Dr Eva Hyde

Senior lecturer

School of Biosciences (/schools/biosciences/index.aspx)

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About

Eva Hyde's research interests are how proteins recognise their appropriate ligands; this includes the recognition of substrates by enzymes and the binding of proteins to their cognate operator DNA and to other proteins. She has extensive experience in NMR spectroscopy and complementary biophysical techniques, such as fluorescence and circular dichroism, and, more recently, in small angle X-ray and neutron scattering. She has worked at the University of Birmingham since 1989 and teaches enzyme kinetics and protein structural techniques. She helped develop and implement the MSci, fourth year undergraduate programme in Biosciences at the University.

Qualifications

- 1977-1981: Ph.D. (C.N.A.A.) 1981. National Institute for Medical Research, London.
  NMR studies of coenzyme binding to dihydrofolate reductase from Lactobacillus casei.
- 1973-1977: B.A. Chemistry 1977; University of Oxford (Somerville College)

Biography

After her PhD in NMR studies of coenzyme binding to the enzyme dihydrofolate reductase, in NIMR, London, Eva Hyde spent seven months at the University of Cambridge and then went to the University of Washington for five years. She studied tRNA structure using NMR spectroscopy, for two years, and was in the Dept. of Microbiology studying transcription by B.subtilis RNA Polymerase for three years. She returned to England in 1986, obtaining an MRC project grant for NMR studies on the structure of Trp repressor, at the University of Leicester.

In 1989 she obtained a Welcome Trust Senior fellowship and shortly thereafter became a lecturer at the University of Birmingham. Here she continued her research on the structures and interactions of DNA-binding proteins, including Trp repressor, MelR, NifA and KorA. She also determined the structures of two snake toxins with similar function but very different 3D structure, by NMR spectroscopy. She is currently studying the kinetics and specificity of nitroreductase, an enzyme with potential for cancer gene therapy, and also the structure and interactions of the DNA-partitioning protein, KorB.

She is also extensively involved in the undergraduate Biochemistry course. She was Biochemistry programme co-ordinator for eight years and helped design and implement several undergraduate modules. She helped initiate and run the MSci fourth year course for five years, and is currently a module organiser for a second year undergraduate course on proteins and a fourth year course on research developments.

Teaching

- Undergraduate and MSci - enzyme kinetics and enzyme action, NMR in Biosciences, protein structure.
- Module organiser for 2nd year undergraduate Biochemistry course on ‘Proteins and Enzymes’.
- Module organiser for MSci course ‘Research Developments and Funding’
- MSc workshop on NMR in Biosciences.

Postgraduate supervision

For a list of possible PhD projects offered by Dr Hyde **www.findaphd.com/search/customlink.asp?inst=birm-Biol&supersurname=Hyde (http://www.findaphd.com/search/customlink.asp?inst=birm-Biol&supersurname=Hyde)**

Research

Research Theme within School of Biosciences: Molecular Microbiology - structural biology.

Short research description:

Biophysical studies of protein structure, function and dynamics, in particular using NMR spectroscopy and, more recently, Small angle scattering. Areas of interest include protein-ligand specificity, protein-DNA interactions and disordered proteins. Current research is on (1) the structure and interactions of KorB, a protein controlling DNA-partitioning and gene regulation, and (2) the substrate specificity of nitroreductase enzymes which have potential for cancer gene therapy.

Full research description

Protein structure, function and kinetics: Use of NMR and other biophysical techniques to examine KorB, a protein controlling DNA-partitioning and gene regulation, and to optimise nitroreductase activity for cancer gene therapy.
I am using a variety of biophysical techniques, including NMR spectroscopy, Circular dichroism and small angle X-ray and neutron scattering, to examine how proteins recognise their appropriate ligands; this includes the recognition of substrates by enzymes and the binding of proteins to their cognate operator DNA.

My current research is on two systems:

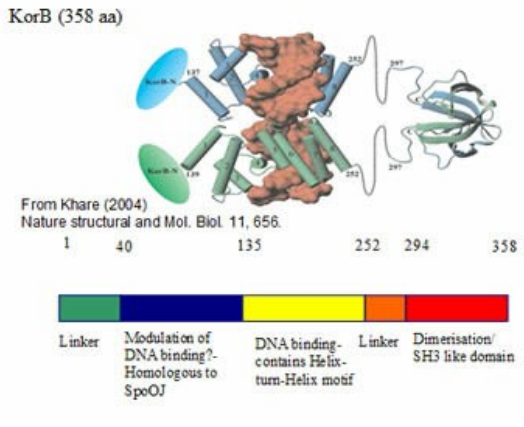
- (a). Determination of the 3D structure and interactions of the partitioning protein KorB
- (b). Comparison of the specificity and action of two E. coli nitroreductase enzymes NfsA and NfsB, which have potential for cancer gene therapy

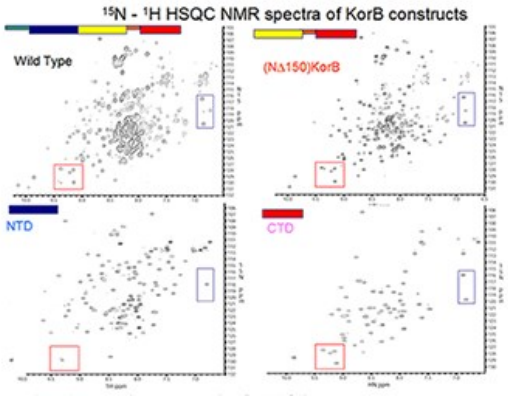
a) KorB from IncP1 plasmid, RK2, is involved in two essential functions. It belongs to the ParB family of proteins, encoded both on bacterial chromosomes and on certain plasmids, which are critical for DNA partitioning and cell division. In addition to this role, KorB controls gene expression in RK2, interacting co-operatively with other proteins including the repressor KorA and RNA polymerase. The plasmid RK2 has been extensively characterised, as a small model genome, as an expression vector and as a carrier of multiple antibiotic resistance genes between bacterial species.

Understanding DNA partitioning and gene regulation at a molecular level, is a key first step to developing new targets for antibiotics to stop the spread of this group of plasmids, as well as targeting bacterial partitioning in general. To date there is no detailed 3D structure of an intact ParB protein because of flexible domains within the proteins, which are likely to contribute to both partitioning and gene regulation.

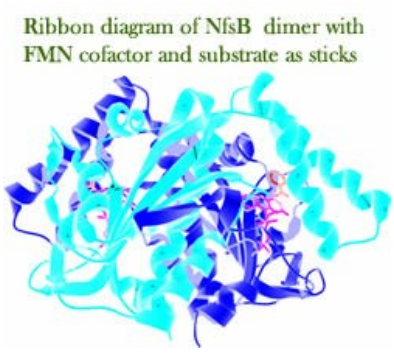
Disordered regions have been found for many biologically important proteins and innovative approaches are required to describe and determine their structures and flexibility. The NMR spectra of KorB constructs below show that the structure of the free KorB protein is modular, with little interaction between the domains. In collaboration with Scott White, and Chris Thomas, both in Biosciences at the University of Birmingham and Dave Scott, at the University of Nottingham, we have combined this NMR data with X-ray crystallography and Small Angle X-ray Scattering data to give a model of the full length KorB. We have also determined the structure of KorA in the presence and absence of operator DNA and mapped its site of interaction with KorB.

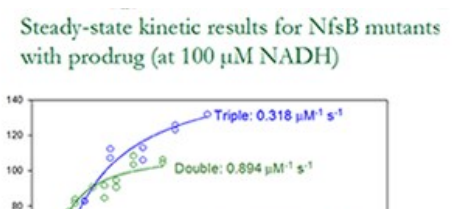
We now intend to examine KorB complexes with DNA and with KorA using similar methods, and to examine the structural and thermodynamic roles of the flexible regions. The methods developed and the conclusions from this study will be directly applicable to numerous proteins with disordered regions, which are involved in key functions such as cell signalling, regulation and cancer biology.

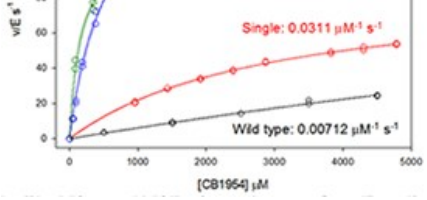




b) The NfsA and NfsB nitroreductases from E. coli are flavoenzymes with broad substrate specificity. One of their substrates is the prodrug CB1954 that is converted by the enzymes into a cytotoxic agent, and NfsB is in trials for cancer gene therapy. In collaboration with Peter Searle, Cancer Studies, and Scott White, Biosciences, we aim to improve the catalytic activity and selectivity of the protein for prodrugs. We have determined the structure of the NfsB protein complexed to different substrate analogues, by X-ray crystallography. We have also obtained excellent NMR spectra of the protein.







Based on the crystal structure, we have made a series of mutations at several amino acids around the active site of NfsB. Some of the mutants with greatly enhanced activity have been purified and their steady-state kinetics measured with different substrates. We intend to characterise the most interesting mutants by X-ray crystallography, and then examine the internal motion of the proteins by NMR spectroscopy, to see if this correlates with catalytic activity. We are also comparing the structures and activities of wild type and mutant NfsB enzymes with those of NfsA to understand substrate selectivity. This will allow us to design further mutations in the hope of improving the enzyme further.

Publications

- Rajasekar, K.V.; Tul Muntaha, S.; Tame, J.R.H.; Kommareddy, S.: Morris, G.; Wharton, C.W.; Thomas, C.M.; White, S.A.; Hyde, E.I. and Scott D.J. (2010). Order and disorder in the domain organisation of the plasmid partition protein KorB. J. Biol. Chem. 285 (20) 15440-15449.
- Jaberipour M., Guise, C.P., Grove, J.I., Knox, R.J., Hu, L., Hyde, E.I. and Searle, P.F. (2010) Testing double mutants of the enzyme nitroreductase for enhanced cell sensitization to prodrugs: effects of combining beneficial single mutations. Biochem. Pharmacol. 79 (2) 102-111.
- Jarrom, D., Jaberipour M., Guise, C.P., Daff, S., White, S.A., Searle, P.F., and Hyde, E.I. (2009) Steady-state and stopped-flow kinetic studies of three *E. coli* NfsB mutants with enhanced activity for the prodrug CB1954. Biochemistry 48, (32) 7665-7672.
- Vass, S.O., Jarrom, D., Wilson, W.R., Hyde E.I., and Searle, P.F. (2009) E. coli NfsA; an alternative nitroreductase for prodrug activation gene therapy in conjunction with CB1954. Br. J. Cancer 100, 1903-1911.
- Bingle, L.E.H., Rajasekar, K.V., tul Muntaha, S., Nadella, V., Hyde, E.I., and Thomas, C.M. (2008) A single aromatic residue in transcriptional repressor protein KorA is critical for cooperativity with its coregulator KorB. Mol. Micro 70 (6) 1502-1514.
- Smith, K.J., Baillie, G.S., HydeE.I., Li Xiang, Li, Houslay, T.M., McCahill, A., Dunlop, A.J.; Bolger, G.B., Klussmann, E., Adams, D.R., and Houslay, M.D. (2007) Functional and structural characterisation of a cAMP-specific phosphodiesterase-4D5 (PDE4D5) N-terminal region peptide that disrupts PDE4D5 interaction with the signalling scaffold proteins, b-arrestin and RACK1. Cellular Signalling, 19, 2612-2624.
- Race, P.R., Lovering, A.L., White, S.A, Grove, J.I., Searle, P.F., Wrighton, C.J., and Hyde, E.I. (2007) Kinetic and structural characterization of *Escherichia coli* Nitroreductase mutants showing improved efficacy for the prodrug substrate CB1954. J. Mol. Biol., 368, 481-492
- Guise, C.P., Grove, J.I., Mountain, A., Hyde, E.I. and Searle, P.F. (2007) Direct positive selection for improved nitroreductase variants. Gene Therapy, 14 , 690-698.
- Rajasekar, K. V., Bingle, L.E.H., Thomas, C.M. and Hyde, E.I. (2006) Letter to the Editor: ^{1}H, ^{13}C and ^{15}N assignments of the KorA global transcriptional repressor protein from the low copy number IncP-1 plasmid, RK2. J. Biomol. NMR 36, 71