EUROPEAN SYNCHROTRON RADIATION FACILITY

INSTALLATION EUROPEENNE DE RAYONNEMENT SYNCHROTRON

ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Solution structures of the SCR domains in complement factor H and related proteins	**Experiment number**: SC-2521
Beamline: ID02	**Date of experiment**: **3-5 Dec** 2008 (2 days)	**Date of report**: 1st Sep 2009
Shifts: 6	**Local contact(s)**: Dr Anuj Shukla	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

(1) Okemefuna, A. I. *, Nan, R. *, Gor, J. & Perkins, S. J. * (UCL)

(2) Okemefuna, A. I. *, Li, K. *, Nan, R. *, Gor, J., Ormsby, R. J., Sadlon, T., Gordon, D. L. & Perkins, S. J. * (UCL; Flinders, Adelaide, Australia)

(3) Bonner, A. *, Almogren, A., Furtado, P. B. *, Kerr, M. A. & Perkins, S. J. * (UCL; Riyadh, Saudi Arabia; Leeds University

Report:

Publication: Okemefuna, A. I., Nan, R., Gor, J. & Perkins, S. J. (2009). Electrostatic interactions contribute to the folded-back conformation of wild-type human Factor H. J. Mol. Biol. **391**, 98-118.

Abstract: Factor H (FH) is a major serum regulator of C3b in the complement alternative pathway, being composed of 20 short complement regulator (SCR) domains. Previous solution structures for FH showed that this has a folded-back domain arrangement and existed as oligomers. To clarify the molecular basis for this, analytical ultracentrifugation and X-ray scattering studies of native FH were performed as a function of NaCl concentration and pH. The sedimentation coefficient for the FH monomer decreased from 5.7 S to 5.3 S with increase in NaCl concentration, showing that weak electrostatic inter-domain interactions affect its folded-back structure. FH became more elongated at pH 9.4, showing the involvement of histidine residue(s) in its folded-back structure. Similar studies of partially deglycosylated FH suggested that oligosaccharides were not significant in determining the FH domain structure. The formation of FH oligomers decreased with increase in NaCl concentration, indicating that electrostatic interactions also affect this. X-ray scattering showed that the maximum length of FH increased from 32 nm in low salt to 38 nm in high salt. Constrained X-ray scattering modelling was performed to generate significantly improved FH molecular structures at medium resolution. In 50 mM NaCl, the modelled structures showed that inter-SCR domain contacts are likely, while these contacts are diminished in 250 mM NaCl. Our results show that the conformation of FH will be affected by its local environment, and this may be important for its interactions with C3b and when bound to polyanionic cell surfaces.

Publication: Okemefuna, A. I., Li, K., Nan, R., Ormsby, R. J., Sadlon, T., Gordon, D. L. & Perkins, S. J. (2009). Multimeric interactions between complement Factor H and its C3d ligand provide new insight on complement regulation. J. Mol. Biol. **391**, 119-135.

Abstract: Activation of C3 to C3b signals the start of the alternative complement pathway. The C-terminal SCR-20 domain of factor H (FH), the major serum regulator of C3b, possesses a binding site for C3d, a 35 kDa physiological fragment of C3b. Size distribution analyses of mixtures of SCR-16/20 or FH with C3d by analytical ultracentrifugation in 50 mM and 137 mM NaCl buffer revealed a range of discrete peaks, showing that multimeric complexes had formed at physiologically-relevant concentrations. Surface plasmon resonance studies showed that native FH binds C3d in two stages. An equilibrium dissociation constant K_{D1} of 2.6 μM in physiological buffer was determined for the first stage. Overlay experiments indicated that C3d formed multimeric complexes with FH. X-ray scattering showed that the maximum dimension of the C3d complexes with SCR-16/20 at 29 nm was not much longer than that of the unbound SCR-16/20 dimer. Molecular modelling suggested that the ultracentrifugation and scattering data are most simply explained in terms of associating dimers of each of SCR-16/20 and C3d. We conclude that the physiological interaction between FH and C3d is not a simple 1:1 binding stoichiometry between the two proteins that is often assumed. Because the multimers involve the C-terminus of FH which is bound to host cell surfaces, our results provide new insight on FH regulation during excessive complement activation, both in the fluid phase and at host cell surfaces decorated by C3d.

Publication: Bonner, A., Almogren, A., Furtado, P. B., Kerr, M. A. & Perkins, S. J. (2009). The non-planar secretory IgA2 and near-planar secretory IgA1 solution structures rationalize their different mucosal immune responses. J. Biol. Chem. **284**, 5077-5087

Abstract: Secretory IgA (SIgA) is the most prevalent human antibody and is central to mucosal immunity. It exists as two subclasses, SIgA1 and SIgA2, where SIgA2 has a shorter hinge joining the Fab and Fc regions. Both forms of SIgA are predominantly dimeric and contain an additional protein called secretory component (SC) that is attached during the secretory process and is believed to protect SIgA in harsh mucosal conditions. Here we locate the five SC domains relative to dimeric IgA2 within SIgA2 using constrained scattering modelling. The X-ray and sedimentation parameters showed that SIgA2 has an extended solution structure. The constrained modelling of SIgA2 was initiated using two IgA2 monomers which were positioned according to our best-fit solution structure for dimeric IgA1. SC was best located along the convex edge of the Fc-Fc region. The best-fit models showed that SIgA2 is significantly non-planar in its structure, in distinction to our previous near-planar SIgA1 structure. Both the shorter IgA2 hinges and the presence of SC appear to displace the four Fab regions out of the Fc plane in SIgA2. This may explain the noncovalent binding of SC in some SIgA2 molecules. This non-planar structure is predicted to result in specific immune properties for SIgA2 and SIgA1. It may explain differences observed between the SIgA1 and SIgA2 subclasses in terms of their interactions with antigens, susceptibility to proteases, effects on receptors, and their distribution in different tissues. The different structures accounts for the prevalence of both forms in mucosal secretions.