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Visualization of the Annealing of Complementary Single-stranded DNA Catalyzed by the Herpes Simplex Virus Type 1 ICP8 SSB/Recombinase

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The rate of annealing of long linear complementary single-stranded (ss) DNAs can be increased greatly by certain DNA-binding proteins including the herpes simplex virus type 1 ICP8 SSB/recombinase. Using electron microscopy, we have investigated the DNA–protein structures involved in ICP8-mediated DNA annealing. We show that the formation of superhelical ICP8–ssDNA filaments is required for annealing. Two superhelices interact with each other to form a coiled-coil, which is the intermediate in annealing. In this process, the superhelices likely rotate and translocate relative to each other. Psoralen/UV photocrosslinking studies revealed that meta-stable contacts form at sites of limited sequence homology during the annealing. Partial proteolysis of ICP8 in the protein–ssDNA complexes showed that Mg^{2+} induces conformational changes in the N-terminal region (amino acid residues 1–305) of ICP8. In addition to Mg^{2+}, Ca^{2+} and, to a significantly lesser extent, Cu^{2+} and Mn^{2+}, were found to induce superhelix formation of the ICP8–ssDNA filament and to facilitate annealing. Mechanisms for how the coiled-coil structures facilitate annealing are discussed.

Keywords: ICP8; herpes simplex virus; SSB; electron microscopy; single-stranded DNA annealing

Introduction

A general property shared by many single-strand (ss) binding proteins (SSBs) and recombinases is the ability to increase markedly the annealing kinetics of complementary single strands of DNA. In a typical experiment, duplex DNA of several kilobases is melted and allowed to anneal alone or in the presence of one of these proteins and appropriate cofactors. In the latter case, annealing to fully duplex DNA may be increased many-fold.[1–7]

Herpes simplex virus type 1 (HSV-1) ICP8 is a 128 kDa protein encoded by the HSV-1 UL 29 gene.[8,9] It is one of seven HSV-1 gene products required for origin-dependent replication of the viral genome,[10] and was identified originally as a single-strand binding protein (SSB)[11] and, in this capacity, facilitates HSV-1 replication reactions *in vitro*.[11–13] ICP8 binds preferentially and cooperatively to ssDNA,[11,14,15] and holds it in an extended conformation.[14] Each ICP8 monomer binds 10–15 nt of ssDNA.[16–18] ICP8 also destabilizes poly[(dA)-(dT)] duplexes[19] and short duplex DNAs,[20] and is able to displace oligonucleotides annealed to long ssDNA.[21,22] This activity is independent of ATP and Mg^{2+}, shows no preferential directionality, and requires saturating amounts of ICP8.[20]

ICP8 shares properties with recombinases and can catalyze the strand invasion of linear ssDNA into a complementary supertwisted double-stranded (ds) DNA.[21,22] It will also catalyze full strand transfer between a 7 kb tailed linear dsDNA and a complementary circular ssDNA.[23] ICP8 resembles RecA and Rad51 in the formation of highly regular helical filaments on ssDNA[14] and, like RecA,[24,25] ICP8 forms helical protein filaments in the absence of DNA,[12] and filamentous crystals on lipid monolayers.[26]

One class of proteins that promotes DNA annealing has been termed single-strand annealing

Abbreviations used: SSB, single-strand binding protein; EM, electron microscopy; HSV-1, Herpes simplex virus type 1; ssDNA, single-stranded DNA; MALDI-MS, matrix-assisted laser desorption/ionization mass spectrometry; MS/MS, tandem mass spectrometry; SSAP, single-strand annealing protein.

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proteins (SSAPs) and includes *Escherichia coli* RecT protein,[2] bacteriophage lambda Recβ protein,[4,5] bacteriophage P22 Erf protein,[27] and eukaryotic Rad52,[7,28] which belong to three different groups of proteins sharing little homology.[3] However, each binds ssDNA and promotes the pairing of complementary ssDNA. The annealing reactions induced by this class of proteins are highly dependent on protein concentration and Mg^{2+} but not ATP.

In addition to SSAPs, some SSBs, including bacteriophage T7 gene 2.5 protein,[29,30] bacteriophage T4 gene 32 protein,[31] *E. coli* SSB in combination with RecO,[32] and RPA in combination with Rad52,[28] also promote the annealing of complementary ssDNA. However, the efficiency of catalysis by these SSBs is less than that of the SSAPs and, in some cases, requires the presence of other factors, such as Rad52 for RPA,[28] or RecO for *E. coli* SSB.[32]

The ability of ICP8 to catalyze the annealing of complementary ssDNA has been examined in detail by Dutch and Lehman, who showed that its activity depends on pH, salt, temperature and Mg^{2+}.[1] Variation of the concentration of Mg^{2+} (in the presence of 50 mM NaCl) from 0 to 30 mM $MgCl_2$ showed an optimum at 6 mM, where a sixfold stimulation was observed. In addition to Mg^{2+}, annealing required monovalent cations, with optimal annealing occurring between 50 mM and 100 mM NaCl. Annealing occurred over a broad pH range and the temperature dependence was optimal between 37 °C and 40 °C.

While the annealing of complementary ssDNAs appears to be a common property of many SSBs and recombinases, and much is known about the cofactor dependence of these reactions, almost nothing is known about the physical mechanism by which the process occurs. In this study, we examined the structure of the protein–ssDNA filaments generated during ICP8-catalyzed strand annealing. Using electron microscopy (EM) we show that the annealing activity of ICP8 depends on the organization of the protein–ssDNA filaments into superhelices. Two such superhelices then form a paired coiled-coil structure within which homologous pairing is tested, and if the DNAs are complementary, progresses to generate fully duplex DNA. Other metal ions, including Ca^{2+} and, to a lesser extent, Cu^{2+} and Mn^{2+}, were found to induce superhelix formation and facilitate annealing. Biochemical analysis suggested that the N terminus of ICP8 undergoes a Mg^{2+}-dependent change within the ssDNA filaments. A model for how annealing occurs within these super-molecular structures is described.

Results

ICP8–ssDNA filaments form superhelical coils under conditions optimal for strand annealing

A detailed biochemical analysis of the ICP8-catalyzed annealing of complementary ssDNA has been described by Dutch and Lehman.[1] In the work described here, we examined the morphology of the ICP8–ssDNA filaments formed over the range of concentrations of Mg^{2+} and salt that they found to be optimal for DNA annealing. When ICP8–ssDNA filaments were formed (see Materials and Methods) and visualized unfixed by negative staining in the absence of Mg^{2+}, EM visualization revealed nucleoprotein filaments identical with those described before,[20] in which assembly appears to initiate at multiple sites along the ssDNA (Figure 1(a)–(c)). Assembly appeared to be cooperative,[14,15] with ICP8 holding the ssDNA in a relatively stiff, extended configuration with 0.13 nm per base.[14] The appearance of these nucleoprotein filaments remained the same, even when the samples were incubated for 60 min.

When ICP8 was assembled onto ssDNA in the presence of 6 mM Mg^{2+}, the nucleoprotein

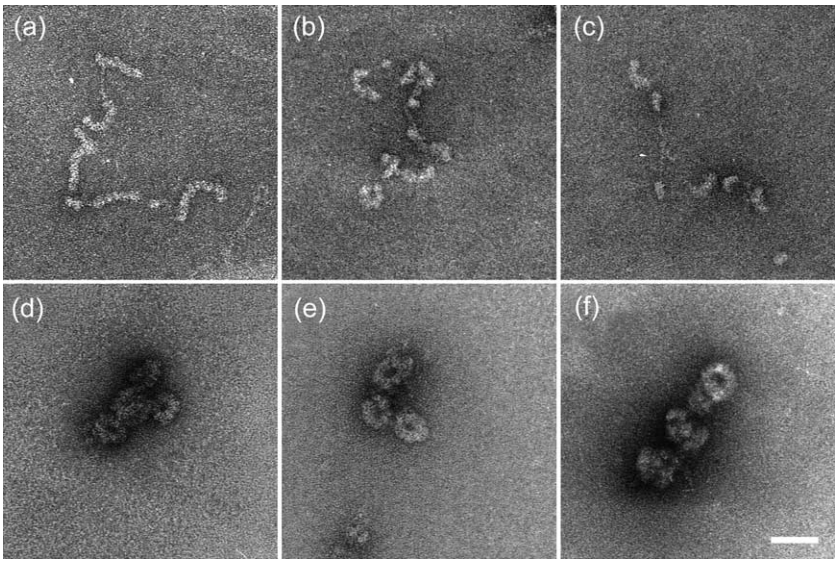

Figure 1. Electron micrographs of ICP8 bound to linear ssDNA. ICP8 was incubated with linear ssDNA for (a)–(e) 5 min or (f) 10 min at 37 °C (a)–(c) in the absence or (d)–(f) in the presence of 6 mM Mg^{2+}. The complexes were mounted onto thin glow-charged carbon foils and negatively stained with 2% uranyl acetate. The scale bar represents 40 nm.