonal Australia we developed a test with immobilized monoclonal anti-haemoglobin reagents that could selectively bind to intact haemoglobin prior to development with hydrogen peroxide. The antibody was absorbed on nylon membrane with the face of immobilization opposite to the application filter. Hence any haemoglobin in the sample could filter through the membrane and then by diffusion be captured by the monoclonal antibody. The gum guaiac and hydrogen peroxide were added separately to ensure that any production of chromophore would be localized to the area of immobilization.



Once you can get over the yuk factor of manipulating faeces – as Ed Breen queried "what are the brown patches around the outside of the test for?" it is actually an easily used format and being immuno-based has significantly stronger specificity than equivalent tests.

The performance of the MonoHaem test compared to Hemoccult demonstrated a strong positive predictivity in subjects undergoing controlled and uncontrolled diets for detection of colorectal cancer (12% positive predictivity compared to 3-4% with Hemoccult). Although this test is still marketed in Australia it wasn't pushed enough in my opinion by the management (after Alex left) to gain much of a marketing foothold in Australia

1994-2001 **ICT Diagnostics Sydney**

The experience in immobilizing reagents on membranes was then applied to lateral-flow tests which are the main route to rapid point-of-care diagnostic tests at present.

At this point I also became involved in linking antibodies (and antigens) to colloidal gold suspensions which are the main detection agent used in the

lateral-flow tests. Latex beads linked to dyes are also used but these agents cannot be so well-controlled for size as the colloidal gold suspensions.

The two diagnostic tests that I did most work on where for dengue fever and malaria diagnosis.

Dengue fever is an arbovirus (i.e. it is an arthropod-borne virus most commonly passed on by a blood-sucking insect) which has the unusual feature that it has four serotypes that only partially cross-react. Hence infection by one serotype does not give complete immunity to infection by the other serotypes but does raise an immune reaction. As the immune response does not clear the secondary infection there is the problem of overstimulation of the immune system and anaphylactic shock. The way the primary and secondary infections can be differentiated is by the levels of immunoglobulinG and immunoglobulinM. The primary infection is characterized by almost exclusive IgM production with the subsequent IgG levels being quite low in common with normal immune status. However, the secondary infection by another serotype involves the normal IgM response followed by high IgG levels and this was the basis of the test that we were working on.

Human IgG and human IgM were captured in an initial flow of the serum along a membrane and then a reverse flow with a monoclonal antibody directed against the dengue virus coat (a general antibody against a mixture of the four serotypes) linked to colloidal gold could detect whether the immunoglobulins captured by the immobilized anti-human Ig antibodies were bound to viral antigens by capturing the colloidal gold detector. If the infection was a primary infection only IgM showed a positive reaction while if it was a more serious secondary infection both the IgM and IgG were positive.

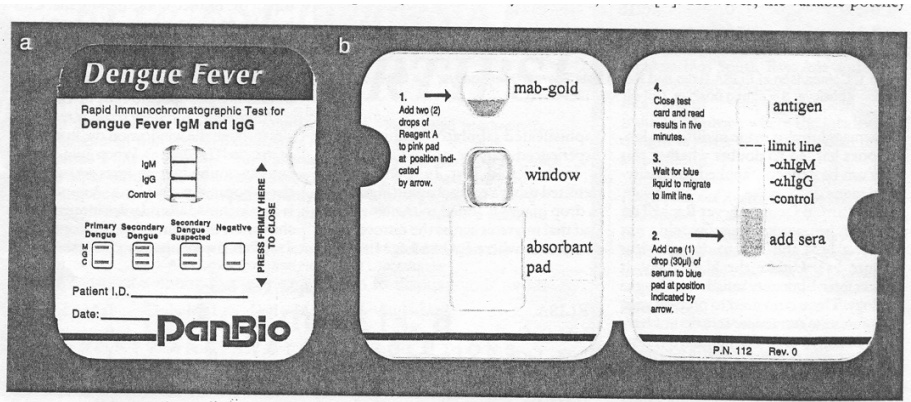


Figure 1. (a) The result of a PanBio Dengue Rapid Test using serum taken from a patient with secondary dengue infection (IgM, IgG and control lines visible). Interpretation criteria are printed on the front; (b) inside view showing the antigen, gold-conjugate and absorbent pads, as well as the anti-human IgG, anti-human IgM, control, and limit lines.

The malaria test that was developed by Mary Garcia at ICT had immobilized antibodies against both *Plasmodium falciparum* and

Plasmodium vivax. The lateral-flow test was designed to pass lysed blood over the capture antibodies and then the reverse flow completed the sandwich with different monoclonal antibodies linked to colloidal gold suspensions. The necessity to detect two different agents led on to linking the two antibodies to the same colloidal gold suspension which was shown to be reasonably successful with the lateral-flow format but of course is much more suited to the flow-through format.

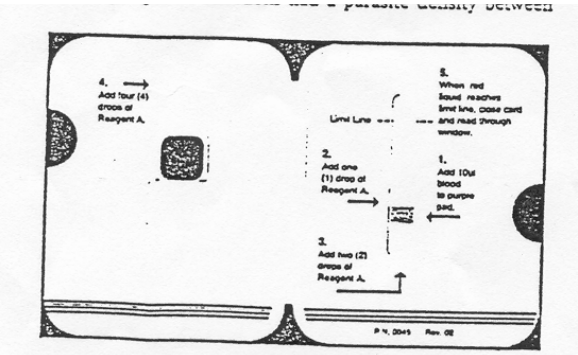


Figure: Photograph of two opposing faces of ICT Malaria Pf test card and sequential procedural steps of test

Based on the technique for doubly-linking the reagents to the gold colloid presented a route to manipulate the reagents for the WheatRite test developed in my time at Proteome Systems. This technique is quite valuable in that it can be used to match up quite accurately to the nature of the membrane support in these tests. This has always been problematic in these rapid tests and can be a valuable QC measure in place prior to manufacture.

I will wind up there as most of you have heard talks on the work at Proteome on these tests but I will finish by making the point that although I have been in the biochemical/organophysical chemistry field for many years I have never got bored or tired with the opportunities offered by science and particularly working hands-on at the laboratory bench.

It may sound somewhat arrogant – actually it is arrogant, but I am considered one of the experts in the field of immunogold colloid chemistry. Teresa Howard at the MacFarlane Burnet Institute at one time, when she wanted some immunogold reagent prepared, said that as far as immunogold chemistry in Australia was concerned I was it! To give all scientists hope that you never stop innovating, I only started in the field of immunogold chemistry at the age of 54!

I will finish by listing all the people in my career that I have worked and published with below. As you may see in the last few years there has been some crossover with the several institutes.

University of Exeter
Prof. H N Rydon
K G Orrell
P M Hardy
J C Haylock
H T Storey
R C Thompson

Harvard Medical School
Prof E R Blout (President of US Academy of Sciences)
D A Laufer
T M Chapman
V M Vaidya

Imperial College
J Gibson
Prof D O Hall
R Cammack

MRE Porton Down
K A Cammack
D S Miller
A I McMullen

Papanicolaou Institute
J W Ryan
U S Ryan
A R Day
A Chung
G H Fisher

John Curtin School
P D Jeffrey
G B Treacy
A B Roy
C I Prosser

Monoclonal Australia
A Pucci
M Williams
H Mclean
M B Slade
K Tynan
A Smithyman
M Garcia

ICT Technology
R Cole
M Garcia
G Mearns
M Wareing
R Taylor
L Seroa

Proteome Systems
K Williams
J Harry
R Cole
L Seroa
R Buls
& many others