Interactions of the intact FsrC membrane histidine kinase with its pheromone ligand GBAP revealed through synchrotron radiation circular dichroism (SRCD)

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Introduction

Enterococcus faecalis is a significant agent of hospital-acquired infection, accounting for over 20 % of cases in the UK. Adherence to host tissue and organs is a key feature of these infections and one of the most important virulence factors that contributes to the adherence properties is gelatinase (GelE) which is under the control of the quorum sensing Fsr signal transduction pathway. Quorum sensing has therefore been identified as a promising target for the design of novel antibacterial agents.

During the past year, we have been developing SRCD methods to stabilise and characterise intact FsrC, the membrane sensor kinase component of the FsrC pathway, including its molecular interactions with the activating pheromone signalling ligand, GBAP. Use of SRCD (rather than CD) permits low volume measurements which are important for studies of low abundance membrane proteins. The work has led to the determination of the first quantitative (k_d) binding data for any intact membrane protein using CD methods, and paves the way for obtaining binding data for other ligands including inhibitors. We suggest that SRCD may also serve as a useful method to confirm stability of FsrC and other membrane proteins purified within detergent micelles prior to undertaking other methodologies such as crystallisation which depend on stability for success.

Results

Using SRCD, we identified suitable conditions required to stabilise purified FsrC within detergent micellar complexes. Once these conditions were established, stabilised protein was used in SRCD studies in the far- and near-UV regions to investigate binding by its native quorum ligand, gelatinase biosynthesis-activating pheromone (GBAP). Whilst GBAP binding did not significantly affect FsrC secondary structure (as revealed by measurements in the far-UV region – Fig. 1), nor the thermal stability of FsrC (data not shown), measurements in the

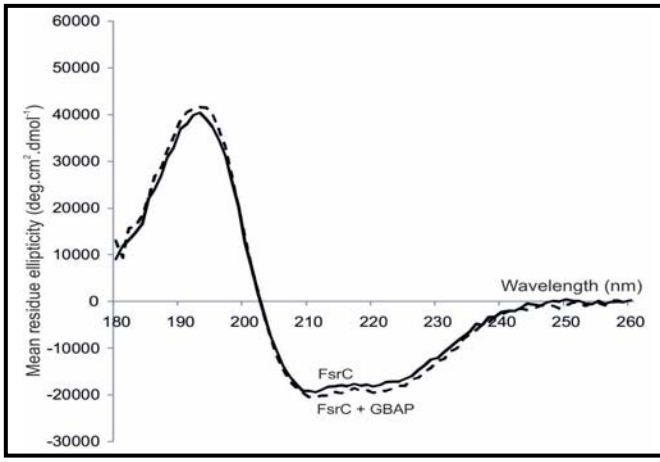

Figure 1: the effect of two-fold GBAP on FsrC revealed through SRCD in the far-UV region of the spectrum at 20 °C. Solid line, 6 µM FsrC (plus equivalent acetonitrile); dashed line, 6 µM FsrC in the presence of two-fold GBAP (dissolved in acetonitrile). Average of four scans.

near-UV region revealed that the tertiary structure in the regions of the Tyr and Trp residues was significantly affected (Fig. 2a). Titration experiments using several GBAP:FsrC molar ratios within the range of 0:1 and 5:1 revealed a calculated k_d value of 2 µM (Fig. 2b), indicative of relatively loose binding of GBAP to FsrC, which is consistent with its role in signal transduction in which rapid responses to changing GBAP levels are required.

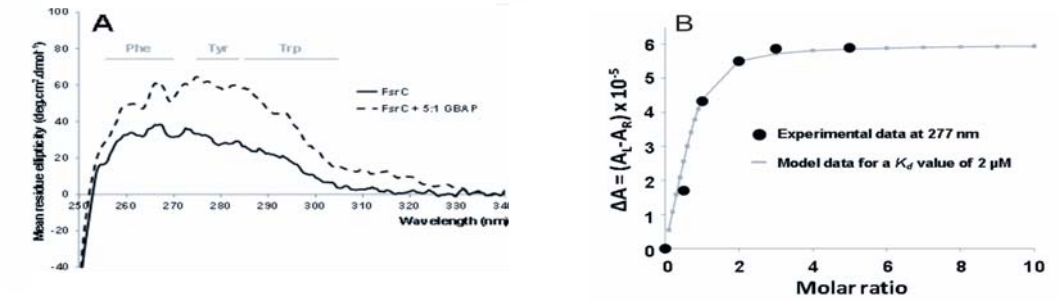

Figure 2: GBAP binding to FsrC revealed through SRCD measurements in the near-UV region at 20 ^{o}C. **(a)** 20 µM FsrC in the absence (solid line) and presence (dashed line) of five-fold GBAP. Data corrected for buffer/acetonitrile/GBAP signals by subtraction of control spectra; **(b)** Determination of binding affinity (K_d value) for GBAP binding using data from titration data analysed using the programme CD Titration v1.6.

Publications

Ma, P., Nishiguchi, K., Yuille, H.M., Davis, L.M., Nakayama, J. and Phillips-Jones, M.K. (2011) Anti-HIV siamycin I directly inhibits autophosphorylation activity of the bacterial FsrC quorum sensor and other ATP-dependent enzyme activities. *FEBS Lett*. **585:**2660-2664.

Funding

This work was funded by the BBSRC, Diamond Light Source and the University of Leeds. SGP acknowledges the EU EDICT Consortium (contract FP7 201924) for financial support on other projects.

Collaborators

This work was accomplished in collaboration with Dr P. Ma (ITQB, Portugal), Drs G. Siligardi and R. Hussain (Diamond Light Source, Oxfordshire) and Professor J. Nakayama (Kyushu University).