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Crystallographic evidence for deviating C3b structure?

Bert J.C. Janssen[1], **Randy J. Read**[2], **Axel T. Brunger**[3], and **Piet Gros**[1]

[1]Crystal and Structural Chemistry, Bijvoet Center for Biomolecular Research, Faculty of Sciences, Utrecht University, Padualaan 8, 3584 CH Utrecht, The Netherlands [2]Department of Haematology, University of Cambridge, Cambridge Institute for Medical Research, Wellcome Trust/MRC Building, Hills Road, Cambridge CB2 0XY, U.K. [3]Howard Hughes Medical Institute, and Departments of Molecular and Cellular Physiology, Neurology and Neurological Sciences, Structural Biology, and Stanford Synchrotron Radiation Laboratory, Stanford University, Stanford CA 94305-5432, USA

Abstract

Activation of C3 into C3b is a critical step in the complement immune response against pathogenic, immunogenic and apoptotic particles. Ajees *et al.*1 report a crystal structure of C3b, which deviates from the one reported by Janssen *et al.*2 and Wiesmann *et al.*3. Analysis of the data deposited by Ajees *et al.*1 reveals features that are inconsistent with known physical properties of macromolecular structures and their diffraction data. Therefore, Ajees *et al.* do not provide substantial evidence for the deviating crystal structure of C3b.

Three structures of the 12-domain protein C3b were reported1-3. In the structure of C3b reported by Ajees *et al.*1 the CUB domain adopts an unfolded conformation and the TED domain has a C3-like (instead of an activated, C3d-like) conformation and is positioned away from the main body of the molecule. The structure of the remaining 10 domains is similar among the three structures and resembles the C3c structure as determined by Janssen *et al.* in 20054. The conformation and locations of the CUB and TED domains are of specific interest, because they are critical to the biological functions of this central molecule of the complement system.

While analysing the structural differences among the three structures of C3b, we noticed that the deposited coordinates of Ajees *et al.* (PDB entry 2HR0) do not form a connected network of molecules in the crystal lattice. The crystal structure forms layers that are separated by a large void in the c-direction (an approximately 30-40 Å thick slab that spans the entire unit cell). First, we considered whether this highly unusual, and unreported, feature was due to a book-keeping error. We reproduced the reported refinement statistics using the diffraction data deposited in the PDB using two independent computer programs (Refmac[5] and CNS 1.26). We conclude that the published statistics, the deposited coordinate model and diffraction data are consistent with each other. Second, we considered the possibility of a missing protein molecule. Re-determination by molecular replacement using the program Phaser7 resulted in the same overall molecular arrangement as observed by Ajees *et al.* We found no evidence for potentially missing protein molecules, neither by features in the electron density map nor by increased scores in the log-likelihood function when searching for additional components in Phaser. In addition to the absence of crystal contacts in the c-direction, we noticed other physically unrealistic features. The deposited diffraction data do not show the features that should arise from the presence of bulk solvent

Correspondence to: Piet Gros, Email: p.gros@chem.uu.nl.

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(Figure 1), while the molecular arrangement indicates large regions not occupied by protein molecules. In other words, the diffraction data are consistent with protein molecules in vacuum, but not with molecules surrounded by disordered solvent as always seen for macromolecular crystals8. The B-factors of the model (both the deposited B-factors as well as the B-factors obtained by re-refinement) do not display any significant variations throughout the molecule, even though long segments of the chain are almost completely solvent exposed (see Figure 2). B-factors describe the size of displacements available to the atoms, so they are correlated with disorder for surface-exposed residues and rigid-body like motion of domains9. The R_{free} and R distributions are exceptionally low at low resolution, and the R_{free}-R difference is unusually small for a structure refined at 2.3-Å resolution using an amplitude-based target function (Figure 1B). Given these physically unrealistic features we question the validity of the deposited diffraction data and the associated model presented in the paper by Ajees *et al*. Only with the experimental diffraction images made available can the deviating C3b model be either verified or falsified.

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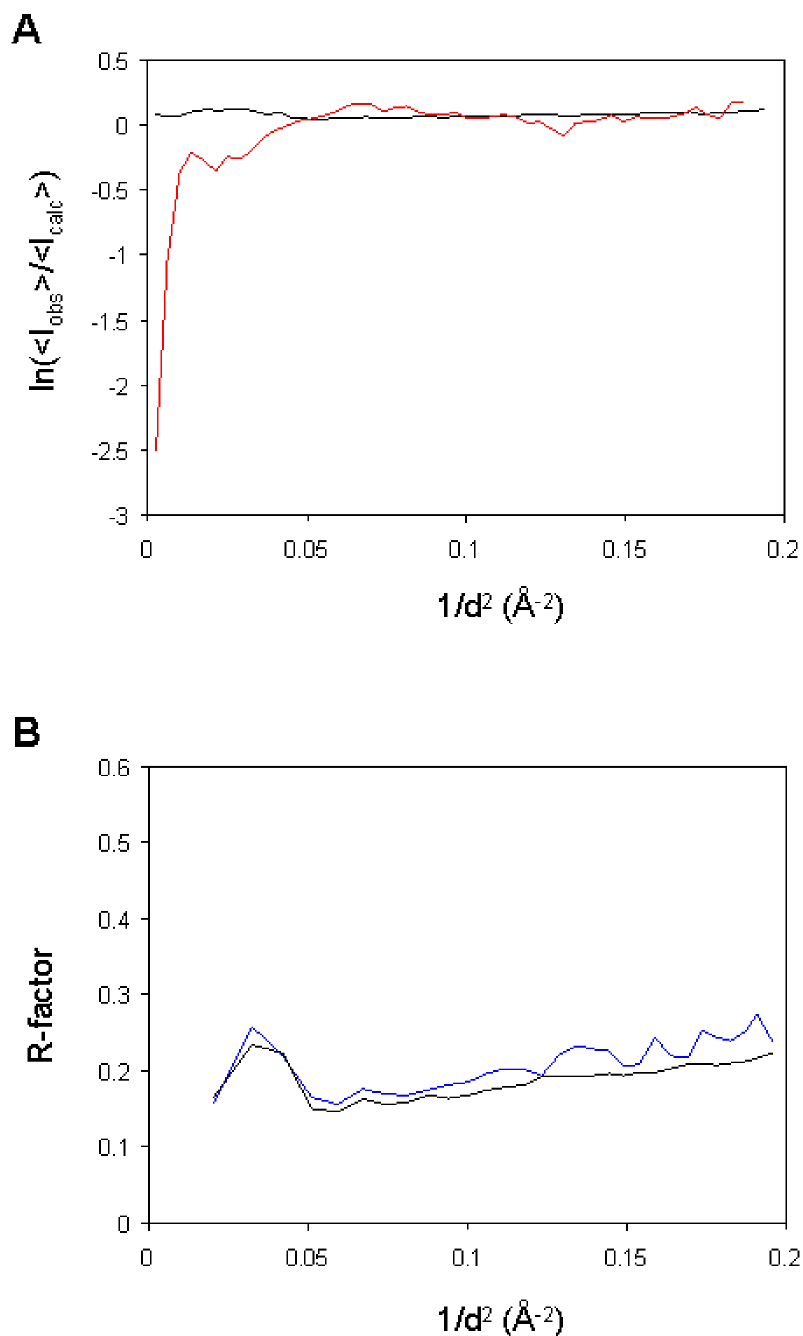

Figure 1.

Lack of bulk solvent in the deposited diffraction data. (A) Plot, as a function of resolution, of the logarithm of the ratio between average observed intensities and average intensities calculated from a model lacking a bulk solvent contribution. The black line shows the results for the structure from Ajees *et al.* (2HR0), while the red line shows results for a control experiment with data from PDB entry 1H1810, which has a similar size and resolution limits. The plot is expected to fall off at low resolution, as seen for 1H18, because the presence of disordered solvent reduces the contrast and hence the average diffraction intensity. (B) R (black line) and R_{free} (blue line) plotted as a function of resolution, calculated with CNS 1.2 using the data and the structure of Ajees *et al.* (2HR0) without a

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bulk solvent model. In the absence of a bulk solvent model a large discrepancy is expected at low resolution (with R-factors beyond 0.5), because the contribution of the bulk solvent in the crystal to the diffraction data is not accounted for in the model8.

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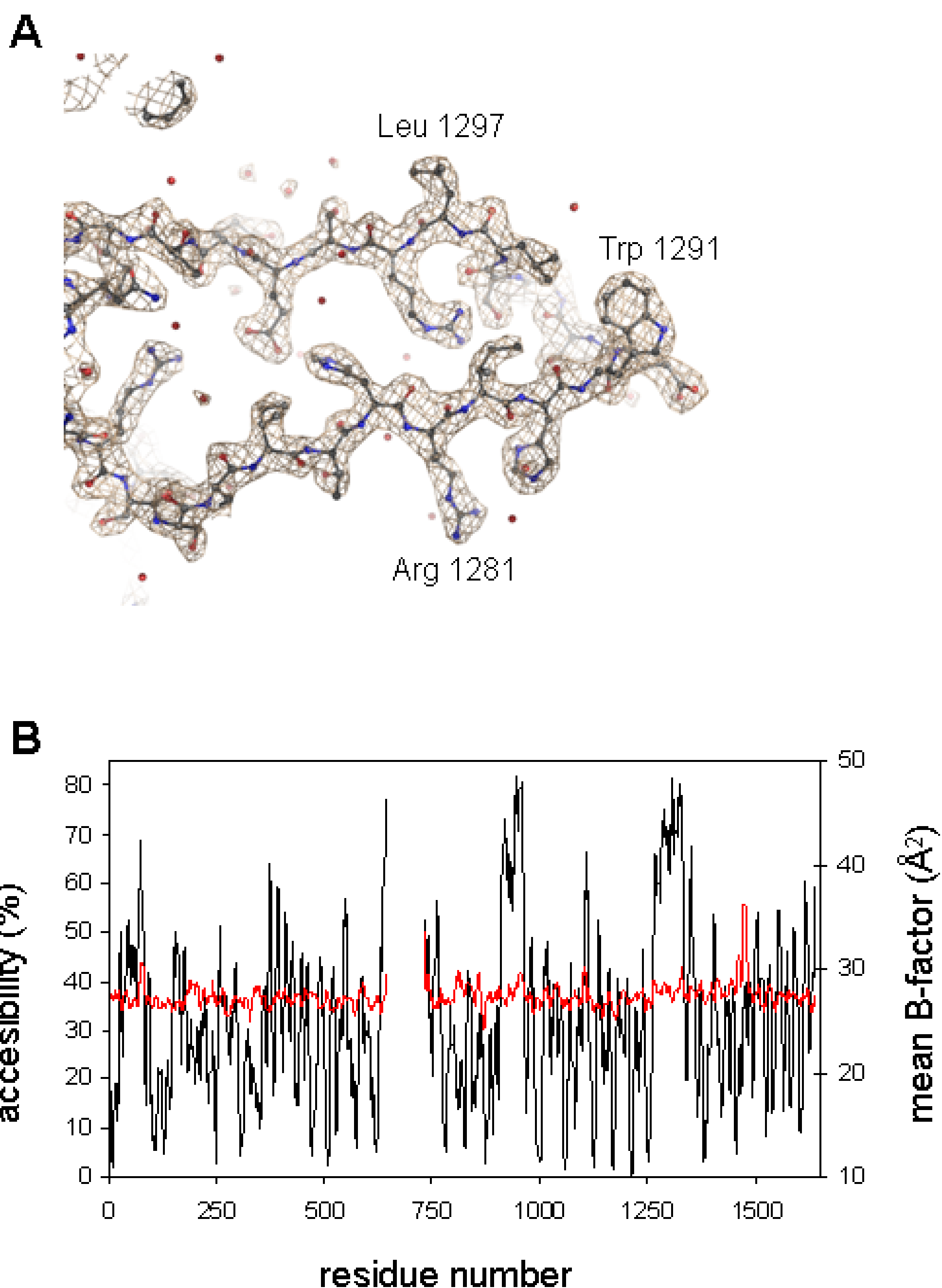

Figure 2.

Lack of correlation between surface exposure and disorder. (A) The electron density (calculated with coefficients $2mF_{obs}$-DF_{calc}, φ_{calc}) of a region in the unfolded CUB domain is contoured at 2.5 σ (0.55 e/Å^3). (B) Plot of percent surface accessibility (black, computed with NACCESS11) and atomic B-factors (Å^2) after re-refinement (red), both averaged over a window of 9 residues. Normally the two measures are highly correlated.