Characterisation of the protein ligand binding site in protein disulphide-isomerase (PDI) and its homologues.

BBSRC joint project grant between the University of Warwick and the University of Kent.

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We are seeking two post-doctoral researchers, one based in Warwick on a fixed term contract of up to 2 years and one based in Kent on a fixed term contract of up to 3 years to work on the above project. The researcher at Warwick will generate catalysts and substrates and characterise their interaction using a range of chemical and biophysical methods, and the researcher at Kent will focus on collecting high-field NMR structural data as well as generating structural information on these bimolecular systems.

You will require a first degree in biological or physical science and a PhD in biochemistry, biophysics or equivalent. Depending on the position of interest, you will ideally have experience with a range of physical methods for protein characterization and high field biological NMR, preferably including protein structure determination and/or protein dynamics studies.

Informal enquiries for the Warwick based position can be made to Professor Robert Freedman, +44 (0) 2476 523516 (r.b.freedman@warwick.ac.uk) and informal enquiries for the Kent based position can be made to either Dr Richard Williamson, +44 (0) 1227 827155 (r.a.williamson@kent.ac.uk) or Dr Mark Howard +44 (0) 1227 824730 (m.j.howard@kent.ac.uk).

To apply for the Warwick post please visit www.warwick.ac.uk/jobs. For a hard copy pack, please contact Personnel Services, on +44 (0) 2476 523685 (24 hour answer phone), or by e-mail to Recruit@warwick.ac.uk.

To apply for the Kent post please contact the Personnel Office, on +44 (0) 1227 827837 (24 hours) or visit http://www.kent.ac.uk/jobs/index.htm

Deadline to receive applications for either position is 7 July 2006

Salary range for each position: £20,044 - £30,002 p.a.

Further details of the project are shown on the following page.

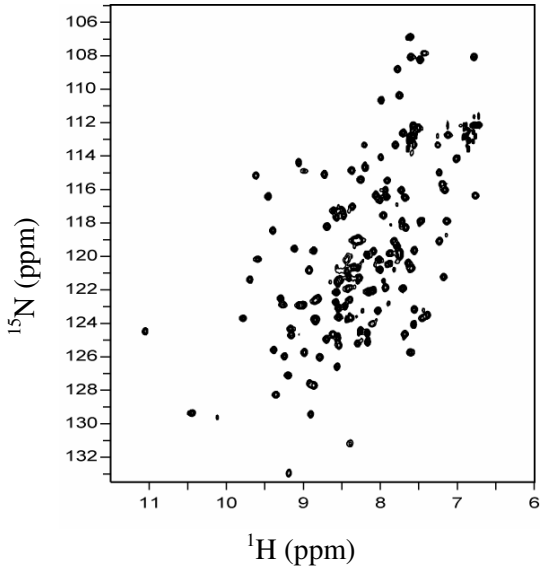
Project Summary

Proteins carry out most key biological activities in all organisms, and the individual biological activity of each protein is crucially dependent on its specific 3-dimensional structure. One of the most significant discoveries in cell biology in the past 20 years has been the recognition that there is extensive and complex machinery in cells devoted to ensuring that newly-made proteins fold up and assemble correctly to their specific and unique 3-dimensional structure. This machinery comprises 'folding catalysts' which ensure that proteins fold rapidly and correctly and 'molecular chaperones' which prevent misfolding. This machinery is now quite well-defined - in terms of the identification of its component parts - but we know very little about how these folding catalysts and chaperones work in molecular detail. The aim of this proposal is to increase our detailed molecular-level understanding of one part of the cellular protein folding machinery.

Protein disulphide-isomerase (PDI) is a folding catalyst and chaperone which has been known for many years. It is absolutely required for the folding and assembly of proteins that contain disulphide bonds. Disulphide bonds provide proteins with additional stability and are found in almost all proteins which are secreted from cells or exposed at the extracellular surface of cells. Since this group of proteins includes most protein hormones and other intercellular messengers, hormone receptors, digestive enzymes, antibodies, blood clotting proteins, and (in other species) venom toxins, plant storage proteins etc. this is a very significant class of proteins. For example, most of the human protein drugs which are currently used in therapy (such as insulin, interferons, growth hormones, antibody fragments, blood clotting factors etc.) are disulphide-bonded proteins. To the best of our knowledge, PDI or a closely related member of the PDI family, is required for the correct folding of all such proteins. As a result more detailed understanding of PDI would be significant not only for basic cell biology but also for medical, veterinary and biotechnological applications.

Surprisingly, after almost 30 years of study, the detailed structure of mammalian PDI is not known at the molecular level, and so we cannot picture precisely how it acts to assist newly-made proteins to fold and form correct disulphide bonds. There appear to be some difficulties which have frustrated conventional approaches using x-ray crystallography. Preliminary work that we and our collaborators have done over the past 2-3 years suggests that we now understand the basis of these difficulties and makes it possible to plan how to determine the structure of PDI bit-by-bit.

We plan to start with the 'domain' of PDI which is most interesting to us, because we know that it is the key domain for the 'chaperone' properties of PDI. We will determine the structure of this domain alone and in combination with a neighbouring domain, as a step towards determining the whole structure. We will not focus simply on a static picture but also determine the flexibility of these parts of PDI, in order to picture the various molecular motions they undergo on various timescales. Finally we will study these domains of PDI in combination with various small proteins and even smaller fragments (peptides) aiming to understand how PDI and the proteins on which it acts bind to each other and how each influences the detailed structure and dynamics of the other. This will finally give us some insight into how PDI works, in molecular terms.



NMR spectrum of the chaperone domain of PDI

In the naturally occurring protein this domain is too flexible to give sharp data and the peaks appear weak and very broad. Here we show the results from a more stable form of the domain where we have replaced just one of its 130 amino acids. This more stable form will allow us to determine a 3-dimensional model for this part of the protein and so help us to understand how this part binds to newly-made proteins which it then helps to fold.