Arrangement of RecA protein in its active filament determined by polarized-light spectroscopy

Katsumi Morimatsu^{*†}, Masayuki Takahashi[‡], and Bengt Nordén^{*§}

^{*}Department of Physical Chemistry, Chalmers University of Technology, S-41296 Gothenburg, Sweden; and [‡]FRE 2230, Université de Nantes and Centre National de la Recherche Scientifique, F-44322 Nantes Cedex 3, France

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Linear dichroism (LD) polarized-light spectroscopy is used to determine the arrangement of RecA in its large filamentous complex with DNA, active in homologous recombination. Angular orientation data for two tryptophan and seven tyrosine residues, deduced from differential LD of wild-type RecA vs. mutants that were engineered to attenuate the UV absorption of selected residues, revealed a rotation by some 40° of the RecA subunits relative to the arrangement in crystal without DNA. In addition, conformational changes are observed for tyrosine residues assigned to be involved in DNA binding and in RecA–RecA contacts, thus potentially related to the global structure of the filament and its biological function. The presented spectroscopic approach, called “Site-Specific Linear Dichroism” (SSLD), may find forceful applications also to other biologically important fibrous complexes not amenable to x-ray crystallographic or NMR structural analysis.

Many proteins, like RecA, actin, etc., and also prions, display biological activities related to their ability to assemble into large filamentous structures. Whereas such assemblies are generally not amenable to structure analysis by x-ray crystallography or NMR, linear dichroism (LD), i.e., anisotropy to absorption of polarized light, may often provide valuable structural information about their internal organization (1). The basic principle is that absorption is maximum when the light polarization is parallel to the transition moment, i.e., the molecular “antenna” responsible for the interaction with the radiation field. Measurement of LD, defined as the absorption differential between orthogonal polarizations, can thus provide information about the angles between the respective transition moments and the orientation direction of the sample; a prerequisite is that the latter is macroscopically oriented. Knowledge about how the transition moments are directed within the molecular framework of a chromophoric residue, for example, obtained from quantum chemical calculations or experiments on small molecules (2) then allows conclusions about the orientation of the particular molecular residue in the structure. LD measured on flow-oriented DNA solutions has been used for a long time to determine binding angles for small ligand molecules, a measurement that may generally quickly discriminate between different binding modes, such as groove binding or intercalation (1). Also, average tilt and roll angles of nucleobases, electrophoretic orientation and flexibility of DNA helices have been studied (3). In a few cases, detailed binding geometries have been possible to deduce, including small angular distortions in diastereomeric complexes (4). From such directional information (i.e., in principle, two angular coordinates for each chromophore), a three-dimensional structure should be possible to determine by assistance from molecular modeling. Here, we present a study in which the RecA protein of *Escherichia coli* has been systematically modified to allow determination by LD of the orientations of a set of amino acid residues, a technique we call “Site-Specific Linear Dichroism” (SSLD; Fig. 1).

We have previously used LD to study the DNA base orientation in the complex of DNA and RecA protein, which plays a crucial role in the DNA repair in *E. coli* (5, 6). RecA regulates expression and activity of DNA repair enzymes by its co-

Fig. 1. Schematics of SSLD. LD spectra are measured on a wild-type protein system (Left) and on a protein modified so that a selected chromophore is replaced by a “transparent” residue (Right). The difference LD spectrum—the SSLD—corresponds to the LD from the selected chromophore (Center).

protease activity for the LexA repressor and facilitates DNA repair by catalyzing the strand exchange between homologous DNA strands (7–9). For these activities, RecA forms a filamentous complex with a single DNA strand by cooperative binding. Electron microscopy (EM) image analysis reveals a helical organization of RecA monomers around the DNA (10). The three-dimensional structure of RecA itself and in complex with ADP has been determined by x-ray crystallography (11), but the structure of the active RecA–DNA–ATP complex is not yet known at the atomic level. EM as well as small-angle neutron scattering (SANS) observations indicate significant structural variations between pure RecA, as helically stacked in crystal, and in its helical filament when binding ATP and DNA (10, 12, 13).

We now focus on the organization and conformation of the RecA subunits of the fiber by analyzing the LD signals from a set of aromatic residues (Tyr and Trp) of the protein. These residues absorb light in the near UV region and provide LD signals that reveal their orientations relative to the filament axis. Combined with three-dimensional data of RecA determined by x-ray crystallography, the orientation data allow us to assess the orientation of the RecA subunits in the filament and also to get indications of potential conformational changes in the protein connected to its biological function.

Abbreviations: ATPγS, adenosine 5′-O-(3-thiotriphosphate); poly(deA), poly(1,N⁶-ethenodeoxyadenosine); LD, linear dichroism; SSLD, site-specific LD; ssDNA, single-stranded DNA; LD′, reduced LD.

[†]Present address: Department of Microbiology, University of California, Davis, CA 95616.

[§]To whom reprint requests should be addressed. E-mail: norden@phc.chalmers.se.

Materials and Methods

Materials. Wild-type RecA protein was prepared as described (14). Adenosine 5'-O-(3-thiotriphosphate) (ATP γ S) was purchased from Roche Molecular Biochemicals. Poly(1,*N*⁶-ethenodeoxyadenosine) [poly(dεA)] was made by terminal transferase (Roche Molecular Biochemicals)-mediated extension of deoxyadenosine with oligo(dA)₁₂ (Pharmacia) as a primer, followed by modification with chloroacetaldehyde (15). The concentrations of these materials were photometrically determined by using $\epsilon_{278} = 21,500 \text{ M}^{-1}\text{cm}^{-1}$ for RecA protein, $\epsilon_{254} = 15,000 \text{ M}^{-1}\text{cm}^{-1}$ for ATP γ S, and $\epsilon_{257} = 5670 \text{ M}^{-1}\text{cm}^{-1}$ for poly(dεA).

Preparation of Modified RecA Proteins. The site-directed mutagenesis was performed on an M13 phage containing *recA* gene under the T7 promoter control (see additional text, which is published as supporting information on the PNAS web site, www.pnas.org). The modified RecA proteins were expressed by infecting $\Delta recA$ *E. coli* strain cells AK101 (16) carrying a plasmid pTI1219 that expresses T7 RNA polymerase (a personal gift from T. Ito, Shinshu University, Nagano, Japan), with the modified M13 phage in LB-medium containing 100 $\mu\text{g}/\text{ml}$ isopropyl- β -D-thiogalactopyranoside. The proteins were then purified as described (14). Their concentrations were photometrically determined by using $\epsilon_{278} = 20,100 \text{ M}^{-1}\text{cm}^{-1}$ for RecA-Y65F, RecA-Y103F, RecA-Y218F, RecA-Y264F, RecA-Y271F, RecA-Y291F, and RecA-Y293F proteins; $\epsilon_{278} = 15,800 \text{ M}^{-1}\text{cm}^{-1}$ for RecA-W290H and RecA-W308H proteins; and $\epsilon_{278} = 10,100 \text{ M}^{-1}\text{cm}^{-1}$ for RecA-W290H-W308H protein.

LD Measurement. The LD spectra were measured in a Couette flow device (17) as described elsewhere (5). The sample containing 4 μM RecA protein, 20 μM ATP γ S, and 12 μM poly(dεA) was preincubated for 2 h at 22°C before loading into the system. The buffer contained 20 mM sodium phosphate (pH 6.8), 1 mM MgCl₂, and 50 mM NaCl. The spectra were averaged over four scans (scan rate, 50 nm/min; bandwidth, 2 nm; shear gradient, 1,200 s⁻¹). As a baseline, the signal obtained with very slow rotation of the cell (shear gradient, 90 s⁻¹) was used to minimize influence of optical defects of the cell.

Results

Principles of SSLD. The normal (total) LD of the filamentous complex is a function of wavelength, λ , with contributions from all chromophoric residues in it: $LD(\lambda) = \sum_i LD_i(\lambda)$, where $LD_i(\lambda)$ is the LD spectrum due to the *i*th residue. SSLD evaluates $LD_i(\lambda)$ to get the orientation of *i*th residue of the filament from the difference in LD signal observed when the *i*th residue in a protein is replaced with another amino acid (Fig. 1). The aromatic residues tryptophan, tyrosine, and phenylalanine all provide suitable targets for SSLD by having distinct absorptions in the UV wavelength region and transition moments with established directions.

For a uniaxial orientation distribution of protein units around the filament axis, one has the following relation between total LD at wavelength λ and the respective angles α_{iu} between filament axis and transition moment of transition *u* of residue *i*:

$$LD^r(\lambda) = LD(\lambda)/A(\lambda) = \sum_i \left[\sum_u LD_{iu}^r(\lambda) A_{iu}(\lambda) \right] / \left[\sum_i \left[\sum_u A_{iu}(\lambda) \right] \right], \quad [1a]$$

$$\text{where} \quad LD_{iu}^r(\lambda) = S(3/2)(3 \cos^2 \alpha_{iu} - 1) \quad [1b]$$

and *S* is the degree of orientation of filament particles, $A_{iu}(\lambda)$ the absorption component because of transition *u* of residue *i* and

$A_i(\lambda)$ the total absorption of residue *i*, $A_i(\lambda) = \sum_u A_{iu}(\lambda)$. In the case of negligible overlap between absorptions of different transitions, i.e., if $A_{ik}(\lambda) = 0$ for all *k* different from *u*, one has simply

$$LD^r(\lambda_u) = S(3/2)(3 \cos^2 \alpha_{iu} - 1) \quad [2]$$

at the wavelength, λ_u , where only transition *u* absorbs light. For the situation when a protein and its mutant are studied, one has, obviously, for the differential LD spectrum between the two oriented systems:

$$\Delta LD(\lambda) = \left[\sum_i \sum_u S_w(3/2)(3 \cos^2 \alpha_{iuw} - 1) A_{iuw}(\lambda) \right] - \left[\sum_j \sum_v S_m(3/2)(3 \cos^2 \alpha_{jvm} - 1) A_{jvm}(\lambda) \right], \quad [3]$$

where subscript *w* refers to wild-type and *m* to mutant (α_{iuw} angle for transition *u* in residue *i* in wild-type protein, etc.). If the orientations of wild-type and mutant samples are the same, or information on *S* is available so that one may normalize LD spectra with respect to orientation, e.g., by multiplying the mutant LD spectrum by the factor S_w/S_m , Eq. 3 simplifies further:

$$\Delta LD(\lambda) = S(3/2) \left[\sum_u (3 \cos^2 \alpha_{iuw} - 1) A_{iuw}(\lambda) - \sum_v (3 \cos^2 \alpha_{jvm} - 1) A_{jvm}(\lambda) \right]. \quad [4]$$

Contributions from all residues except the substituted (*i*) and the substituent (*j*) residues thus vanish. The idealized situation when there is no absorption overlap between different transitions is even simpler: if mutant residue *j* is transparent at the wavelength λ_u where transition *u* of residue *i* absorbs [$A_{jvm}(\lambda_u) = 0$], one has:

$$\Delta LD(\lambda_u) = S(3/2)[(3 \cos^2 \alpha_{iuw} - 1)A(\lambda)_{iuw}]. \quad [5]$$

Here, $A(\lambda)_{iuw}$, the absorption spectrum of the replaced residue, is simply the difference between wild-type and mutant absorptions, which allows determination of α_{iuw} , the angle of the particular transition moment in the substituted residue, provided one may independently determine the orientation factor *S*.

Modification of the RecA Protein for SSLD Analysis. We have chosen the two tryptophan and seven tyrosine residues of RecA protein for SSLD analysis. Because a prerequisite for our spectroscopic approach is that the residue substitutions do not significantly affect the structure of RecA itself or its complex with DNA, these residues should be replaced by structurally similar residues. The tryptophan residues, one at a time, were replaced by histidine (the imidazole ring of histidine being considered half of indole). Correspondingly, each tyrosine residue was replaced by phenylalanine, which is like removing one oxygen from tyrosine. The histidine and phenylalanine chromophores are, compared with the tryptophan and tyrosine chromophores, practically transparent in the UV region. Hence, the differential LD between wild-type and mutant complex will to a good approximation be the pure LD spectrum of the respective substituted tryptophan and tyrosine residue, revealing by a rather simple analysis the orientation of the selected residue itself. Thus, we prepared modified RecA proteins containing W290H, W308H, Y65F, Y103F, Y218F, Y264F, Y271F, Y291F, and Y293F alter-

Fig. 2. LD spectra of wild-type and modified protein complexes with poly(dεA) and SSLD spectra of tryptophan and tyrosine residues. (a and b) LD spectra of RecA complexes with poly(dεA) presented after normalization with respect to LD intensity (set to -1) at 320–350 nm. (c and d) SSLD spectra of each tryptophan and tyrosine residues computed by subtraction: (LD of wild-type RecA complex) – (LD of modified RecA complex). In c, the SSLD spectra for Trp-290 and Trp-308 were computed in two ways as described in the text. In d, “Background” represents the differential spectrum between the two wild-type LD spectra that were measured first and last in a series of measurements. For SSLD spectra of the Tyr-271, Tyr-291, and Tyr-293, see Fig. 6 (which is published as supporting information on the PNAS web site).

ations, respectively. A modified protein with double modifications, W290H and W380H, was also made.

We have verified that the modified proteins can form active filaments with single-stranded DNA (ssDNA) by measuring their ssDNA-dependent ATPase activities. Except for Y103F and W290H-W380H, where a reduction by some 50% in activity was observed, all of the RecA mutants showed full activity, with similar dependence on DNA concentration (Table 3, which is published as supporting information on the PNAS web site). The results thus demonstrate an overall retained activity for the modified RecA proteins to bind ssDNA and to display the conformational change required to hydrolyze ATP.

LD Spectra of RecA Protein Complexes with Poly(dεA) and SSLD Spectra of Tryptophan and Tyrosine Residues. Flow LD spectra of wild-type and modified RecA protein-DNA complexes were measured with the ethenoadenine polynucleotide poly(dεA) as ssDNA. The ethenoadenine chromophore displays an extra absorption band at 320 nm, where neither RecA nor nucleotide cofactor absorbs light, with a transition moment polarized in the plane of the chromophore (18). Assuming that the structure of DNA complexes with modified RecA is effectively the same as that of wild-type RecA, we can exploit the LD signal of ethenoadenine as an internal standard for normalization of the flow orientation parameter S .

LD spectra of DNA complexes with modified RecA proteins, in which either or both tryptophans are replaced by histidine, displayed in Fig. 2a after normalization with respect to the LD signal from poly(dεA) absorption at 320–350 nm, differ clearly from the LD spectrum of the wild-type complex: the difference represents the differential SSLD according to Eq. 4. The SSLD, centered at 280 and 225 nm (Fig. 2c), fits well with LD signals expected from the B , L_a , and L_b tryptophan transition moments absorbing at 225 nm, 270 nm, and 290 nm, respectively (19). The SSLD spectra of Trp-290 and Trp-308 were assessed in two ways

(Fig. 2a): the Trp-290 SSLD spectrum was obtained by subtracting both the RecA-W290H LD spectrum from the wild-type RecA spectrum and the RecA-W290H-W380H spectrum from the RecA-W380H spectrum. Correspondingly, the Trp-308 SSLD spectrum was determined by subtracting the RecA-W380H LD spectrum from the wild-type RecA spectrum or the RecA-W290H-W380H spectrum from the RecA-W290H spectrum. These spectra of the tryptophan residues are obviously very similar to each other. Furthermore, the Trp-290 spectrum obtained here is very similar to that obtained by another modified RecA protein and method (20). These results support our strategy for determining SSLD spectra. LD spectra of some RecA proteins in which one of the tyrosine residues is replaced by a phenylalanine are also shown (Fig. 2b). The SSLD spectra display peaks at 280 nm and 230 nm characteristic of transitions of tyrosine (Fig. 2d).

The overall consistency between SSLD spectra and transition moment directions of the tryptophan and tyrosine residues justifies the use of poly(dεA) as an internal standard of the flow orientation (orientation factor S).

Determination of Angles of Tryptophan and Tyrosine Residues in Active RecA Filament. The angular orientation α of a transition moment may be determined once the specific reduced LD (LD^r) value and the S factor are known: the angle correlates in a simple way with the value of the ratio LD^r/S as indicated by Eq. 1 (2, 5). The LD^r/S spectra of all aromatic residues are determined from the SSLD spectra (Fig. 3), and corresponding residue-specific absorption spectra for each protein and α values are calculated with the assumption that the base orientation of poly(dεA) is $70^\circ \pm 10^\circ$ (5). In Fig. 3a and b, showing the LD^r/S spectra determined, the LD^r/S value was found almost constant from 260 to 280 nm for the tryptophan residues and from 270 to 285 nm for the tyrosine residues. These wavelength regions correspond to the broad absorption because of the dominant L_a

Fig. 3. LD^r/S spectra of tryptophan and tyrosine residues. The reduced LD, LD^r (which equals LD/A_{iso}), spectrum calculated by dividing the SSLD spectrum by the absorption spectrum of the corresponding tryptophan or tyrosine residue, obtained as the difference (Abs wt protein/DNA complex) – (Abs modified protein/DNA complex). Orientation factor S estimated from LD intensity at 335 nm at the assumption that the bases in poly(dεA) in RecA-poly(dεA)-ATPγS are oriented approximately 70° from the fiber axis (5).

transition (around 260 nm) somewhat overlapped by L_b at longer wavelength (around 290 nm) for tryptophan and the L_b transition (275 nm) of tyrosine. The observed constancy justifies that the LD^r/S values may indeed be used to calculate the respective transition moment angles, because any overlap between transitions of markedly different polarizations would have made LD^r/S vary strongly with wavelength. Table 1 shows the LD^r/S values determined and the corresponding angles of transition moments. The possibility of some 10° uncertainty in the orientation of ethenoadenine at α = 70° in poly(dεA) was considered but generally had no radical effect on the resulting orientation

Table 1. Angles of transition moments of tryptophan and tyrosine residues in active RecA filament

Transition moment	Wavelength, nm	LD ^r /S	Angle α, °	Confidence interval, °	Angle in crystal, °
p(dεA)	320–350	−0.974	70*	80–60*	—
Tyr65-B	227	−1.39	81	90 ⁺ –62	73
Tyr65-L _b	278	0.62	47	44–52	40
Tyr103-B	227	0.0	55	55–55	81
Tyr103-L _b	278	0.70	46	42–51	32
Tyr218-B	227	1.71	32	21–46	20
Tyr218-L _b	278	−0.26	58	60–56	80
Tyr264-B	227	−0.59	63	67–58	82
Tyr264-L _b	278	1.37	37	29–48	12
Tyr271-B	227	−0.32	59	61–56	71
Tyr271-L _b	278	−1.27	77	90 ⁺ –62	70
Tyr291-B	227	−1.26	77	90 ⁺ –62	78
Tyr291-L _b	278	0.38	50	48–53	19
Tyr293-B	227	2.87	10	0 ⁺ –40	26
Tyr293-L _b	278	−0.76	66	72–59	65
Trp290-B	227	−1.45	84	90 ⁺ –63	80
Trp290-L _a	270	2.56	18	0 ⁺ –42	54
Trp290-L _b	289	2.52	19	0 ⁺ –42	32
Trp308-B	227	0.87	43	39–50	21
Trp308-L _a	270	0.39	50	48–53	30
Trp308-L _b	289	−0.34	59	61–57	69

*Angles of transition moments determined at the assumption that poly(dεA) in the filament exhibits the angle 70° (see text).

†LD^r/S value out of range (see equation for LD).

angles. Table 1 also contains the corresponding angles calculated from the RecA-ADP crystal structure (11), for the directions of these transition moments relative to the axis of the helical RecA aggregate in crystal. As seen from Table 1, the orientation angles determined for aromatic residues in the RecA-DNA structure deviate significantly from those of the crystal structure of RecA. In conclusion, our results indicate that the arrangement of RecA monomers in the crystal filament differs significantly from that in the active filament.

Determination of Orientation of RecA in Active RecA-DNA Filament and Conformational Changes. Differences in orientation of Trp and Tyr residues of RecA protein between the structure in the crystal

Fig. 4. Determination of orientation of RecA in active RecA-DNA filament. (a) To judge how much the RecA monomers are rotated on forming the RecA-DNA complex, relative to their orientation in the crystal structure, different trial axes of the protein were considered. The long axis of the crystal structure filament (z axis) was rotated and tested to fit the angles of transition moments for the various residues as determined from the SSLD analysis. (b) The long axis of the RecA crystal filament (z axis) is first rotated (around the y axis), followed by a rotation around the z axis and a check for fit to LD data. The axes that reproduce the LD data of either of two amino acid residues (in total, four transition moments) were plotted (Table 2). The vertical scale (left side) and the scale around the circle are showing the rotation in degrees around the y and z axes, respectively. Circles indicate two accumulation regions (see text). (c) Each rotated axis was checked with respect to coincidence with the LD results of Tyr-218, Tyr-291, Try293, Trp-290, and Trp-308. A higher value corresponds to higher probability for this axis orientation in the active RecA filament. The highest value is obtained for 36° rotation around y followed by 351° rotation around the z axis.

Table 2. Axes fitting LD results

	Tyr65	Tyr103	Tyr218	Tyr264	Tyr271	Tyr291	Tyr293	Trp290	Trp308
Tyr65		(43,315)	(31,356)	(49,328)	(29,302)	(30,303)	(11,275)	(27,300)	(26,307)
Tyr103	(43,315)		(66,269)	(28,148)	(51,185)	(44,310)	(15,88)	(41,319)	(35,325)
Tyr218	(31,356)	(66,269)		(49,254)	(29,1)	(40,348)	(32,356)	(30,359)	(36,345)
Tyr264	(49,328)	(28,148)	(49,254)		(49,330)	(40,350)	(36,22)	(38,37)	(38,350)
Tyr271	(29,302)	(51,185)	(29,1)	(49,330)		(32,301)	(13,339)	(16,345)	(35,300)
Tyr291	(30,303)	(44,310)	(40,348)	(40,350)	(32,301)		(41,349)	(36,355)	(41,142)
Tyr293	(11,275)	(15,88)	(32,356)	(36,22)	(13,339)	(41,349)		(36,8)	(37,350)
Trp290	(27,300)	(41,319)	(30,359)	(38,37)	(16,345)	(36,355)	(36,8)		(30,325)
Trp308	(26,307)	(35,325)	(36,345)	(38,350)	(35,300)	(41,142)	(37,350)	(30,325)	

Axes with maximum probability values fitting the SSLD results of pairs of aromatic residues displayed in table. (θ_y , θ_z) indicates θ_y rotation of the axis in crystal around y -axis followed by θ_z rotation around z -axis. Red and blue colors indicate data belonging to Groups 1 and 2, respectively (see Fig. 4b).

without DNA and in the complex with DNA, determined here, could be due to changes in local conformation of these residues on interaction with DNA and/or a change in orientation of the whole RecA unit in the filament. In the latter case, we expect a concerted rotation of all residues relative to their orientations in RecA in the crystal structure. To examine the possibility of a concerted movement of residues, we determine the rotations of RecA that fit the change in orientation for each residue. The rotation of a RecA unit can be described by a rotation θ_y of the z axis (the long axis of crystal filament) around an orthogonal y axis, followed by a rotation θ_z around the z axis (see Fig. 4a). This manner defines a trial RecA axis vector $\vec{a} = (x, y, z)$ with:

$$x = \sin(\theta_y)\cos(\theta_z), \quad y = \sin(\theta_y)\sin(\theta_z), \quad z = \cos(\theta_y).$$

Because of the cosine-square dependence of LD (e.g., Eq. 1b) there is a sign ambiguity in the determination of angle. With two transition moments for each residue, there are still four possible rotations for each residue (the weaker L_b transition of tryptophan was in a first approach ignored because the absorptions of the L_a and L_b transitions of tryptophan overlap (19), so that two transition moments, B and L_a , were used for tryptophan). We therefore combined pairs of residues, and for each pair determined the trial angle that best fitted the LD data. The fit was judged by computing $n/\sum |(\alpha - \alpha')|$ where n is the number of transition moments, α is the effective tilt angle of each transition moment investigated by SSLD, and α' is the tilt angle between a trial axis vector ($\vec{a}$) and the respective transition moment vector in the crystal structure. If none of the residues undergoes any local rotation, i.e., there are no conformational changes, one would expect the same trial angles for all of the pairs. In fact, the computation showed for a number of pairs of residues that were located in the core of RecA or known not to be involved in the DNA binding, like Trp-290 and Trp-308, that they clustered around the same trial angle position (Fig. 4b and Table 2; marked as “Group 1” including Tyr-218, Tyr-291, Tyr-293, Trp-290, and Trp-308), indicating that these residues do not undergo any local conformational change. By contrast, the trial angle values of other pairs of residues departed markedly from this cluster (Fig. 4b), indicating that several residues change conformation on DNA binding. Among such residues, we find Tyr-103, which interacts with DNA and ATP (14). Another clustering of trial angles, marked “Group 2” (Fig. 4b and Table 2), including Tyr-65 and Tyr-271, is considered only an accidental clustering for several reasons: the position of Group 1 fits the rotation of the whole RecA unit calculated from the SSLD of Trp 290, a residue established to be inflexibly embedded in the protein structure and not be interacting with DNA. By contrast Tyr-65, of Group 2, has been shown to change its environment by interaction with DNA bases and is therefore not likely to be a fixed residue representing the rotation of the whole RecA unit.

Therefore, we computed the rotation of RecA by using the data of the residues of Group 1 and obtained the best fit with the rotation of (36°, 351°), i.e., 36° rotation around y axis followed by 351° rotation around z axis (Fig. 4c). If the orientation of the bases of poly(dεA) was varied between 65° and 75°, the best-fit axes of the Group 1 changed only from the axis (42°, 349°) to (31°, 339°), with a maximum difference of 8.3° to the axis vector (36°, 351°) (data not shown). In conclusion, there is a 36° ± 8° rotation of the RecA monomer on the rearrangement of active RecA filament with DNA compared with its orientation as assembled in the crystal.

Comparison between the angular parameters of RecA in the active filament in complex with DNA and RecA alone in the crystal filament suggests that the DNA interaction does not cause any significant conformational changes around residues Tyr-218, Tyr-291, Tyr-293, Trp-290, and Trp-308, but suggests some alterations of the conformations of other tyrosine residues. Thus, comparison between the SSLD signals of Tyr-65, Tyr-103, Tyr-264, and Tyr-271 and the tilt angles of their respective transition moments relative to the concluded orientation axis of

Fig. 5. Comparison between the crystal and active filament structures. The three-dimensional structure of the active RecA filament (b) was constructed by a modification of the crystal organization of RecA (a) (11): each RecA monomer was first rotated by the rotation vector (36°, 351°) determined by SSLD data. Then, the RecA monomers were aligned to make a 9.6-nm pitch filament containing 6 monomers per turn (10, 12). The proposed DNA binding regions, Tyr-65 (green), Tyr-103 (red), both sides of loop2 (residues 194 and 210, orange), Arg-243 (yellow), and Tyr-264 (blue), were spacefilled and colored, respectively. A red line shows the helix axis (z) of the filament. The RASMOI program (version 2.6) was used for view.

active RecA filament indicates the following local conformational changes of RecA on formation of the active filament: (i) the aromatic ring of Tyr-65 rotates 24° around its long axis from the β -carbon toward the hydroxyl-group; (ii) the Tyr-103 bends 15° around its α - or β -carbon together with a 10° rotation around the long axis of its aromatic ring; (iii) the aromatic ring of Tyr-264 rotates 11° around its long axis; and (iv) the Tyr-271 bends 23° around its α - or β -carbon (see Fig. 7, which is published as supporting information on the PNAS web site). The significant conformational change of Tyr-65 is very likely related to the interaction of this part of RecA with the DNA (21, 22). The Tyr-103 and Tyr-264 residues are close to the nucleotide co-factor, and the Tyr-103 stacks with the adenine ring of ADP (11, 14). A large conformational change at Tyr-103 is in harmony with a previous observation that Tyr-103 interacts with ATP in a different way than when stacking with ADP in crystal (14). In addition, these conformational changes around Tyr-103 and Tyr-264 may relate to interactions with DNA inferred from fluorescence measurements of tyrosine and tryptophan (14, 23, 24). The conformational change indicated for Tyr-271 does not correlate to any so far reported function of this part of RecA, but may be related to the conformational change at Tyr-264.

Construction of a Structural Model of Active RecA-DNA Filament.

From the determined rotation of the RecA monomer (36°, 351°) and the previous information about the active RecA filament (9.6 nm filament pitch, 6 monomers per turn) (10, 12), a structural model of the active filament was constructed (Fig. 5). Interestingly, the proposed regions for DNA binding, Tyr-65 (21), Tyr-103 and Tyr-264 (14), Arg-243 (25, 26), and the loop2 (11, 22, 27, 28), appear rather clustered in the active filament structure whereas these regions are not significantly clustered in the crystal filament without DNA. These regions align on the inner cabinet of the active filament, supporting the reliability of the model structure because the first and second DNA strands have been shown to be located close to the central filament axis (10, 12). The proposed third DNA binding site spanning residues 268–330 (29) is on the outside of the filament in the model, suggesting that the third DNA strand be located outside the active filament. The nucleotide binding site including Tyr-103 and Tyr-264 (11) was found close to the monomer–monomer interface. This observation may indicate that the local conformational change at the nucleotide binding site is somehow

coupled to the monomer–monomer interaction and, thus, to the change in global structure (pitch) of the filament visualized in Fig. 5.

Discussion

Normalizing the LD spectrum by its intensity in the ethenoadenine absorption region is crucial for the accurate determination of SSLD because the orientation factor S may vary significantly between modified and unmodified protein DNA complexes depending on experimental conditions. For the case of tryptophan substitution in the RecA protein, this chromophore is responsible for about 25% of the total protein absorption at 278 nm. Therefore, a rough estimate of the orientation factor S , such as from small-angle neutron scattering measurements (20), is enough for normalizing the LD spectrum to sufficient accuracy. However, for the SSLD of RecA's tyrosine residues, a more precise normalization is required because each tyrosine residue contributes only some 7% of the total protein absorption. Using an inserted probe chromophore, like in poly(dεA), that displays its LD outside the absorption region of the protein is here a solution to the problem of more precise determination of the orientation factor. Because the LD spectrum itself is used for the determination of S , this method can eliminate most of the experimental error of the S factor. Thus, using an internal standard with separable absorption and strong LD is effective for the SSLD analysis.

Phenylalanine may also be analyzed by SSLD if replaced by tyrosine (although one then gets instead the orientation of the tyrosine residue in the mutant). The cysteine with reduced side chain may also be studied; measuring its absorption at 235 nm, it may be replaced by alanine or glycine for SSLD. The histidine residue absorbing at 211 nm is currently difficult to use for LD measurement.

The combined protein engineering-spectroscopic analysis presented here could be applicable also to other filamentous structures not amenable to x-ray crystallography or NMR, such as amyloids of prion and β -amyloid proteins that cause amyloid diseases.

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