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-2790
Beamline:	**Date of experiment**: from: 02 Dec 2009 to: 04 Dec 2009	**Date of report**: 1st Sep 2010
Shifts:	**Local contact(s)**: Dr Shirley Callow	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

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(1) Li, K. *, Gor, J. & Perkins, S. J. * (UCL)

(2) Abe, Y. *, Gor, J., Bracewell, D. G., Perkins, S. J. * & Dalby, P. A. (UCL)

(3) Nan, R. *, Ward, G., Gavigan, L., Miller, A. *, Gor, J., McKay, A. R., Lengyel, I. & Perkins, S. J.* (UCL)

Report:

Publication: Li, K., Gor, J. & Perkins, S. J. (2010). Self-association and domain rearrangements between complement C3 and C3u provide insight into the activation mechanism of C3. *Biochem. J.* **431**, 63-72.

Abstract: Component C3 is the central protein of the complement system. During complement activation, the thioester group in C3 is slowly hydrolysed to form C3u, then the presence of C3u enables the rapid conversion of C3 to functionally-active C3b. C3u shows functional similarities to C3b. To clarify this mechanism, the self-association properties and solution structures of C3 and C3u were determined using analytical ultracentrifugation and X-ray scattering. Sedimentation coefficients identified two different dimerisation events in both proteins. A fast dimerisation was observed in 50 mM NaCl that was removed in 137 mM NaCl. Low amounts of a slow dimerisation was observed for C3u and C3 in both buffers. The X-ray radius of gyration R_G values were unchanged for both C3 and C3u in 137 mM NaCl, but depend on concentration in 50 mM NaCl. The C3 crystal structure gave good X-ray fits for C3 in 137 mM NaCl. By randomisation of the TED/CUB domains in the C3b crystal structure, X-ray fits showed that the TED/CUB domains in C3u are extended and differ from the more compact arrangement of C3b. This TED/CUB conformation is intermediate between those of C3 and C3b. The greater exposure of the TED domain in C3u (which possesses the hydrolysed reactive thioester) accounts for the greater self-association of C3u in low salt. This conformational variability of the TED/CUB domains would facilitate their interactions with a broad range of antigenic surfaces. The second dimerisation of C3 and C3u may correspond to a dimer observed in one of the crystal structures of C3b.

Publication: Abe, Y., Gor, J., Bracewell, D. G., Perkins, S. J. & Dalby, P. A. (2010). Masking of the Fc region in human IgG4 by constrained X-ray scattering modelling: implications for antibody function and therapy. *Biochem. J*. in press.

Abstract: Of the four human IgG antibody subclasses IgG1-IgG4, IgG4 is of interest in that this does not activate complement and exhibits atypical self-association including the formation of bispecific antibodies. The solution structures of antibodies are critical to understand function and therapeutic applications. Thus IgG4 was studied by synchrotron X-ray scattering. The Guinier X-ray radius of gyration R_G increased from 5.0 nm to 5.1 nm with increase of concentration. The distance distribution function *P(r)* revealed a single peak at 0.3 mg/ml, which are resolved into two peaks that shifted to smaller *r* values at 1.3 mg/ml, even though the maximum dimension of IgG4 was unchanged at 17 nm. This indicated a small concentration dependence of the IgG4 solution structure. By analytical ultracentrifugation no concentration dependence in the sedimentation coefficient of 6.4 S was observed. Constrained scattering modelling resulted in solution structural determinations that showed that IgG4 has an asymmetric solution structure in which one Fab-Fc pair is closer together than the other pair, and the accessibility of one side of the Fc region is masked by the Fab regions. The averaged distances between the two Fab-Fc pairs change by 1-2 nm with change in IgG4 concentration. The averaged conformation of the Fab regions appear able to hinder complement C1q binding to the Fc region and the self-association of IgG4 through the Fc region. These results clarify IgG4 function and provide a starting point to investigate antibody stability.

Publication: Nan, R., Ward, G., Gavigan, L., Miller, A., Gor, J., McKay, A. R., Lengyel, I. & Perkins, S. J. (2010). Self-association and folded-back solution structures of the wild-type Tyr402 and the disease-related His402 allotypes of complement Factor H. Submitted.

Abstract: Factor H (FH) is a major regulator of complement activation and is related to age-related macular degeneration (AMD) through a Tyr402His polymorphism. Knowledge of the self-association and solution structures of the 20 short complement regulator (SCR) domains in the purified Tyr402 and His402 allotypes is important for interpreting its physiological interactions. Surface plasmon resonance showed that the His402 risk allotype of the SCR-6/8 fragment self-associated more than the Tyr402 allotype, in confirmation of earlier ultracentrifugation data. Surface plasmon resonance and analytical ultracentrifugation showed that the full-length FH His402 and Tyr402 allotypes formed similar 12% amounts of oligomeric structures. Mass spectrometry indicated that both FH allotypes exhibited monomer, dimer and possibly trimer forms, and X-ray scattering also showed that both FH allotypes self-associated. In relation to solution structures, X-ray scattering showed that the maximum length of both monomeric FH allotypes is 28 nm. Constrained scattering modelling fits gave improved best-fit structures that were more folded-back in their structure than those previously determined. With these models, sedimentation modelling without conformational changes now accounted for the reversible association of the monomer, dimer and trimer forms of FH. In conclusion, our data identify the participation of His402 in one of at least two self-association sites in FH, a limited degree of flexibility between the 20 SCR domains of FH is indicated, and the inhibition of FH self-association by native C-reactive protein is clarified. These observations may be relevant for the development of deposit formation in the Bruch's membrane in AMD.