Conformational flexibility of RecA protein filament: Transitions between compressed and stretched states

ARTICLE *in* PROTEINS STRUCTURE FUNCTION AND BIOINFORMATICS · NOVEMBER 2006

Impact Factor: 2.63 · DOI: 10.1002/prot.21116 · Source: PubMed

7 AUTHORS, INCLUDING:

Michael Petukhov
Petersburg Nuclear Physics Institute
39 PUBLICATIONS **431** CITATIONS
SEE PROFILE

Dmitry V Lebedev
Petersburg Nuclear Physics Institute
36 PUBLICATIONS **138** CITATIONS
SEE PROFILE

Akhmed Islamov
Joint Institute for Nuclear Research
69 PUBLICATIONS **521** CITATIONS
SEE PROFILE

PROTEINS: Structure, Function, and Bioinformatics 65:296–304 (2006)

Conformational Flexibility of RecA Protein Filament: Transitions between Compressed and Stretched States

Michael Petukhov,[1,2]* Dmitry Lebedev,[1,2] Valery Shalguev,[1] Akhmed Islamov,[3] Aleksandr Kuklin,[3] Vladislav Lanzov,[1,2] and Vladimir Isaev-Ivanov[1,2]

[1]*Division of Molecular and Radiation Biophysics, Petersburg Nuclear Physics Institute, the Russian Academy of Sciences, Gatchina/St. Petersburg, Russia*

[2]*Research and Education Center "Biophysics", PNPI RAS and St. Petersburg State Polytechnical University*

[3]*Frank Laboratory of Neutron Physics, Joint Institute for Nuclear Research, Dubna, Russia*

ABSTRACT **RecA protein is a central enzyme in homologous DNA recombination, repair and other forms of DNA metabolism in bacteria. It functions as a flexible helix-shaped filament bound on stretched single-stranded or double-stranded DNA in the presence of ATP. In this work, we present an atomic level model for conformational transitions of the RecA filament. The model describes small movements of the RecA N-terminal domain due to coordinated rotation of main chain dihedral angles of two amino acid residues (Psi/Lys23 and Phi/Gly24), while maintaining unchanged the RecA intersubunit interface. The model is able to reproduce a wide range of observed helix pitches in transitions between compressed and stretched conformations of the RecA filament. Predictions of the model are in agreement with Small Angle Neutron Scattering (SANS) measurements of the filament helix pitch in RecA::ADP-AlF$_4$ complex at various salt concentrations. Proteins 2006;65:296–304.** © 2006 Wiley-Liss, Inc.

Key words: SANS; homologous recombination; molecular modeling; RecA mobile N-terminal domain; RecA filament helix pitch

INTRODUCTION

RecA-like proteins belong to a larger protein family that includes the nearly ubiquitous RecA proteins in bacterial species, the Rad51 and Dmc1 proteins of eukaryotes, and the archaeal RadA proteins.[1,2] RecA, being a central enzyme of homologous recombination and recombinational DNA repair in bacteria, plays a pivotal role in genome reproduction and the maintenance of genome integrity.[3–7]

RecA functions as a helical filament polymerized on single-stranded DNA (ssDNA) in the presence of ATP and Mg^{2+} ions. This activated form of RecA filament is capable for interaction with double-stranded DNA (dsDNA), followed by homologous pairing and strand exchange between dsDNA and ssDNA that are two main steps of homologous recombination.

Several different RecA crystal structures at high resolution have been published to date. These include the RecA filaments from *Escherichia coli, Mycobacterium tuberculosis, Mycobacterium smegmatis*, and hyperradioresistant *Deinococcus radiodurans*.[8–13] All the RecA proteins have high levels of sequence identity to that of *E. coli* (62% for *M. tuberculosis* and *M. smegmatis* and 56% for *D. radiodurans*). Moreover, all of them have similar overall topologies, filament structures, and intersubunit interfaces, although their filament helix pitches vary in a wide range from 67–83 Å. Despite many efforts, no RecA crystal structures at high resolution have been obtained for the activated form of RecA filaments in complex with DNA.

The RecA–DNA complexes have been studied by electron microscopy (EM) and small-angle neutron scattering (SANS). These studies show that in the presence of DNA and ATP, the RecA filament is converted into an active form with conformation that is distinctly different from the inactive one. Particularly, both EM[14–16] and SANS studies[17–19] show that, being in its active form, the RecA::ssDNA filament has an increased helix pitch (from 67–83 to 91–95 Å) and shortened cross-sectional radius of gyration (38 vs. 33 Å).[19] The RecA::dsDNA complexes have also been examined by atomic force microscopy (AFM).[20] The AFM images reveal as large as an ≈100-Å helix pitch in the RecA nucleoprotein filaments. Also, substantial movements of the RecA C-terminal domain have been observed by EM.[14]

Although precise positions and conformations of ssDNA and dsDNA in active RecA nucleoprotein filaments are still unknown, the molecular mechanism of DNA stretching, homologous pairing, and strand exchange between dsDNA

Grant sponsor: the Russian Foundation for Basic Research; Grant number: 02-04-49259-a; Grant sponsor: Russian Academy of Science programs "Physics of Elementary Particles" (subprogram "Neutron physics," direction "Studies of structure, dynamics and nonordinary properties of matter by neutron scattering), by the Ministry of Education and Science of Russian Federation; Grant number: RNP.2.2.1.1.4663; Grant sponsor: St. Petersburg Scientific Center, the Russian Academy of Sciences and the Fogarty International Research Collaboration Award; Grant number: 2 R03 TW00 1319-04 (to Dr. Michael M. Cox).

*Correspondence to: Michael Petukhov, Division of Molecular and Radiation Biophysics, Petersburg Nuclear Physics Institute, the Russian Academy of Sciences, Orlova roscha, Gatchina, 188300, St. Petersburg, Russia. E-mail: pmg@omrb.pnpi.spb.ru

Received 2 November 2005; Revision 2 March 2006; Accepted 29 March 2006

Published online 14 August 2006 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/prot.21116

and ssDNA have been examined in several experimental and theoretical works. Approximately 1.5 times longer relative to the classic B-form, the extended conformation of bound ssDNA in RecA filament has been determined by NMR.[21] This DNA structure contains a novel stacking between deoxyribose and base where, instead of the conventional Watson-Crick stacking of adjacent bases, the deoxyribose is placed above the base of the adjacent residue, which results in spacing out the ssDNA bases by ~5 Å. Based on this study of extended ssDNA bound to RecA filament, the molecular model of DNA stretching has been developed, and two types of extended DNA structures have been proposed.[22] One structure is a duplex with the N-type sugar pucker and with a helix pitch of ≈95 Å (18.6 bp/turn) corresponding to that in an active RecA filament. Another structure has the S-type sugar pucker with a helix pitch of 64 Å (12.5 bp/turn) that corresponds to the inactive filament. The molecular modeling shows a possibility of conformational transitions between these two forms of sugar puckers that can rotate bases horizontally while maintaining the base stacking interaction. It has been suggested that this base rotation enables base pair switching between dsDNA and ssDNA facilitating homologous pairing and strand exchange.

In addition to the proposed mechanism of DNA extending, a molecular mechanism for RecA filament extending should also exist. In this work, based on analysis of two RecA protein crystal structures and on molecular modeling of the RecA N-terminal domain flexibility, we present a simple model reproducing a possible molecular mechanism behind a wide range of experimentally observed changes in the geometry of RecA filaments. The model predictions are in a good agreement with experimental data available in the literature as well as those obtained in this work by SANS measurements.

MATERIALS AND METHODS

Experimental Procedures

SANS data were acquired on the YuMO spectrometer, Frank Laboratory of Nuclear Physics, Joint Institute for Nuclear Research in Dubna, Russia. The standard geometry was used as described,[23] with a collimated beam diameter of 14 mm. The time-of-flight method was used for the scattering curve acquisition, with the range of neutron wavelengths from 0.7 to 6 Å. The raw data were processed and normalized as described,[23] so that the absolute value (in cm^{-1}) for the scattering intensity could be determined. Two detectors were used for simultaneous acquisition in the range of Q from 0.008 to 0.3 $Å^{-1}$.[23] Samples were placed in 2-mm Helma cuvettes and maintained in a temperature-stabilized box during measurements.

The *E. coli* RecA protein was purified as described.[24] The protein concentration was determined by absorption at 280 nm using an extinction coefficient of $2.23 \times 10^4\ M^{-1}\ cm^{-1}$. The ATPase activity of RecA protein was measured by registering the rate of NADH oxidation (photometry at 340 nm) in the presence of lactic dehydrogenase/pyruvate kinase system in Tris-Mg^{2+}-DTT buffer (pH 7.5) as described.[25] SANS measurements were performed at 15°C in 20 mM Tris-HCl buffer (pH 7.5), 2 mM Mg containing >99.8% D_2O acetate and 2–5% glycerol under conditions of different ionic strengths. A blank sample of the buffer containing the same amount of glycerol was used for a reference. ADP–AlF_4 [2.5 mM ADP, 5 mM NaF, and 0.5 mM $Al(NO_3)_3$] was used as a nonhydrolyzable ATP analog. Glycerol, ATPγS, ADP, Tris, NaF, $Al(NO_3)_3$ and Mg acetate were purchased from Sigma. We observed no substantial difference between ADP–AlF_4 and ATPγS (0.5 mM) in their effect on structure parameters of the two RecA protein complexes under both low-salt and high-salt conditions. ADP–AlF_4 was added immediately prior to the experiment with subsequent incubation for 15 min at 37°C. Salt titration was done by addition of NaCl (to 0–1.8 M final concentration). RecA concentration was 6 mg/mL; the acquisition time for each curve was 20–40 min. Replicate measurements, taken on each sample at the beginning and at the end of each experiment, did not show significant difference in the scattering curves, indicating that the protein structure is not degraded during the time of measurements. Structure parameters (the helical pitch and the cross-sectional radius of gyration) of RecA polymer were determined by fitting SANS data to the model for scattering on a long helical filament, as described.[19]

Molecular Modeling and Conformational Analysis

Search for dynamics domains in *E. coli* RecA protein and its hinge axis was done with DynDom program[26] using two crystal RecA protein structures.[9,13] The molecular modeling was done using ICM-Pro, the commercially available package of programs (MolSoft LLC, USA),[27] which uses the ECEPP/3 force field.[28,29] All atoms in the proteins were treated explicitly. Bond lengths and bond angles were fixed at their standard values during the energy calculations and minimization. The van der Waals, electrostatic, hydrogen bond, and torsion potentials were included in the energy calculations. The protein surface based solvation energy term was modeled by the continuum approximation model for protein solvent interactions as implemented in ICM.

The model RecA protein dimer was used for molecular modeling of possible N-terminal domain movements in RecA filament. Initial conformation of the filament was taken from RecA protein crystal structure.[8] It was regularized according to standard ICM protocol to eliminate minor steric clashes existing in all protein crystal structures.[27] To account for electrostatic energy contributions from charged residues of disordered loops L1 (residues 157–164) and L2 (residues 195–209) as well as of long C-terminal tail (residues 329–352), these amino acid residues were added to the molecular model. Ends of the L1 and L2 loops were fixed at their regularized crystal structure positions in energy minimization of amino acids residues of the L1 and L2 loops. The residues of the disordered C-terminal tail were initially set in extended conformation (all dihedral angles = 180°) and energy minimized. During modeling of the loops and the

C-terminal tail, the rest of RecA protein was fixed in its regularized crystal structure conformation.

The energy maps of N-terminal movements were calculated for RecA dimer conformations obtained by rigid body domain rotation around two main-chain dihedral angles (Psi23 and Phi24) of Lys23 and Gly24 on a grid with step of 1°. The position of the second RecA subunit was kept fixed relatively the N-terminal domain of the first RecA subunit. Conformations of other parts of the RecA protein main chain were fixed in the regularized crystal form. To model a certain degree of protein flexibility conformations of amino acid side chains were allowed to vary using energy minimization by conjugate gradient method at every node of the grid. RecA filament containing two helix turns (13 RecA subunits) was build based on each dimer conformation on the grid. The helix axis, helix pitch, and cross-sectional radius of gyration were calculated as described earlier[30] using special scripts written in the ICM shell program language. The cross-sectional radius of gyration, R_g, was calculated using the following standard formula:

$$R_g^2 = \frac{\sum\limits_{\text{atoms}} w_i d_i^2}{\sum\limits_{\text{atoms}} w_i}$$

where d_i is the distance of atom i to helix axis and w_i is its standard atomic weight. The scripts are available from the corresponding author upon request.

To evaluate electrostatic properties of the model molecular system and its dependencies on salt concentrations due to Debye-Huckel charge screening, the numerical solution of the Poisson-Boltzmann equation was carried out using APBS computer program.[31] The linearized form of Poisson-Boltzmann equation (LPBE) was solved using grid lengths 120 × 120 × 120 Å with a 0.62-Å grid spacing with multiple Debye-Hukel boundary conditions. Values for dielectric constants were set at 2 and 80 for the solute and the solvent, respectively. The calculations were done for low (0.1 M) and high (1.8 M) NaCl salt concentrations on a personal computer equipped with AMD Athlon-64X2/4400+ processor and 2Gb RAM using Windows version of APBS taken from author's Internet site. All other parameters for APBS calculations were set to their recommended values. Special scripts in ICM shell language allowing the use of APBS from ICM environment were prepared. The scripts are available from the corresponding author upon request.

RESULTS AND DISCUSSION

Stretched Conformations of the RecA Filament Observed by SANS

Two distinct states (active and inactive) of RecA filament complex with ssDNA has been observed in a number of studies.[14,17] It has been suggested that the intrinsic state of DNA may be responsible for the conservation of the stretched filament forms across evolution.[32] In this study, we investigated the changes in the structure of RecA self-polymer complex with a nonhydrolyzable ATP analogue in the absence of ssDNA that occurs under high ionic strength conditions. Such conditions are known to induce the RecA protein ATPase activity, normally seen only for the RecA presynaptic complex in its active conformation.[25] Figure 1a shows the SANS scattering spectra of RecA protein self-polymer complex with ADP–AlF_4 in the absence and in the presence of 1.8 M NaCl. Compared to low-salt conditions, in 1.8 M NaCl we observed a substantial shift in the geometric parameters of the filament (increase in the helix pitch on average from 78 ± 3 to 86 ± 1 Å and decrease in the cross-sectional radius of gyration from 39.9 ± 0.2 to 34.7 ± 0.7 Å, $n = 3$) toward those obtained for the presynaptic complex of the enzyme with ssDNA.[17,19] Thus, the high salt appears to induce a transition of the RecA filament structure from inactive to active (stretched) form.

To further investigate this transition, the geometric parameters of the filament at intermediate salt concentrations were obtained. SANS spectrum of RecA complex with ADP–AlF_4 in 0.8 M NaCl is shown in Figure 1b. As seen from the fit of the data to scattering by the long helical filament, the geometric parameters of the filament obtained by fitting the data to scattering by a long helical object (the helix pitch of 85 Å, the cross-sectional radius of gyration of 37.6 Å) were different from those seen in both active and inactive RecA forms.

The model used to obtain the above results assumes a system of ordered helical filaments and would not be valid for polydisperse or disordered filaments. The existence of the diffraction maximum near 0.08 $Å^{-1}$ (Fig. 1b) indicates that the RecA helix structure is not disordered, as would likely be the case of a heterofilament comprised of a mixture of RecA monomers in two stable conformations. On the other hand, the SANS spectrum presented in Figure 1b can be fitted to a linear combination of the scattering spectra obtained at 0.0 and 1.8 M NaCl (Fig. 1b, dashed line), which may point to a mixture of filaments of two distinct geometries (approximately 65% active and 35% inactive filaments).

Despite the marked difference from the inactive conformation in the SANS spectrum, RecA protein in 0.8 M NaCl exhibited fairly low rate of ATP hydrolysis ($k_{cat} < 3$ min^{-1}), while the stretched RecA conformation assumed in 1.8 M NaCl had much higher ATPase activity ($k_{cat} \sim 12$ min^{-1}). The ATP hydrolysis measurements were in agreement with the earlier work[25] that shows only a slight increase in the salt-induced DNA-independent ATPase activity of RecA protein at NaCl concentrations below 1 M. These measurements are inconsistent with the interpretation of SANS data by a mixture of active and inactive filament forms (because such a mixture would have had a higher ATPase activity than observed), and would rather imply the existence of a RecA stable conformation with the intermediate filament geometry and low ATPase activity. Variability in the geometric parameters of inactive RecA self-polymers, such as approximately 10% difference in the helical pitch between RecA self-polymers and its complexes with ATPγS at low salt conditions, has been observed previously.[19]

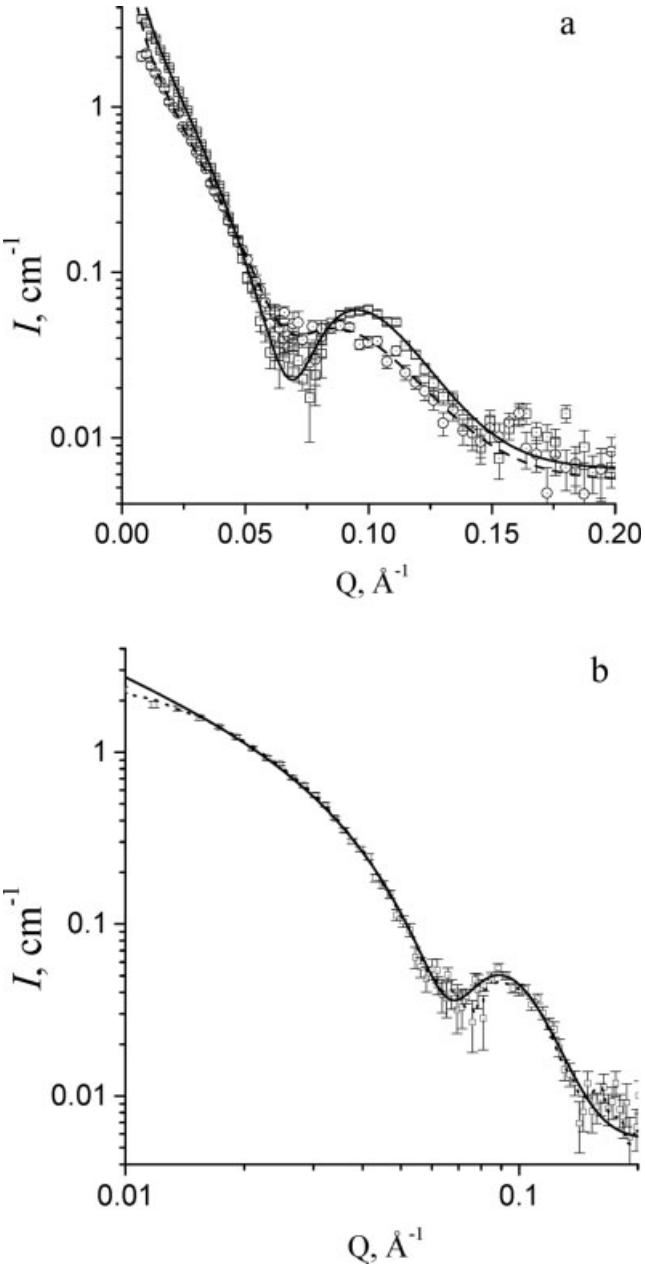

Fig. 1. **a:** Scattering spectra of *E. coli* RecA complex with ADP-AlF4 in D_2O Tris-HCl buffer (pH 7.5) under the salt absence (squares) and in the presence of high salt concentrations (1.8 M NaCl, circles). Data fitted to the long helical filament model (solid and dashed lines, respectively), yielding the helical pitch of 76 Å under low salt and 87 Å under high salt conditions. The cross-sectional gyration radiuses were 40.1 and 35.5 Å, respectively. **b:** Scattering spectrum of RecA complex with ADP-AlF4 in D_2O Tris–HCl buffer (pH 7.5) containing 800 mM NaCl. Data fitted to the long helical filament model (solid line, the pitch of 85 Å, the radius of gyration of 37.6 Å, $\chi^2 = 1.88$) and to the linear combination of the two spectra shown in (**a**) (dashed line, $\xi^2 = 1.15$).

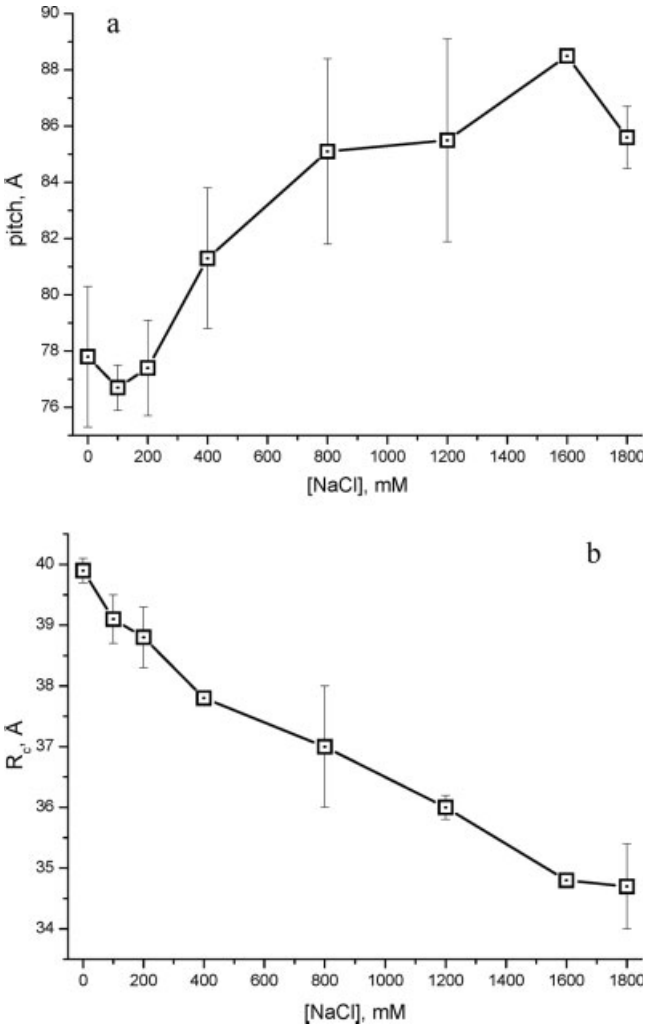

Fig. 2. The dependence of filament helix pitch in RecA::ADP-AlF_4 complex (**a**) and its radius of gyration (**b**) on NaCl concentrations obtained by SANS measurements of *E. coli* RecA filaments.

Figure 2a and b show, respectively, the dependence of RecA filament helix pitch and its radius gyration on salt concentration as measured by SANS. These results indicate that under conditions of graduate increase of the salt concentration, and hence Debye-Huckel's scaling down of the electrostatic contribution to RecA filament free energy, that the RecA filament is capable for a variety of stabile intermediate conformations between inactive and active stretched forms found in presynaptic complex with ssDNA.[15,19] Most of these intermediates are characterized by low rates of ATP hydrolysis (data not shown), extended filament helix pitches, and decreased radiuses of gyration.

The changes in the helical pitch and the filament radius of gyration do not appear to occur in parallel. Most of the helical pitch increase occur in a range of concentration of NaCl from 200 to 800 mM, while the gyration radius exhibited more gradual decrease over the entire range of salt concentration up to 1.8 M. One can also note that although the maximal filament helix pitch of RecA filament in complex with ADP–AlF_4 at high-salt concentration was slightly below of that found in RecA presynaptic complex with ssDNA (~91–95 Å), its radius of gyration was no different from that found in the presynaptic complex by SANS.[19] Thus, the observed decrease in the radius of gyration is unlikely to be directly associated with filament stretching. Among the possible conformational changes

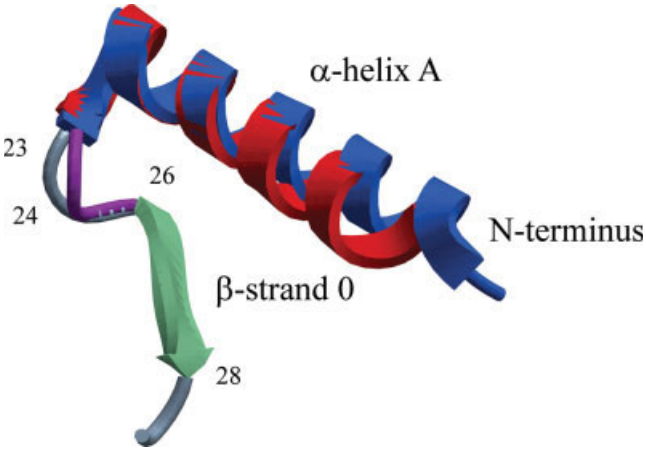

Fig. 3. Spatial superposition of N-terminal domains of crystal[8] (α-helix A shown in blue) and compressed[13] (α-helix A shown in red) structures of *E. coli* RecA protein. Residue numbers are indicated in the figure. The conformations of β-strands 0 (shown in green) in both crystal structures are almost identical.

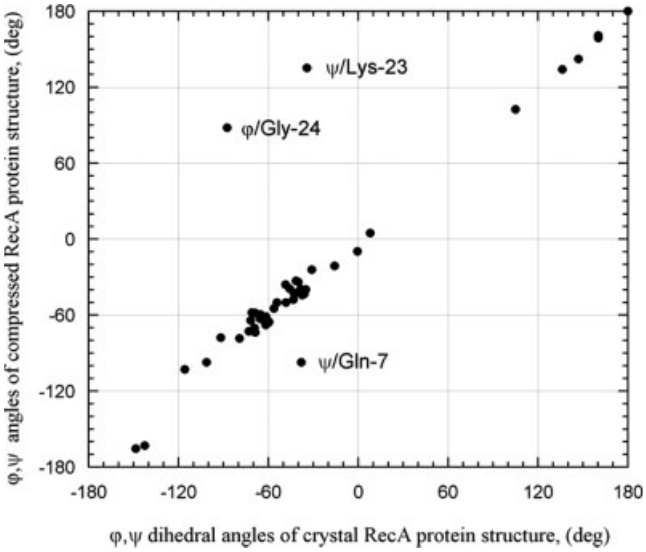

Fig. 4. Correlation between main-chain dihedral angles of the first 30 amino acid residues from RecA protein N-terminus of crystal[8] and compressed[13] *E. coli* RecA crystal structures.

that may affect the radius of gyration are C-terminal domain rotation observed by EM[14,15] and/or a binding of unstructured C-terminal tail consisting of 25 regulatory amino acids within RecA filament structure.[33]

Identification of Amino Acids Residues Acting as a Hinge in the RecA Filament

Recently, a new crystal structure of uncomplexed *E. coli* RecA protein with four extra residues Gly-Ser-His-Met at its N-terminus has been published (PDB code: 1U94).[13] The protein maintains all standard RecA activities and crystallizes as a right-handed helical filament with significantly shorter pitch (≈74 Å) compared to the wild-type RecA protein (≈83 Å). The DynDom search[26] for dynamical domains based on two different *E. coli* RecA protein crystal structures[9,13] revealed that the N-terminal α-helix A moves relative to β-strand 0 rotating around a spatial axis that passes close to the main-chain atoms of Lys23 and Gly24. The spatial superposition of the N-terminal domains of crystal and compressed crystal structures of RecA protein shown in Figure 3 illustrates a major change of the protein main-chain conformation around Lys23 and Gly24 resulting in rotation of the N-terminal α-helix A.

This observation is confirmed by correlation analysis of main-chain dihedral angles of the first 30 amino acid residues from the RecA N-terminus as shown in Figure 4. One can see that, with exception of three dihedral angles (Psi/Gln7, Psi/Lys23, and Phi/Gly24), that there is a fairly good agreement between the main-chain dihedral angles, of the N-terminal residues. Visual inspection of the crystal structures showed that, unlike the crystal RecA structure,[8] the first turn of α-helix A in compressed filament is distorted so that Gln7 is no longer a part of the α-helix. Obviously, due to crystal environment and N-terminal sequence modifications this minor N-terminal fraying of α-helix A has no connection to mechanisms of RecA filament extension and compression.

Another significant change in the main-chain dihedral angles between crystal and compressed RecA crystal structures, which maybe responsible for a large change of RecA filament structure, was associated with a couple adjacent dihedral angles Psi/Lys23 and Phi/Gly24 (see Fig. 3). Flexibility of these two angles can cause changes in relative dispositions of α-helix A against the rest of RecA protein structure in a way that intersubunit interface of the filament is maintained and, consequently, the relative disposition of α-helix A and neighboring RecA protein subunit is unchanged. Additionally, Gly residues are known to have elevated backbone flexibility compared to that of amino acids with side chains. Therefore, Gly residues are the best candidates for hinge residues in proteins. Conformational analysis of different model dipeptides showed that, unlike the standard (φ_i, ψ_i) pair of dihedral angles in a given residue, the protein flexibility of (ψ_i, φ_{i+1}) dihedral angles was much less restricted (data not shown). Finally, a small movement of the RecA protein N-terminal domain observed in compressed RecA structure was sufficient for shortening the filament helix pitch in the compressed filament by 9 Å. All these data suggest that mechanism of the RecA filament transitions into stretched conformation with much longer helix pitch (91–95 Å) can be due to a relatively small rotation of two main-chain dihedral angles in residues Lys23 and Gly24, which are likely candidates for flexible hinge formation in the RecA structure.

Low-Energy Conformations of the Stretched RecA Filaments

To estimate a possible degree of the N-terminal domain flexibility due to changes in Psi/Lys23 (Psi23) and Phi/Gly24 (Phi24) dihedral angles, we built the Ramachandran-like energy map for RecA dimer in a full range (from −180 to 180°) of angle changes using low- and high-salt concentrations (see the Materials and Methods

section). Figure 5a depicts a fraction of the map that contains the low-energy area without steric clashes in the RecA dimer structure (shown in gradations of gray). As seen from this figure, the low-energy area of Psi23/Phi24 dihedral angle space is a deep narrow groove protruding along strait line Phi24 = −0.952*Psi23–124.0 (shown by broken line on the map).

One can translate the conformations of RecA dimers into longer filaments with any number of protein subunits and calculate experimentally measurable parameters including the filament helix pitch and the radius of gyration along the low-energy groove. Figure 5a also shows values of the RecA filament radiuses of gyration in contour lines. These calculations show the possibility of a minor decrease in the filament radius of gyration (~1 Å) resulting from stretching the filament from its crystal structure (the helix pitch of ~83 Å) to the stretched filament structure (the helix pitch of ~91 Å). Thus, the model predicts that the 5-Å decrease of the radius of gyration observed by SANS for RecA filament at high-salt concentrations (see Fig. 2b) as well as for the presynaptic RecA–ssDNA complex at low-salt concentrations[19] appears to be not completely due to RecA filament stretching. This prediction of the model is in agreement with the results of SANS measurements for the RecA::ADP–AlF_4 complex at different salt concentrations (Fig. 2a and b) where minor changes in the helix pitch of RecA filament in the range of salt concentrations of 800–1800 mM were accompanied by a significant decrease of the filament radius of the gyration (37 to 34.7 Å).

Modeling of Electrostatic Interactions at Low- and High-Salt Concentration

Several distance-dependent models for the dielectric constant ϵ have been successfully employed to mimic the water screening of electrostatic interactions in proteins. The choice of dielectric constant is always a problem in molecular-mechanical calculations due to solvent polarization by protein charges. However, a low value of dielectric constant (ϵ ~2–4) was found to be a reasonably good approximation for internally buried groups of globular proteins, while the effective dielectric constant of bulk water (ϵ = 81) is more appropriate for solvent-exposed groups.[34] In this study, we chose numerical solution of linearized form of the Poisson-Boltzmann equation with the Debye-Huckel charge screening to describe the effect of high salt concentrations on total electrostatic energy of the model RecA protein dimer, as described in the Materials and Methods section.

Figure 5b shows energy profiles calculated for RecA dimer conformers along the straight line Phi24 = −0.952*Psi23–124.0 crossing the low-energy area presented in Figure 5a. The sum of nonbonded interactions excluding electrostatic contributions (marked by ECEPP/3-Ele) shows smooth unsymmetric U-shaped curve with minimum in close proximity to the position for crystal conformation of RecA protein filament (marked with sign "+"). Two other curves [marked as ECEPP/3 + APBS_Ele (0.1 M NaCl) and ECEPP/3+APBS_Ele (1.8 M NaCl)]

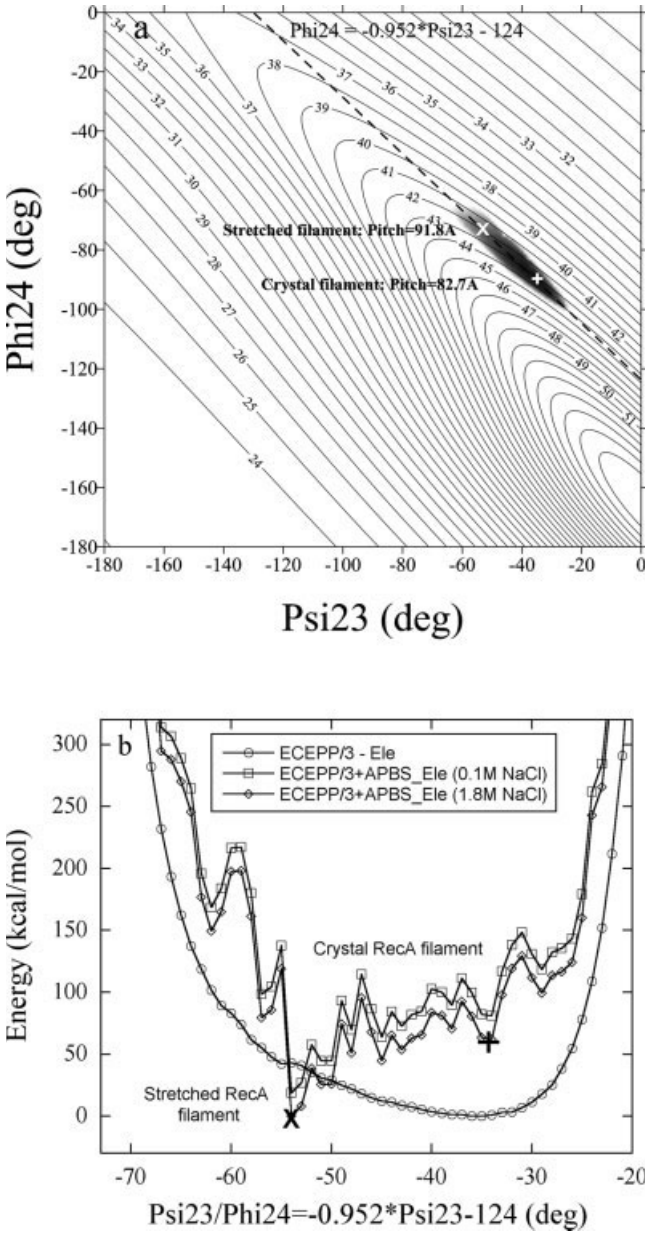

Fig. 5. The energy maps for rotamers of *E. coli* RecA protein dimer with two variable dihedral angles: Psi/Lys23 and Phi/Gly24. **a:** Low energy area (100 kcal/mol above a global energy minimum) of sterically allowed conformations of RecA dimer. Gradations shown in gray represent a nonbonded energy of RecA dimer excluding electrostatic interactions. Contour lines show the RecA filament radius of gyration (in Å) corresponding to each pair of Psi/Lys23 and Phi/Gly24 main-chain dihedral angles. The RecA crystal structure[8] is indicated by "+" mark (shown in white); the lowest energy conformation is marked by "×" (shown in white). **b:** Energy profile within sterically allowed area of Psi23/Phi24 for the nonbonded energy excluding electrostatic interactions (marked by ECEPP/3-Ele, the same as in **a**) and that including electrostatic interactions calculated for low and high salt concentrations [marked as ECEPP/3+APBS_Ele (0.1 M NaCl) and ECEPP/3+APBS_Ele (1.8 M NaCl) correspondingly]. The RecA protein crystal structure[8] and the lowest energy conformation found in this study are shown by marks "+" and "×," respectively.

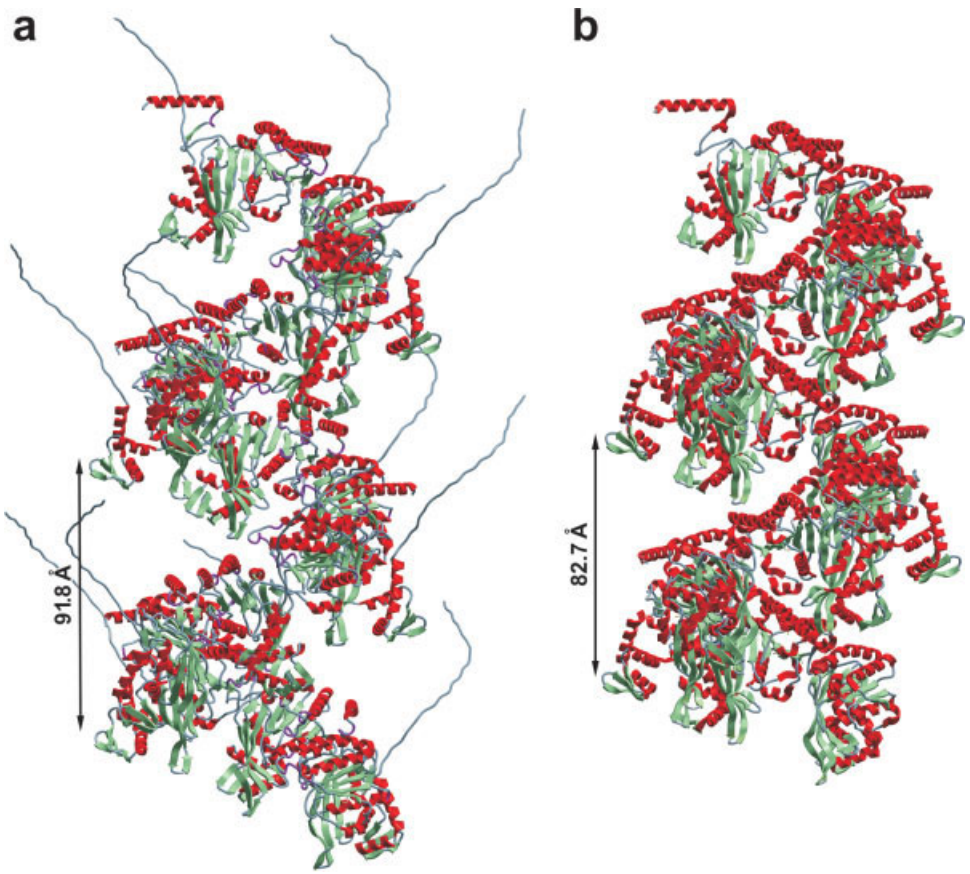

Fig. 6. The comparison of crystal and stretched RecA filaments built by molecular modeling. **a:** The model of RecA protein filament in minimal energy conformation (the helix pitch of 91.8 Å). **b:** The crystal structure of RecA filament (PDB code: 2REB) with the helical pitch of 82.7 Å.

show the sum of all nonbonded interactions including electrostatic interactions calculated by the APBS method for low- and high-salt concentrations, respectively.

Unlike in the former case, inclusion of electrostatic interactions in the two later cases produced several distinct energy minima in a sterically allowed area of the deep groove in the Psi23/Psi24 energy map. It is remarkable that one of the low-energy minima in the right-hand part of the low-energy area corresponds to conformation of RecA filament seen in crystal.[8,9] However, the lowest energy minimum (marked with the sign "X") is shifted to the left-hand part of the energy groove at a distance of approximately 20° from the crystal structure conformation. Here, the RecA filament has its helix pitch of 91.8 Å. Figure 6 shows the comparison of two RecA filament structures, the spatial structure of minimal energy conformation of the RecA filament with the helix pitch of 91.8 Å and that seen in the crystal.[8,9]

The results of calculations at low- and high-salt concentrations show that positions of the corresponding energy minima remain largely unchanged at the two different salt concentrations while the magnitudes of energy minima become significantly lower at high-salt concentration than those at low-salt concentration. It is of interest that despite its relative simplicity, the calculations done with Coulomb electrostatics model with low, high, and distance-dependent dielectric constants also showed a similar disposition of energy minima in the sterically allowed area of the Psi23/Phi24 energy map (data not shown).

Figure 5b indicates that electrostatic interactions in RecA dimer are very sensitive to relative dispositions of interacting protein subunits, and therefore they play a key role in formation of the energy minima allowed for RecA protein subunits in the helical filament structure.

Significance of the Proposed Mechanism for Large-Scale Movements in the RecA Filament

Thus, the results of our modeling confirmed that the small N-terminal domain movements are sufficient to extend the filament pitch of RecA protein from ~83 to ~92 Å. It is remarkable that such a simple model for conformational transitions not only explains a full range of helix pitches observed by SANS but also maintains a perfect structure of the RecA intersubunit interface, a significant restructuring of which is nearly impossible to imagine. However, there are other possibilities for large-scale flexibility in the RecA structure. It seems reasonable to mention about a flexibility of the RecA protein

C-terminal domain as seen by EM,[14] the unstructured loops (L1 and L2) and the C-terminal tail.[8]

Other possibilities that cannot be ruled out are small conformational changes distributed along many residues that can result in necessary change in relative dispositions of neighboring protein subunits. Due to many uncertainties associated with possible localization of amino acids that may participate in the mechanism and lots of degrees of freedom, this problem still is very difficult for theoretical analysis. Recent study[35] of large-scale motions in 24 proteins, having two or more X-ray conformers, showed a limited repertoire of possible domain motions where majority of connecting structures are short local sequences of two or three amino acid residues in terminal regions of α-helices or β-sheets. Nevertheless, there are some cases where connecting elements include as long as 18 residue segments.[35] A few other cases were found where the remote terminal regions of neighboring β-strands acts as a single hinge.[35]

Unfortunately, the RecA filament is too large molecular object for NMR analysis, which otherwise could shed light on dynamics of conformational transitions between its stretched and crystal states. However, such a knowledge could be derived from analysis of multiple crystal forms of the same RecA protein obtained at crystallization conditions (i.e., in elevated salt concentrations) where filament structure can be significantly different from that found both in crystal[9] and compressed forms.[13]

In this study we mainly focused on molecular mechanisms that can maintain intersubunit interface of the RecA filament and have at least some experimental support. Likely, the model presented in this article describes a key element in the mechanism of RecA filament stretching. Because it is quite possible that other elements of RecA structure can also contribute to functionally important flexibility of this protein, more work needs to be done to identify these amino acid residues.

CONCLUSION

The extended structures of RecA/RadA/Rad51-like filaments are critical for activation of these filaments to promote homologous DNA recombination and repair in all organisms from bacteria to humans. The SANS experimental data presented here indicate that the active conformation of RecA filament does not require the DNA presence. These data also show that RecA protein can exist in a number of stable conformations that manifest themselves in different geometric parameters of the protein filament.

We described a simple and experimentally verifiable atomic level model that is able to explain all observed changes in a main filament geometrical parameter, the filament helix pitch. It is noteworthy that this model does not require any significant changes in the intersubunit interface of RecA filament. Because changes in the filament radius of gyration cannot be explained by the mechanism of filament stretching presented here, the model predicts that a sharp decrease of the filament radius of gyration observed by SANS is to be attributed to other possible alterations in RecA protein conformation.

ACKNOWLEDGMENTS

M.P. and V.L. thank Dr. M.M. Cox for interesting and fruitful discussions.

REFERENCES

1. Roca AI, Cox MM. RecA protein: structure, function, and role in recombinational DNA repair. Prog Nucleic Acid Res Mol Biol 1997; 56:129–223.
2. Brendel V, Brocchieri L, Sandler SJ, Clark AJ, Karlin S. Evolutionary comparisons of RecA-like proteins across all major kingdoms of living organisms. J Mol Evol 1997;44:528–541.
3. Cox MM, Goodman MF, Kreuzer KN, Sherratt DJ, Sandler SJ, Marians KJ. The importance of repairing stalled replication forks. Nature 2000;404:37–41.
4. Cox MM. The nonmutagenic repair of broken replication forks via recombination. Mutat Res 2002;510:107–120.
5. Kowalczykowski SC. Initiation of genetic recombination and recombination-dependent replication. Trends Biochem Sci 2000;25: 156–165.
6. Lusetti SL, Cox MM. The bacterial RecA protein and the recombinational DNA repair of stalled replication forks. Annu Rev Biochem 2002;71:71–100.
7. Kuzminov A. Recombinational repair of DNA damage in *Escherichia coli* and bacteriophage lambda. Microbiol Mol Biol Rev 1999;63:751–813, table of contents.
8. Story RM, Steitz TA. Structure of the recA protein–ADP complex. Nature 1992;355:374–376.
9. Story RM, Weber IT, Steitz TA. The structure of the *E. coli* recA protein monomer and polymer. Nature 1992;355:318–325.
10. Datta S, Prabu MM, Vaze MB, Ganesh N, Chandra NR, Muniyappa K, Vijayan M. Crystal structures of *Mycobacterium tuberculosis* RecA and its complex with ADP-AlF(4): implications for decreased ATPase activity and molecular aggregation. Nucleic Acids Res 2000;28:4964–4973.
11. Datta S, Krishna R, Ganesh N, Chandra NR, Muniyappa K, Vijayan M. Crystal structures of *Mycobacterium smegmatis* RecA and its nucleotide complexes. J Bacteriol 2003;185:4280–4284.
12. Rajan R, Bell CE. Crystal structure of RecA from *Deinococcus radiodurans*: insights into the structural basis of extreme radioresistance. J Mol Biol 2004;344:951–963.
13. Xing X, Bell CE. Crystal structures of *Escherichia coli* RecA in a compressed helical filament. J Mol Biol 2004;342:1471–1485.
14. Yu X, Jacobs SA, West SC, Ogawa T, Egelman EH. Domain structure and dynamics in the helical filaments formed by RecA and Rad51 on DNA. Proc Natl Acad Sci USA 2001;98:8419–8424.
15. Yu X, VanLoock MS, Yang S, Reese JT, Egelman EH. What is the structure of the RecA–DNA filament? Curr Protein Pept Sci 2004;5:73–79.
16. VanLoock MS, Yu X, Yang S, Lai AL, Low C, Campbell MJ, Egelman EH. ATP-mediated conformational changes in the RecA filament. Structure (Camb) 2003;11:187–196.
17. DiCapua E, Schnarr M, Ruigrok RW, Lindner P, Timmins PA. Complexes of RecA protein in solution. A study by small angle neutron scattering. J Mol Biol 1990;214:557–570.
18. Timmins PA, Ruigrok RW, DiCapua E. The solution structure of recA filaments by small angle neutron scattering. Biochimie 1991;73:227–230.
19. Lebedev DV, Baitin DM, Islamov A, Kuklin AI, Shalguev V, Lanzov VA, Isaev-Ivanov VV. Analytical model for determination of parameters of helical structures in solution by small angle scattering: comparison of RecA structures by SANS. FEBS Lett 2003;537:182–186.
20. Umemura K, Komatsu J, Uchihashi T, Choi N, Ikawa S, Nishinaka T, Shibata T, Nakayama Y, Katsura S, Mizuno A, Tokumoto H, Ishikawa M, Kuroda R. Atomic force microscopy of RecA–DNA complexes using a carbon nanotube tip. Biochem Biophys Res Commun 2001;281:390–395.

21. Nishinaka T, Ito Y, Yokoyama S, Shibata T. An extended DNA structure through deoxyribose-base stacking induced by RecA protein. Proc Natl Acad Sci USA 1997;94:6623–6628.
22. Nishinaka T, Shinohara A, Ito Y, Yokoyama S, Shibata T. Base pair switching by interconversion of sugar puckers in DNA extended by proteins of RecA-family: a model for homology search in homologous genetic recombination. Proc Natl Acad Sci USA 1998; 95: 11071–11076.
23. Ostanevich YM. Time-of-flight small-angle scattering spectrometers on pulsed neutron sources. J Makromol Chem Macromol Symp 1988; 15:91–103.
24. Namsaraev EA, Baitin D, Bakhlanova IV, Alexseyev AA, Ogawa H, Lanzov VA. Biochemical basis of hyper-recombinogenic activity of *Pseudomonas aeruginosa* RecA protein in *Escherichia coli* cells. Mol Microbiol 1998;27:727–738.
25. Pugh BF, Cox MM. High salt activation of recA protein ATPase in the absence of DNA. J Biol Chem 1988;263:76–83.
26. Hayward S, Berendsen HJ. Systematic analysis of domain motions in proteins from conformational change: new results on citrate synthase and T4 lysozyme. Proteins 1998;30:144–154.
27. Abagyan R, Totrov M. Biased probability Monte Carlo conformational searches and electrostatic calculations for peptides and proteins. J Mol Biol 1994;235:983–1002.
28. Momany FA, McGuire RF, Burgess AW, Scheraga HA. Energy parameters in polypetides. VII. Geometric parameters, partial atomic charges, nonbonded interactions and intrinsic torsional potential for the naturally occurring amino acids. J Phys Chem 1975;79:2361–2381.
29. Nemethy G, Pottle MS, Scheraga HA. Energy parameters in polypeptides 9. Updating of geometrical parameters, nonbonded interactions, and hydrogen-bond interactions for the naturally-occurring amino-acids. J Phys Chem 1983;87:1883–1887.
30. Bansal M, Kumar S, Velavan R. HELANAL: a program to characterize helix geometry in proteins. J Biomol Struct Dyn 2000; 17:811–819.
31. Baker NA, Sept D, Joseph S, Holst MJ, McCammon JA. Electrostatics of nanosystems: application to microtubules and the ribosome. Proc Natl Acad Sci USA 2001;98:10037–10041.
32. Egelman EH. Does a stretched DNA structure dictate the helical geometry of RecA-like filaments? J Mol Biol 2001;309:539–542.
33. Lusetti SL, Shaw JJ, Cox MM. Magnesium ion-dependent activation of the RecA protein involves the C terminus. J Biol Chem 2003;278:16381–16388.
34. Warshel A, Russell ST. Calculations of electrostatic interactions in biological systems and in solutions. Q Rev Biophys 1984;17: 283–422.
35. Hayward S. Structural principles governing domain motions in proteins. Proteins 1999;36:425–435.