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Efficient interaction of recA protein with fluorescent dye-labeled oligonucleotides

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Abstract

Some fluorescein derivatives attached to the 5′-end of oligonucleotides stimulate recA protein–oligonucleotide binding. The complex formation at near stoichiometric DNA/protein ratios is demonstrated for 18-bases-long oligonucleotides. The complexes with dye-labeled oligonucleotides are shown to be active in the reaction of homologous strand exchange. The strand exchange reaction in the presence of adenosine-5′-*O*-(3-thiotriphosphate) proceeds with the formation of a stable complex of recA protein with the double stranded oligonucleotide, which is a product of the strand exchange. The displaced single-stranded oligonucleotide is shown to be bound weakly.

Key words: recA protein; Fluorescent dye-labeled oligonucleotide; recA-oligonucleotide complex; Strand exchange

1. Introduction

RecA protein catalyzes the central stages of the general genetic recombination, DNA synapsis and strand exchange (for review see [1]). For further studies of the recA protein system it would be useful to obtain stable complexes of recA protein with oligonucleotides of a minimum length. However, the binding of recA protein with short oligonucleotides is ineffective, complete oligonucleotide binding being achieved under excess of the protein [2]. In the present paper we demonstrate that recA protein - oligonucleotide binding efficiency increases when fluorescent dye-labeled oligonucleotides were used. The strand exchange in this system and different procedures for obtaining of stable recA–oligonucleotide complexes are also described here.

2. Materials and methods

RecA protein was isolated by the method of Cox et al. [3]. Concentrations of the protein and ss-oligonucleotides were determined using the extinction coefficient $E_{280} = 0.59$ ml·mg^{-1}·cm^{-1} and the conversion factor $1A_{260} = 36$ mg·ml^{-1}, respectively.

The following oligonucleotides were synthesized as described in [4]: (1) 5′-TGT-AAA-ACG-ACG-GCC-AGT, the (+) strand of a universal primer with and without fluorescent label (JOE) at 5′-end; (2) 5′-ACT-GGC-CGT-CGT-TTT-ACA, complementary to the former, the (−) strand of a universal primer.

2.1. Complex formation

Fig. 1 shows an outline of the experimental procedure. Unless otherwise indicated, recA-ss*-oligonucleotide complexes were formed by preincubation of 0.1 μg of oligonucleotide and 5 μg of the protein for 20 min at 37°C in 10 μl of the reaction mixture containing 20 mM Tris-acetate, pH 7.5 5% glycerol, 2 mM $Mg(CH_3COO)_2$, 0.25 mM ATP-S, followed by the addition of equimolar amounts of ss- or ds-oligonucleotide and further incubation for another 5 min ('2nd incubation') at a magnesium concentration of 10 mM. The samples were loaded onto agarose gels or, after addition of SDS up to 0.2%, on PAG.

2.2. Electrophoresis

Unless otherwise stated, electrophoresis was carried out on a 2% agarose gel in 40 mM Tris-acetate buffer (pH 7.5), 2 mM $Mg(CH_3COO)_2$, and 20 mM NaCl. After 15 min of electrophoresis at 4 V/cm ('1st run') the gels were photographed under UV illumination, incubated for 30 min in electrode buffer and electrophoresed for an additional 45 min ('2nd run').

20% PAGE was performed in standard TBE buffer.

3. Results

Preincubation of the ss*-oligonucleotide with recA protein resulted in retardation of the ss*-oligonucleotide electrophoretic band (Fig. 2, lanes 1 and 2, '1st run') demonstrating the recA–ss*-oligonucleotide complex formation. To check the stability of the complex, the gel after the first run of electrophoresis was incubated for 30 min without voltage and then run again. After such an incubation the ss*-oligonucleotides demonstrated the same electrophorecic mobility in both lanes indicating the dissociation of recA–ss*-oligonucleotide complex ('2nd run'). The complex was stabilized by the addition of a complementary ss-oligonucleotide (lane 3). Under similar incubation conditions an addition of ds*-oligonucleotide to recA protein preincubated without

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Abbreviations: ATP-S, adenosine-5′-*O*-(3-thiotriphosphate); JOE, 4′,5′-dichloro-2′,7′-dimethoxy-6-carboxy-fluorescein; PAGE, polyacrylamide gel electrophoresis; ds-, ss-, double- and single-stranded unmodified oligonucleotides respectively; ds*-, ss*-, double- and single stranded dye-labeled oligonucleotides respectively; b., base; bp, base pair; SDS, dodecyl sulfate sodium salt.

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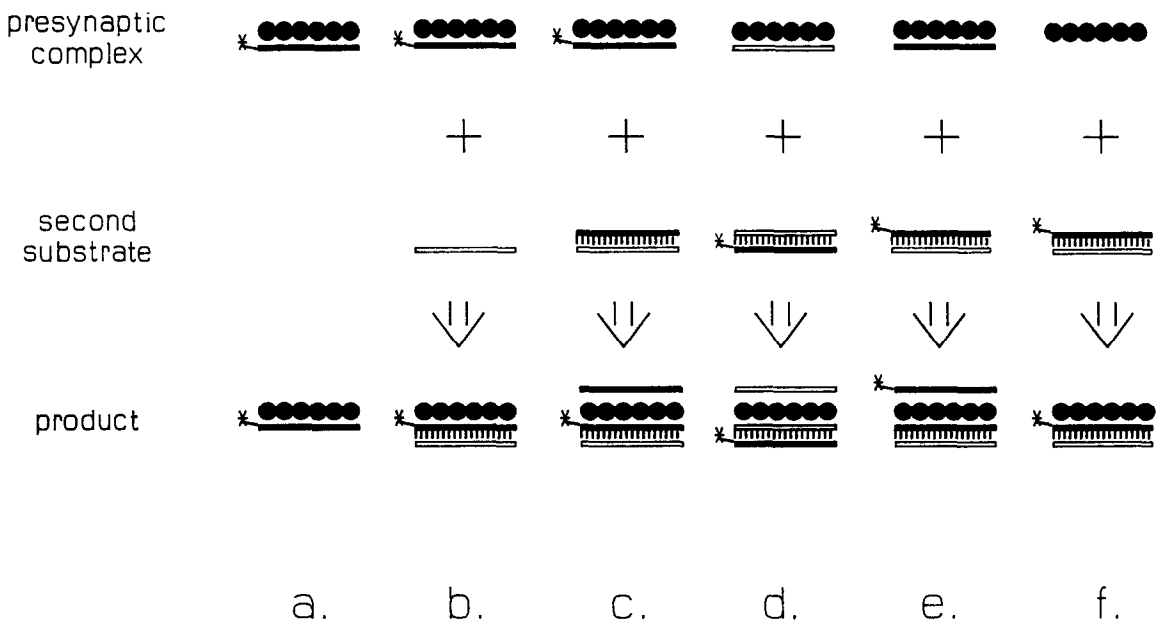

Fig. 1. Experimental design and terminology. Filled and empty bars represent an ss-oligonucleotide with or without fluorescent label (denoted by a star) and a complementary oligonucleotide, respectively; filled circles denote recA monomers. For details see section 2 and legends to Figs. 2–4.

oligonucleotide resulted in a much lower extent of complex formation (lane 4) indicating that the stabilization observed in lane 3 was the result of the interaction of the presynaptic complex with a free ss-oligonucleotide. The recA–ss*-oligonucleotide complex was also stabilized by the addition of a homologous ds-oligonucleotide (lanes 5 and 6).

When the same procedures were used to form recA protein complexes with unlabeled oligonucleotides, only partial binding was observed which demonstrated lower efficiency of the complex formation in the absence of dye (Fig. 2B).

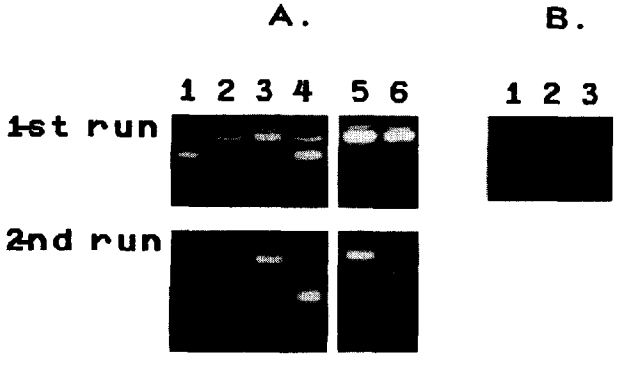

Fig. 2. Electrophoresis of recA–oligonucleotides complexes. (A) Presynaptic complexes were formed with ss*-oligonucleotides. Lane 1, control, no recA added. The 2nd incubation was performed with: lane 2, no addition; lane 3, complementary ss-oligonucleotide; lane 5, homologous ds-oligonucleotide; lane 6, heterologous ds-oligonucleotide. Lane 4 contained the complex formed after the incubation of recA protein (preliminary preincubated in the absence of oligonucleotides) with ds*-oligonucleotide. (B) Presynaptic complexes were formed with ss-oligonucleotides. The 2nd incubation was performed with: lane 2, complementary ss-oligonucleotide; lane 3, homologous ds-oligonucleotide. Lane 1, control, ds-oligonucleotide without recA protein. A single 20 min run of electrophoresis was performed. The gel was stained with ethidium bromide.

The complexes formed after different incubation times of recA–ss*-oligonucleotide presynaptic complexes with a homologous ds-oligonucleotide (Fig. 1c) were deproteinized by addition of SDS and electrophoresed in PAG (Fig. 3a). The results of the electrophoresis demonstrated a progressive conversion of dye-labeled strand from the ss- to the ds-form in the presence of the protein. Consequently, a stable complex observed by agarose electrophoresis (Fig. 2A, lane 5) contained ds*-oligonucleotide as a product of the strand exchange.

Displacement of the labeled strand from ds*-oligonucleotide was observed when the presynaptic complex was formed with an ss-oligonucleotide (Fig. 1e). In this case the complete strand exchange was achieved with about 1.5 equivalent of the prsynaptic complex applied per ds*-oligonucleotide molecule and approximately twice as long time of the second incubation (Fig. 3B).

To control the state of the strands of the recipient ds*-oligonucleotide, presynaptic complexes were formed with different ss-oligonucleotides and incubated with a ds*-oligonucleotide for the time interval sufficient for the complete strand exchange (Fig. 1d,e). An electrophoresis of the resulting complexes demonstrated the stability of ss*-oligonucleotide binding to be depend on its position in stranded exchange reaction (Fig. 4A). When the ss*-oligonucleotide remained a part of the ds*-oligonucleotide it appeared to be firmly bound to the protein (lane 1), while the displaced ss*-oligonucleotide appeared to

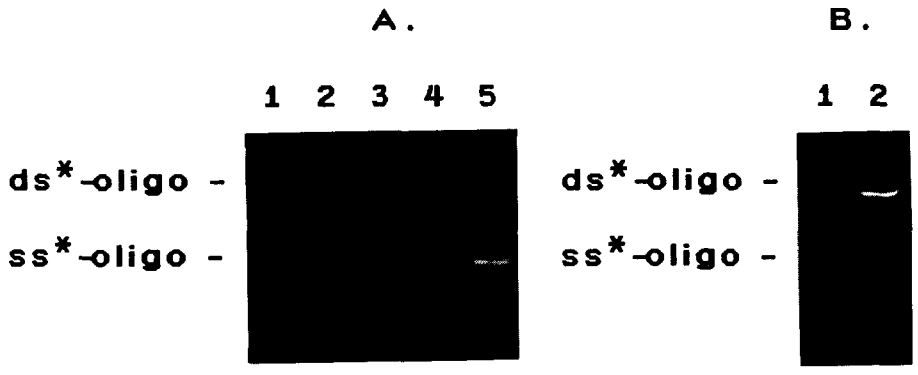

Fig. 3. PAGE of strand exchange reaction products. (A) Incorporation of dye-labeled strand in the ds-form. Lanes 2–4, ss*-oligonucleotide was preincubated with recA protein; 2nd incubation was performed with homologous ds-oligonucleotide added for: lane 2, 3 min; lane 3, 6 min; lane 4, 12 min. Lanes 1,5, ss*-oligonucleotide was preincubated without recA protein; 2nd incubation: lane 1, control, no ds-oligonucleotide added; lane 5, ds-oligonucleotide added for 12 min. (B) Displacement of the dye-labeled strand from the ds-form. 0.15 μg of ss-oligonucleotide (+)strand was preincubated with 7.5 μg (lane 1) or without (lane 2) recA protein. The 2nd incubation was done for 20 min with 0.2 μg of homologous ds*-oligonucleotide.

be bound weakly and released from the complex in the course of prolonged electrophoresis (lane 2).

As seen from Fig. 2A, a short incubation of recA protein with ds*-oligonucleotides did not result in complex formation (lane 4). However, after longer incubation of the recA protein–ds*-oligonucleotide mixture in the presence of ATP-S the complete oligonucleotide binding was observed (Fig. 4B).

4. Discussion

Earlier it was shown that certain fluorescent dyes increase the efficiency of recA protein binding with long ds-DNAs [5,6]. In the present study we demonstrated a stimulating effect of dyes terminally attached to oligonucleotides of recA protein binding to short oligonucleotides and obtained catalytically active presynaptic complexes with 18-b oligonucleotides at near stoichiometric recA/oligonucleotide ratios.

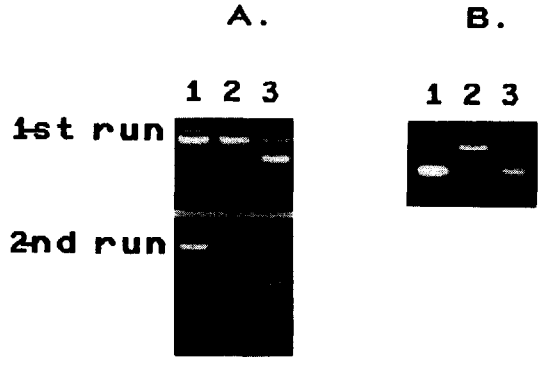

Fig. 4. (A) Complexes formed after preincubation of recA protein with ss-oligonucleotides. 7.5 μg of recA protein was preincubated with 0.15 μg of ss-oligonucleotides: (−)strand (lane 1), (+)strand (lane 2), heterologous (lane 3). The 2nd incubation was done for 20 min with 0.2 μg of ds*-oligonucleotide. (B) Direct recA protein binding to ds*-oligonucleotide. 0.13 μg of ds*-oligonucleotide was incubated for 40 min with: lane 1, control, no recA added; lane 2, 3 μg of recA protein in the presence of 10 mM $Mg(CH_3COO)_2$ and 0.25 mM ATP-S; lane 3, the same but without ATP-S. Electrophoretic conditions as in Fig. 1B.

Much attention is paid to the relation between homology recognition and strand exchange events in the recA promoted reaction [7]. In supercoiled DNA the strand exchange was shown to take place for oligonucleotides containing 26 b of homology, 20 b of homology was insufficient for this reaction, although for homology recognition only 8 b was needed [8]. The use of dye-labeled oligonucleotides allowed us to demonstrate the ability of recA protein to catalyze the strand exchange with as short as 18-b oligonucleotide.

In the present study the displaced strand was shown to interact with the complex of recA protein and heteroduplex product of the strand exchange. This is in agreement with the suggestion that recA protein has a center capable to bind a displaced DNA strand [9].

We suggest that dye-labeled oligonucleotides can be used as convenient substrates for further investigation of recA promoted strand exchange as well as for recA-directed DNA mapping or modification [10,11]. Stable recA–oligonucleotide complexes seem promising for studies on structural aspects of the recA-DNA interaction.

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