Liquid crystal formation of RecA–DNA filamentous complexes†

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Spontaneous optical birefringence of RecA-bound linear and closed circular single-stranded DNA filaments, as well as RecA self-assembled polymer, was observed in aqueous buffer solutions, which demonstrates the formation of lyotropic liquid crystalline phases.

The RecA-like proteins play a crucial role in homologous recombination of DNA genomes and repair of damaged DNA in many organisms.[1] The generality of their functions and the structures of RecA-like proteins prove their importance in biological systems. The *Escherichia coli* RecA protein was the first identified and the most intensively studied member of this group of proteins, which catalyze homologous pairing and strand exchange reaction between two DNA strands. In the presence of ribonucleoside triphosphate cofactors such as adenosine triphosphate (ATP) or adenosine 5′-*O*-thiotriphosphate (ATPγS), RecA protein polymerizes along single-stranded DNA (ssDNA) to form a right-handed helical nucleoprotein filament. The ssDNA within the filament pairs with other double-stranded DNA (dsDNA) and aligns homologous sequences between these two DNAs. A base-pair switching then occurs within the filament, producing a heteroduplex region of the original ssDNA and a complementary strand of homologous dsDNA. Subsequently, the strand exchange between these two DNAs is extended unidirectionally, with the help of ATP hydrolysis, until the exchange of the homologous region is completed. At standard conditions, RecA protein can complete the exchange of 7000 base pairs or more of hybrid DNA within 45 min.[1]

The structure of the RecA filaments has been investigated with X-ray crystallography,[2] electron microscopy,[3–5] and atomic force microscopy (AFM).[6] The RecA filament complexed with ssDNA in the presence of ATP or its analogues was found to extend up to a factor of 1.5 relative to B-form DNA, with a helical pitch of approximately 9.5 nm (6.1 monomers per turn), resulting in significantly stiff rods, which further assemble to form bundles of the filaments without supercoiling.[3] In the absence of ATP, RecA protein also forms a stiff helical filament with ssDNA, which has a helical pitch of 7.6 nm and an axial rise per nucleoside of about 0.21 nm.[4,5] Even without DNA and ATP or its analogues, RecA self-assembles into a helical rod or a ring by concatenation of the RecA protein itself.[4–6]

In spite of the uniqueness of these RecA-based biological filaments, little is known about their liquid crystalline (LC) formations. In biological macromolecules, the mesophase formations of rod-like particles are theoretically predicted and experimentally demonstrated. Such examples include DNA,[7] polysaccharides,[8] polypeptides,[9] some viruses,[10] and actin filament (F-actin)[11] which is formed by a concatenation of globular actin (G-actin) subunits, and many of these exhibits cholesteric LC phases in water due to their stiff backbones composed of their homochiral components.[12]

In this study, we report that RecA–linear and circular ssDNA filamentous complexes, as well as RecA self-assembled polymer, show spontaneous optical birefringence in solution, which demonstrates the formation of lyotropic LC phases (Fig. 1). It is unlikely that the ssDNA is stiff enough to form LC phases, and LC formation of single strand polyribonucleotide has hardly been reported.[13] We anticipated that RecA would stiffen ssDNA when it complexed with ssDNA in the same manner as observed in RecA–dsDNA filaments,[14] resulting in the lyotropic liquid crystallinity.

Fig. 2 shows the typical photographs of RecA self-assembled polymer in buffer solutions in the presence (b–e, 1–20 mM) and absence (a) of Mg^{2+} under a polarizing microscope. The remarkably high birefringence observed in these photographs indicates that there are anisotropic domains in which optical axes

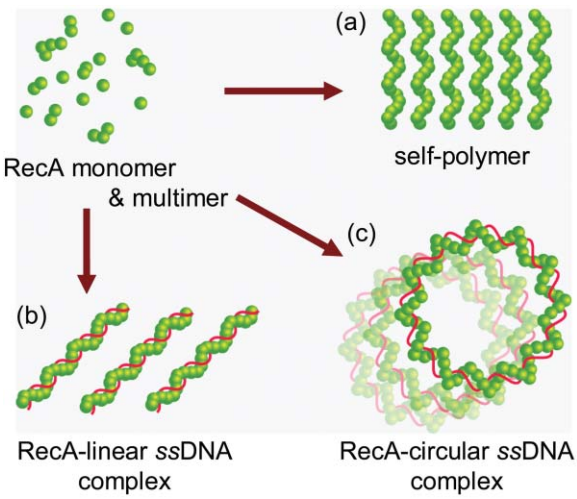

Fig. 1 Schematic illustration of liquid crystal formation of RecA self-assembled polymer (a) and RecA–linear (b) and circular (c) ssDNA filamentous complexes.

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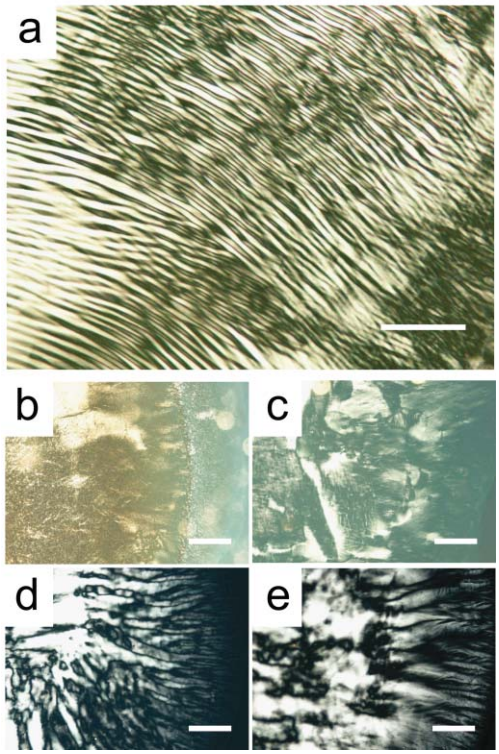

Fig. 2 Polarizing optical micrographs of RecA protein (2.1 mM, 80 mg mL^{-1}) in Tris-acetate (20 mM, pH 7.5) buffer solutions placed on a glass plate without a cover glass to develop the planar texture before observations. The solutions contain 1 mM dithiothreitol, 0.1 mM EDTA, 10% (v/v) glycerol, and $Mg(CH_3COO)_2$: 0 (a), 1 (b), 5 (c), 10 (d), and 20 mM (e). The glycerol was used to prevent solvent evaporation and coagulation of RecA filaments. Scale bars represent 100 μm.

preferentially align parallel to the glass plate, resulting from the LC formation of the concentrated RecA protein solutions. In the absence of Mg^{2+} (Fig. 2a), RecA exhibits a highly birefringent optical texture with characteristic banded patterns; the dark lines between illuminated regions move when the microscope stage is rotated. The optical texture, showing such an alternation of extinction and transmission under crossed polarizers, is caused by undulating molecular orientation, and not by the helical ordering of chiral LC phases.[15]

The birefringence observed in the RecA filament solution was significantly subdued in the presence of a small amount of Mg^{2+} (1 mM (Fig. 2b)). However, the further addition of Mg^{2+} (5–20 mM (Fig. 2c–e)) restored the birefringence, probably because divalent cations such as Mg^{2+} may hamper the rearrangement of the filaments by acting as a bridge between the RecA filaments, or may assist in the transformation of helical rods of the RecA filaments into rings.[6,16] Similarly, the birefringence of the RecA solution totally vanished with the addition of ATPγS (>1 mM). AFM measurements indicated that large aggregations were frequently observed in the sample containing ATPγS, and it appeared to impede the RecA-based LC formation.† Such an aggregation, however, was not detected in the sample without ATPγS.

The RecA protein-based LC formation may support the argument for the "storage form" of RecA protein which has been discussed with respect to its biological significance.[2,5,17] A self-assembled polymeric structure (Fig. 1a) is frequently observed in crystal structures of RecA protein.[2] Story *et al.* argued that the RecA bundle might exist in water solution, regulating the activity of RecA protein while maintaining the storage state when it was not used.[2] In addition, an ordered bundle of filaments was observed by electron microscopy in inclusion bodies of RecA overproducing bacteria.[5,17] The bundle state of filaments may be identical to the LC state demonstrated in this study, and therefore we expect that the LC state likely plays some role in biological functions both *in vitro* and *in vivo*.

The LC formation of genomic ssDNA has not yet been reported, probably because its highly folded configuration, resulting from short runs of intra- and intermolecular base pairing, impedes well-ordered alignments of the ssDNA molecules. RecA protein unwinds the folded structure when complexed with ssDNA, and organizes it into an extended conformation to form a right-handed helical filament with the ssDNA situated at its center of concavity.[18] The persistence length of the RecA filament is about 0.6–1 μm, while that of ssDNA is about 4.0 nm,[14,19] and therefore we expected that the RecA protein would form LC phases when complexed with ssDNA, resulting in the stiff, rigid-rod macromolecular assembly (Fig. 1b). Fig. 3c shows a polarizing optical micrograph of the solution of RecA–linear ssDNA complexes formed in the absence of nucleotide cofactors. The observed bright optical birefringence with smooth texture indicates

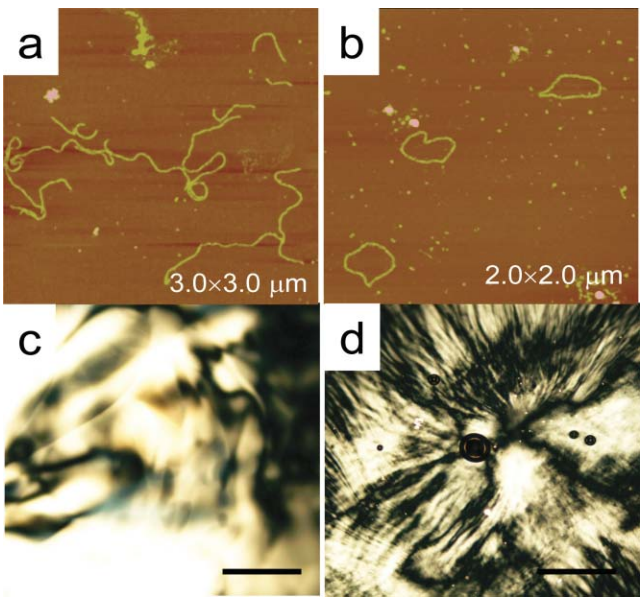

Fig. 3 AFM images (a, b) and polarizing optical micrographs (c, d) of RecA-bound linear (a, c) and circular (b, d) ssDNA filaments. (a, c) RecA protein and M13mp18 linear ssDNA (7249 bases) were concentrated up to 1.0 mM and 2.6 mM, respectively, in 20 mM Tris-acetate buffer containing 25% (v/v) glycerol. (b, d) RecA protein and pUC118 circular ssDNA (3186 bases) were concentrated up to 2.2 and 4.4 mM, respectively, in 25 mM Tris-acetate buffer containing 25% (v/v) glycerol. For AFM observations, the sample solutions were diluted 500 times with the same buffer, loaded onto a gel filtration column, and spread over AP-mica. For polarizing optical microscopic observations, the sample solutions were placed on a glass plate without a cover glass. Scale bars represent 100 μm.

the formation of LC phase resulting from the stiffness of RecA–ssDNA filaments. The rod-like molecular shape was evidenced from AFM measurements of the RecA–linear ssDNA filaments placed on mica functionalized with aminopropyltriethoxysilane (AP-mica) (Fig. 3a).[20] Previous microscopic studies showed that RecA–DNA filaments were seen frequently to aggregate together in side-by-side interactions, producing bundles of RecA filaments coiling about each other.[3] In the LC states, the RecA filaments may align parallel to each other or interwind into multiple bundles, where the base sequence and structure of ssDNA can be designed as a template of bundle scaffolds.

Furthermore, we found that even RecA–circular ssDNA complexes also exhibit optical birefringence in solution (Fig. 3d).[21] The AFM image of the RecA–circular ssDNA complexes showed that the RecA filaments formed a rigid ring, not a self-supercoil (Fig. 3b), as expected from previous microscopic studies.[22] Although the structure of RecA filaments on the mica and in the bulk LC state can be different, it seems likely that the ring-like structure shown in Fig. 3b assembles in the LC phases because the contour length of the RecA–circular ssDNA filaments (about 0.77 μm) was comparable to its persistence length.[14] To our knowledge, this is the first observation of the LC phase of a macrocyclic supramolecular polymer, although the LC formation of self-supercoiling closed circular dsDNA (bacterial plasmid) has been reported.[23]

In summary, we have found that both concentrated RecA protein and RecA-bound linear and circular ssDNA filament solutions formed lyotropic LC phases. This finding suggests that the RecA protein has a property to form liquid crystals by polymerizing itself, and it confers this ability on ssDNA by increasing the stiffness of ssDNA due to the RecA filaments being more than 100 times stiffer than ssDNA.[14,19] In addition, the polymer lengths of the DNA can be precisely controlled, providing a unique chiral LC material composed of RecA-bound DNA with controlled molecular lengths and aspect ratios. The LC states of RecA protein may have biological functions as the storage form, and may regulate the process of homologous recombination by suppressing the activity of RecA reactions both *in vitro* and *in vivo*.[2,5,17]

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