Chemical and biochemical approaches to protein regulation and modification

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A new protein for pantothenate biosynthesis in the Enterobactericeae

The pathway to pantothenate in bacteria has long been thought to have been fully characterised. The extant pathway consists of four enzymes: a hydroxymethyl transferase and a reductase which generate d-pantoate, a decarboxylase to produce β-alanine and an ATP-dependent synthetase to join these fragments. We have now reported the discovery and preliminary characterisation of an essential fifth protein limited to a small subset of enteric Gram-negative bacteria. This protein, PanZ, is required for the activation of the pyruvoyl-dependent aspartate α-decarboxylase (ADC) from the zymogenic form in which it is expressed.

PanZ is a putative Acetyl-CoA dependent acetyl transferase. We have analysed the interaction of a constitutively inactive form of ADC with PanZ using isothermal titration calorimetry and mass spectrometry. These experiments (Figure 1) demonstrated that the proteins interact with nanomolar affinity in a 4:4 complex. We are now using a combination of NMR, X-ray crystallography and small angle X-ray scattering to determine the structure of this complex and how interaction of the two proteins leads to formation of active ADC. In related work, we are using the same set of techniques to investigate the function of other proteins which putatively interact with other components of the pathway.

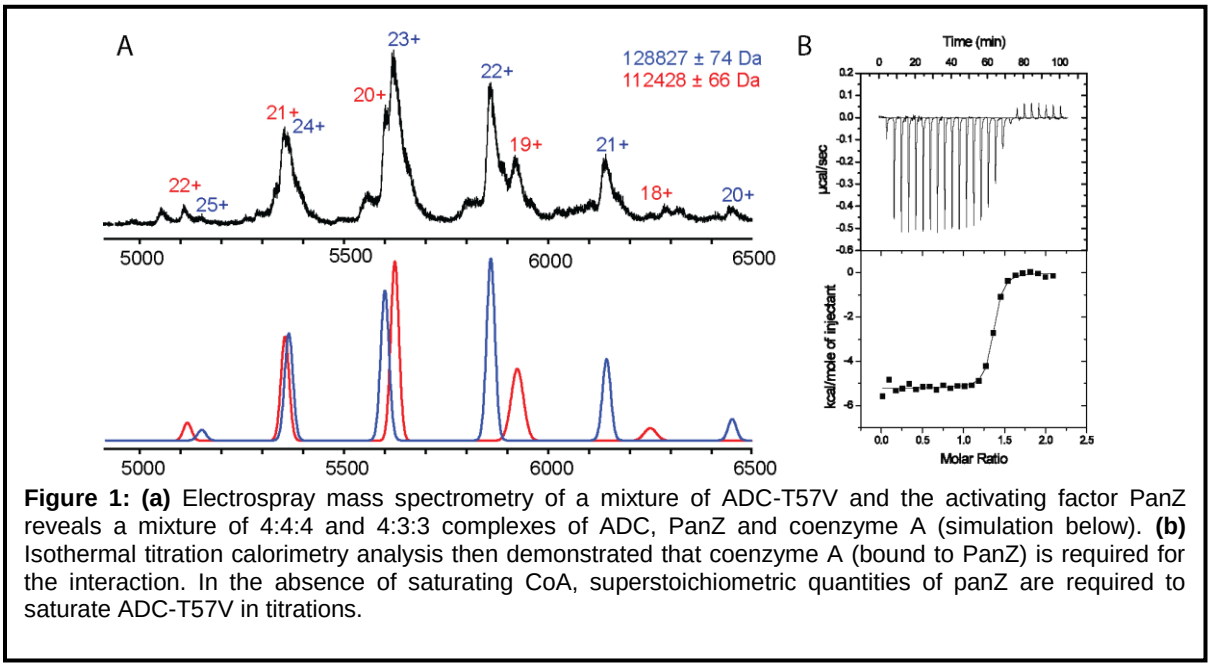

Figure 1: (a) Electrospray mass spectrometry of a mixture of ADC-T57V and the activating factor PanZ reveals a mixture of 4:4:4 and 4:3:3 complexes of ADC, PanZ and coenzyme A (simulation below). **(b)** Isothermal titration calorimetry analysis then demonstrated that coenzyme A (bound to PanZ) is required for the interaction. In the absence of saturating CoA, superstoichiometric quantities of panZ are required to saturate ADC-T57V in titrations.

Development of stable analogues of phosphohistidine

A major continuing interest in our group is the development of stable analogues of phosphohistidine. We propose to use these probes to understand the role of phosphohistidine in the mechanism of an unusual ADP-dependent kinase which we are currently investigating. In addition we are investigating the use of our analogues as haptens for antibody production (with Claire Eyers, Manchester). We have recently reported our optimised synthetic strategy to generate our class of triazole analogues, using P(III) chemistry to produce a key alkynyl phosphonate intermediate as shown below (Figure 2).

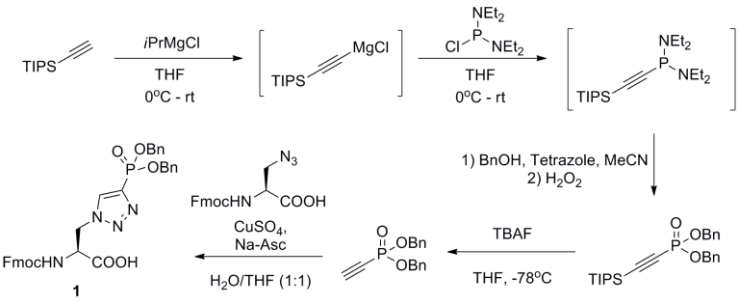

Figure 2: strategy used for the production of the stable phosphonate analogue of phosphohistidine 1 using P(III) chemistry to generate a precursor fully-compatible with the Fmoc-strategy for solid phase peptide synthesis.

Other work

We are working with Dr Ben Murray (School of Earth and Environment) to investigate how biological materials affect the atmosphere via the nucleation of ice in tropospheric clouds. We also collaborate with Bruce Turnbull to develop methodology to apply the transpeptidase sortase A from *Staphylococcus aureus* in the modification of expressed proteins.

Publications

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Funding

This work was funded by the Wellcome Trust, BBSRC, NERC and EPSRC.

Collaborators

External: J. Hibberd (University of Cambridge), C. Eyers (University of Manchester), A. Smith (University of Cambridge), N. Hironori (National Institute for Genetics, Japan), E. Snell (Hauptmann-Woodward Institute, Buffalo, USA).