EUROPEAN SYNCHROTRON RADIATION FACILITY

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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:** Structural determinations of the different forms of IgA	**Experiment number**: WT-41
Beamline: ID02	**Date of experiment**: 19 July 2001, 12 Dec 2001, 27 July 2002, (1, 2, 2 days each)	**Date of report**: 1st Sept 2005
Shifts: 15	**Local contact(s)**: Dr Stephanie Finet	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Furtado, P. B.*, Robertson, A.*, Sun, Z.*, Eaton, J. T.*, & Perkins, S. J.* (UCL)

Whitty, P.W.*, Almogren, A.*, Kerr, M.A. & Woof, J.M. (Dundee University)

Reports:

Solution structure determination of human IgA2 by X-ray and neutron scattering and analytical ultracentrifugation and constrained modelling: a comparison with human IgA1. (2004) *J. Mol. Biol.* **338**, 921-941. Furtado, P. B., Whitty, P.W., Robertson, A., Eaton, J. T., Almogren, A., Kerr, M.A., Woof, J.M. & Perkins, S. J.

Abstract. Immunoglobulin A, the most abundant human immunoglobulin, mediates immune protection at mucosal surfaces as well as in plasma. It exists as two subclasses IgA1 and IgA2, and IgA2 is found in at least two allotypic forms IgA2m(1) or IgA2m(2). Compared to IgA1, IgA2 has a much shorter hinge region which joins the two Fab and one Fc fragments. In order to assess its solution structure, monomeric recombinant IgA2m(1) was studied by X-ray and neutron scattering. Its Guinier X-ray radius of gyration R_G is 5.18 nm and its neutron R_G is 5.03 nm, both of which are significantly smaller than those for monomeric IgA1 at 6.1-6.2 nm. The distance distribution function *P(r)* for IgA2m(1) showed a broad peak with a subpeak and gave a maximum dimension of 17 nm, in contrast to the *P(r)* curve for IgA1 which showed two distinct peaks and a maximum dimension of 21 nm. The sedimentation coefficients of IgA1 and IgA2m(1) were 6.2 S and 6.4 S respectively. These data show that the solution structure of IgA2m(1) is significantly more compact than IgA1. The complete monomeric IgA2m(1) structure was modelled using molecular dynamics to generate random IgA2 hinge structures, to which homology models for the Fab and Fc fragments were connected to generate 10,000 full models. A total of 104 compact best fit IgA2m(1) models gave good curve fits. These best fit models were modified by linking the two Fab light chains with a disulphide bridge that is found in IgA2m(1), and subjecting these to energy refinement to optimise this linkage. The averaged solution structure of the arrangement of the Fab and Fc fragments in IgA2m(1) was found to be predominantly T-shaped and flexible, but also included Y-shaped structures. The IgA2 models show full steric access to the two FcαRI binding sites at the Cα2-Cα3 interdomain region in the Fc fragment. Since previous scattering modelling had shown that IgA1 also possessed a flexible T-shaped solution structure, such a T-shape may be common to both IgA1 and IgA2. The final models suggest that the combination of the more compact IgA2m(1) and the more extended IgA1 structures will enable human IgA to access a broader range of antigens than either acting alone. The hinges of both IgA subclasses appear to show reduced flexibility when compared to their equivalents in IgG, and this may be important for maintaining an extended IgA structure.

Semi-extended solution structure of human myeloma immunoglobulin D determined by constrained X-ray scattering. (2005) *J. Mol. Biol.* In press. Sun, Z., Almogren, A., Furtado, P. B., Chowdhury, B., Kerr, M. A. & Perkins, S. J.

Abstract. Human immunoglobulin D (IgD) occurs most abundantly as a membrane-bound antibody on the surface of mature B cells (mIgD). IgD possesses the longest hinge sequence of all the human antibody isotypes, with 64 residues connecting the Fab and Fc fragments. A novel rapid purification scheme of secreted IgD from the serum of an IgD myeloma patient using thiophilic (T-gel) and lectin affinity chromatography gave a stable, homogeneous IgD preparation. Synchrotron X-ray scattering and analytical ultracentrifugation of IgD identified the solution arrangement of its Fab and Fc fragments, and thereby its hinge structure. The Guinier X-ray radius of gyration R_G of 6.9 ± 0.1 nm showed that IgD is more extended in solution than the immunoglobulin subclass IgA1 (R_G of 6.1-6.2 nm). Its distance distribution function P(r) showed a single peak at 4.7 nm and a maximum dimension of 23 nm. Velocity experiments gave a sedimentation coefficient of 6.3 S, which is similar to that for IgA1 at 6.2 S. The complete IgD structure was modelled using molecular dynamics to generate IgD hinge structures, to which homology models for the Fab and Fc fragments were connected. Good scattering curve fits were obtained with 18 semi-extended best fit IgD models that were filtered from 8,500 trial models. These best-fit models showed that the IgD hinge does not correspond to an extended polypeptide structure. The averaged solution structure arrangement of the Fab and Fc fragments in IgD is principally T-shaped and flexible, with contribution from Y-shaped and inverted Y-shaped structures. Although the linear sequence of the IgD hinge is much longer, comparison with previous scattering modelling of IgA1 and IgA2(m)1 suggests that the hinge of IgA1 and IgD are more similar than might have been expected. Both possess flexible T-shaped solution structures, probably reflecting the presence of restraining O-linked sugars.

Biochemical and structural properties of the complex between human immunoglobulin A1 and human serum albumin by X-ray and neutron scattering and analytical ultracentrifugation. (2005) Submitted for publication. Almogren, A., Furtado, P. B., Sun, Z., Perkins, S. J. & Kerr, M. A.

Abstract. IgA is by far the most abundant antibody in humans, and is unique amongst antibodies in being able to form a range of polymeric structures that may possess important functions in the pathology of specific diseases. Using a novel two-step purification based on thiophilic and jacalin affinity chromatography, the covalent complex between the IgA1 subclass of IgA and human serum albumin (HSA) was purified. The link is formed between Cys471 in IgA1 and Cys34 in HSA. IgA1-HSA binds to IgA receptors on neutrophils and monocytes, and elicits a respiratory burst that is comparable in magnitude to that of monomeric IgA1. The solution arrangement of IgA1-HSA was identified by X-ray scattering and ultracentrifugation. The radius of gyration R_G of 7.5 ± 0.3 nm showed that IgA1-HSA is more extended in solution than IgA1 (R_G of 6.1-6.2 nm). Its distance distribution function P(r) showed two peaks that indicated a well separated solution structure as previously found for IgA1, and a maximum dimension of 25 nm which is greater than that of 21 nm for IgA1. Sedimentation equilibrium showed that the stoichiometry of IgA1 and HSA is 1: 1. Sedimentation velocity resulted in a sedimentation coefficient of 6.4 S and a frictional ratio of 1.87 which is greater than that of 1.56 for IgA1. The constrained modelling of the IgA1-HSA structure using known structures for IgA1 and HSA generated 2432 conformationally-randomised models of which 52 gave good fits to the scattering curve. The HSA structure was located at the base of the Fc fragment in IgA1 in an extended arrangement. Such a structure accounts for the functional activity of IgA1-HSA, and supports our previous modelling analysis of the IgA1 solution structure. The IgA1-HSA complex may represent a new class of targeted therapeutic reagents based on the coupling of IgA1 to carrier proteins.