Experimental Report

Proposal: **8-04-698** **Council:** 10/2012

Title: Spin-echo verification of the molecular dynamics simulations of RecA protein from D.radiodurans

This proposal is resubmission of: 8-04-673

Researh Area: Biology

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Samples: protein
protein-ATP

Instrument	Req. Days	All. Days	From	To
IN15 Standard	12	9	15/03/2013	24/03/2013

Abstract:

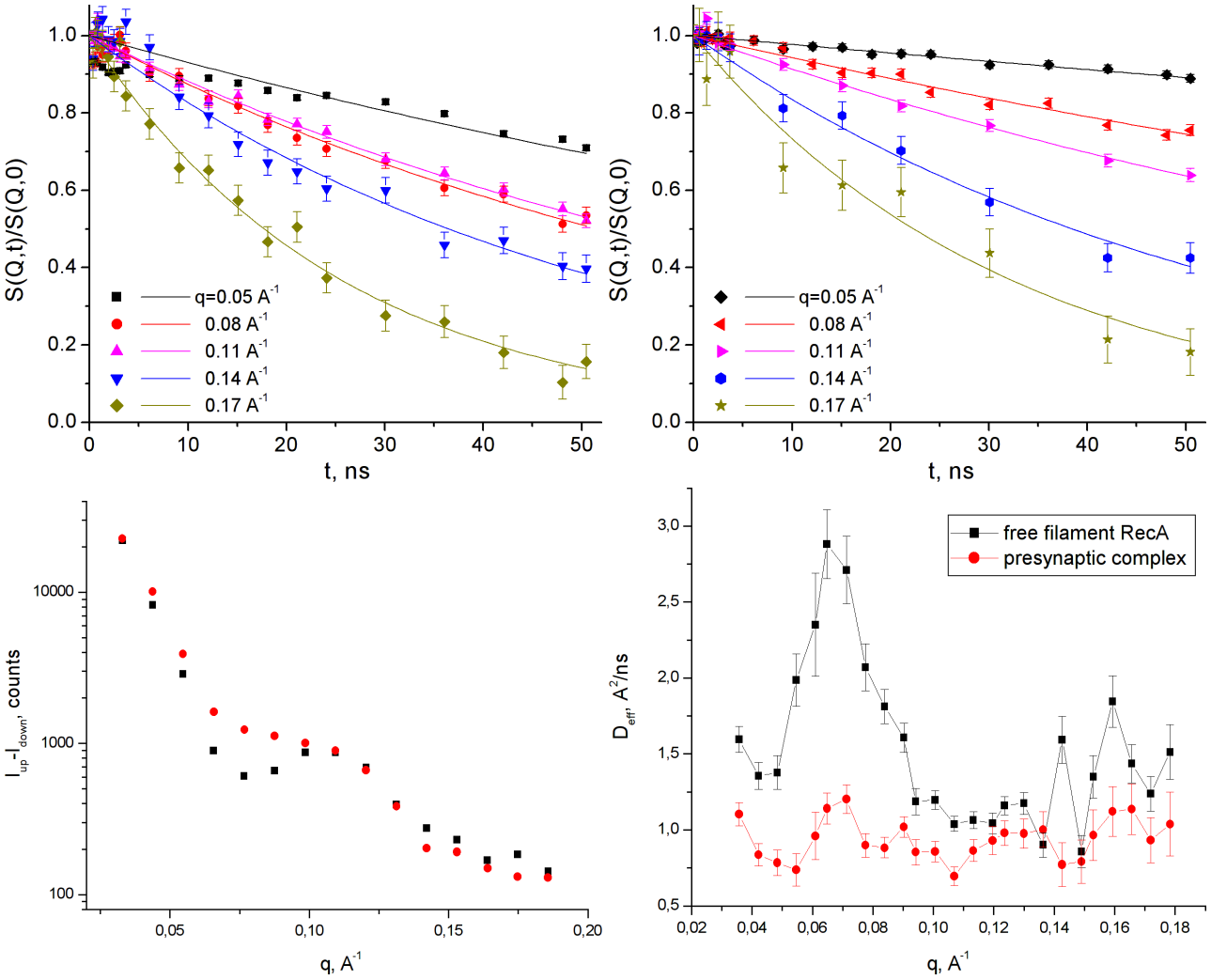
Homologous recombination protein RecA is the key DNA repair enzyme in bacteria. Although the structure and function of a RecA and its analogues (such as RadA in archae and Rad51 in higher eucariotes) are very well studied, the detailed molecular mechanisms for the enzymatic activity of this family of proteins is still uncertain. As this enzyme works in the polymerized form bound to a relatively long stretch of DNA, large-scale conformational flexibility plays an important role in its function.

Functional subdomain motion in RecA protein and its inference on the protein filament flexibility on the larger scale have been identifyed by computer simulation of molecular dynamics. We propose to obtain an experimental verification for the molecular dynamics model by neutron spin-echo.

RecA protein plays key role in homology recombination reaction in bacterial cell. It works by forming right handed filament structure on a single stranded DNA (ssDNA) and subsequently searches for homology region between two DNA molecules: ssDNA inside RecA filament and dsDNA outside it and performs strand exchange reaction between this two homology regions.

Based on the data available in PDB we built a full atomic model of the protein filaments formed by RecA from *E. coli* and *D. radiodurans*, consisting of 12 monomers. To investigate large scale conformational motions of these two proteins a molecular dynamics simulations using GROMACS molecular dynamics package and the corresponding neutron spin echo experiments on IN15 were performed.

NSE relaxation was measured for 12 mg/ml aqueous solution of RecA protein from *D. radiodurans* in a D2O buffer containing ca.5% glycerol. Measurements were performed for the temperatures of 14 and 28°C and the scattering vector magnitude range 0.025 – 0.18 $Å^{-1}$. Neutron wavelength of 10 and 17 Å was used for the range of spin echo times up to 50 ns and 250 ns, respectively. NSE relaxation was measured for both free protein filament and the presynaptic complex with single-stranded phage M13 DNA.



NSE relaxation curves for RecA D.r. (top left) and its presynaptic complex (top right) and the corresponding scattering and effective diffusion coefficient dependency on the scattering vector magnitude.

The relaxation curves could be fitted to a single-exponential decay model. The effective diffusion coefficient of the protein self-polymer seemed to increase over 3-fold near the structure factor minimum (0.07 $Å^{-1}$). The effective diffusion coefficient of the presynaptic complex was lower and did not exhibit the same marked dependency on the scattering angle. Qualitatively similar results (with the quantitative differences consistent with the changes in the solvent viscosity) were obtained in 14˚C measurements, showing no sign of a transition in the dynamics properties of the protein in this temperature range. The effective diffusion of the filaments observed was in both cases much higher than would be expected for the rigid filament (that were over 100 nm in length, as confirmed by SANS measurements on KWS-2 and KWS-3 spectrometers), and, in the case of the self-polymer, was similar to the simulated for a translational diffusion of RecA dodecamer (ca. 20 nm in length). The observed spin-echo relaxation should likely be assigned to the large-scale dynamics (bending modes) of the filament.

The molecular dynamics model of RecA filament yields some valuable predictions about the large-scale motion of the filament, particularly regarding the filament flexibility and its helical pitch (that is in solution decreases from 64 Å (crystallographic data) down to 60 Å in self-polymer and increases to ca.80 Å in the presynaptic complex) and is generally in agreement with the experimental small-angle scattering and NSE data.

Spin echo experiment reveals large-scale dynamics in the protein filament, likely corresponding to the protein bending. Formation of the presynaptic complex significantly reduced the filament mobility. However, further investigation is needed to use NSE data for MD model verification. Particularly, while NSE spectra can be described by the single-exponential decay, the limed spin-echo time range for 10 Å neutron wavelength together with high scatter in the data obtained using 17 Å does not allow to rule out a different relaxation model.

Results were reported at FEBS-2013 conference, July 6-11 2013, St.Petersburg, Russia.