

# Stoichiometry and kinetics of complex formation by the *recA* protein and a double-stranded DNA

A.A. Volodin, V.A. Shepelev and Yu.N. Kosaganov

*Institute of Molecular Genetics, Academy of Sciences of the USSR, Moscow, 123182, USSR*

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## 1. INTRODUCTION

The *recA* protein is involved in the general genetic recombination of *Escherichia coli* [1,2]. In an *in vitro* system this protein catalyzes the homologous pairing of DNA molecules, a process which is now being closely and intensively studied (see [3]). The catalysis of homologous pairing by the *recA* protein is associated with ATP hydrolysis. In the initial stage of the process a complex is formed between the *recA* protein and DNA [4]. But the complexes formed in the presence of ATP degrade with the ATP hydrolysis, thereby eluding research. Therefore we obtained a *recA*-DNA complex in the presence of a non-hydrolyzable ATP analogue, adenosine-5'-*O*-(3-thiotriphosphate) or (ATP- $\gamma$ -S), which gave the complex the necessary stability [4,5]. The *recA* protein-DNA binding in the presence of ATP- $\gamma$ -S occurs cooperatively and causes the formation of nets in the solution incorporating a great amount of DNA molecules complexed with the *recA* protein [5]. Such aggregates were visualized in an electron microscope along with individual DNA molecules completely covered with *recA* protein [5,6]. It was demonstrated in [6] that the formation of the *recA* protein-DNA complex had an effect of elongating the DNA molecules by a factor of 1.5. It was suggested in the same paper that the elongation might be the result of intercalation of the protein's amino acid residues or ATP- $\gamma$ -S molecules between DNA bases. To proceed further with the study of the *recA* protein-DNA interaction one needs more detailed information on the physicochemical properties of such complexes.

The aggregation which accompanies the forma-

tion of complexes between the *recA* protein and DNA sets limits to the application of conventional physicochemical techniques. Therefore we investigated the stoichiometry and kinetics of the *recA* protein-DNA binding using the two following approaches: determination of the complex's sensibility to the action of a micrococcal nuclease and gel filtration of the complex on Sephadex column.

## 2. MATERIALS AND METHODS

The *recA* protein was isolated to an electrophoretically near-homogeneous state as in [7] from the *E. coli* strain KM1842 kindly provided by Dr M.M. Cox. The ATP activity of the resultant preparation was about the same as in [7]. The protein concentration was estimated by spectrophotometry. The extinction coefficient measured by the procedure described in [8] was  $\epsilon^{280} = 0.59 \text{ mg}^{-1} \cdot \text{cm}^{-1} \cdot \text{ml}$ .

We used  $\lambda$  phage DNA and pBR322 plasmid DNA transformed into the linear form with an *EcoRI* restriction endonuclease. An ATP- $\gamma$ -S preparation purchased from Boehringer, Mannheim was used. The *recA* protein-DNA complex was formed at 37°C in a buffer (20 mM cacodylate-HCl (pH 7.4), 10 mM NaCl, 10 mM MgCl<sub>2</sub>, 1 mM CaCl<sub>2</sub>, 0.1 mM EDTA, 0.1 mM dithiothreitol). The mixture of DNA and the *recA* protein was heated in this buffer. Then the complexing reaction was initiated by adding ATP- $\gamma$ -S up to a concentration of 250  $\mu\text{M}$ . Incubation was carried out for the necessary period of time whereupon the complex was treated with a micrococcal nuclease or deposited on a column for gel filtration.

A micrococcal nuclease from Sigma was used. Its concentration was chosen so that in the time of treatment (normally 2 min.): the hydrolysis of free DNA would give rise to an electrophoretic band migrating much faster than the native DNA band.

The hydrolysis was terminated by adding SDS, EDTA and Ficoll to concentrations of 0.6%, 30 mM and 3% respectively, whereupon electrophoresis was carried out in tubes with 1% agarose at 6 V/cm for 2.5 h in a Tris-phosphate buffer. Gels were stained in 1% ethidium bromide for 0.5 h prior to scanning.

Gel filtration was performed at room temperature on a column 0.2 cm in diameter and 9 cm height, filled up with the G-75 superfine Sephadex. The solution was eluted at 20  $\mu$ l/min with the same buffer as that in which the complex had been formed. After the recovery of ATP- $\gamma$ -S the column was washed by passing 25  $\mu$ l of 10% SDS. The optical density at the end of the column was measured by the 'Ob'-4' chromatograph manufactured at the Design Office of Special Electronics and Analytical Instrument Making of the Siberian Branch of the U.S.S.R. Academy of Sciences. Measurements were performed at wavelengths 230, 260 and 330 nm to allow the eluted components.

### 3. RESULTS AND DISCUSSION

The cooperative interaction of the recA protein and DNA gave rise to long DNA fragments protected from the action of the micrococcal nuclease. Fig.1 presents electrophoregrams of  $\lambda$  phage DNA after incubation with the recA protein for different periods of time and standard treatment with the micrococcal nuclease. One can see that the pre-incubation of DNA with the recA protein in the presence of ATP- $\gamma$ -S produced an electrophoretic band (band I; fig.1) migrating much more slowly than the one belonging to nuclease-treated free DNA (band II). The relative intensity of the slower band increases with the incubation time and a growing recA protein:DNA weight ratio. Therefore it was probably a DNA protected by the recA protein from micrococcal nuclease hydrolysis that was moving in band I. To evaluate the fraction of DNA covered with the recA protein, we found the ratio of the area of band I to the aggregate area of bands I and II. The estimate might be underrated if part of the protein formed short clusters on

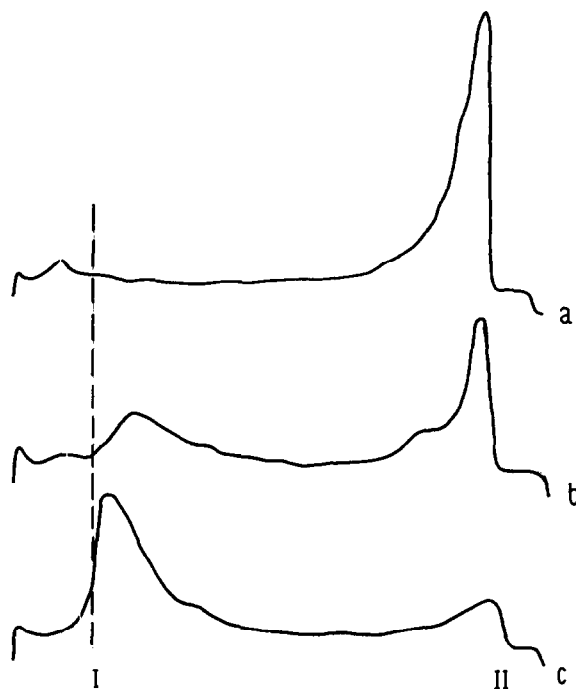


Fig.1. Electrophoregrams of  $\lambda$  phage DNA after incubation with the recA protein and treatment with micrococcal nuclease. The duration of the nuclease treatment is 2 min. (a) Incubation with the recA protein for 120 min without ATP- $\gamma$ -S. The DNA electrophoregram looks the same after incubation with ATP- $\gamma$ -S without the recA protein. (b) recA:DNA weight ratio is 20, the duration of complex formation is 20 min. (c) recA:DNA weight ratio is 60, the duration of complex formation is 120 min. DNA concentration in solution: 9  $\mu$ g/ml (a,b) and 14  $\mu$ g/ml (c). Electrophoresis proceeds from left to right. The dashed line indicates the position of the native DNA band.

DNA, their lengths corresponding to those of the DNA fragments migrating in band II.

Fig.2 shows the relationship between the fraction of DNA migrating in band I and the recA:DNA weight ratio. It took 90–120 min for the recA protein and DNA to form a stable complex. The figure shows that the DNA becomes saturated with the recA protein when the recA:DNA weight ratio is  $\sim$ 30. In this case the DNA fraction protected from the micrococcal nuclease amounts to no less than 80%.

To determine the fraction of protein which remained free during complex formation, gel filtra-

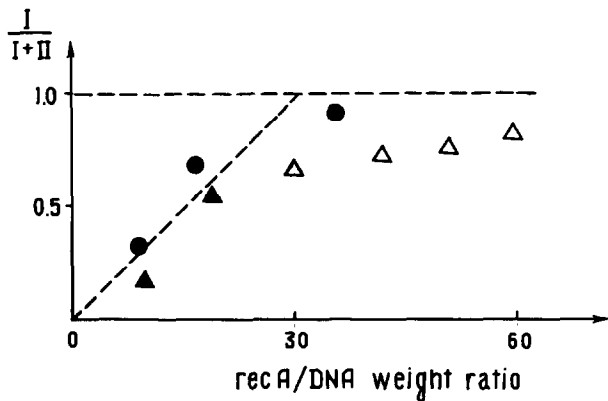


Fig. 2. Saturation of DNA with the recA protein. The duration of complex formation prior to nuclease treatment is 90–120 min. DNA concentration in solution: 40  $\mu\text{g}/\text{ml}$  ( $\bullet$  and  $\blacktriangle$ ) and 14  $\mu\text{g}/\text{ml}$  ( $\triangle$ ). The ordinate is the ratio of the area of peak I to the aggregate area of peaks I and II in the electrophoregram. ( $\blacktriangle$ ) and ( $\triangle$ ) are  $\lambda$  DNA, ( $\bullet$ ) is pBR 322 DNA.

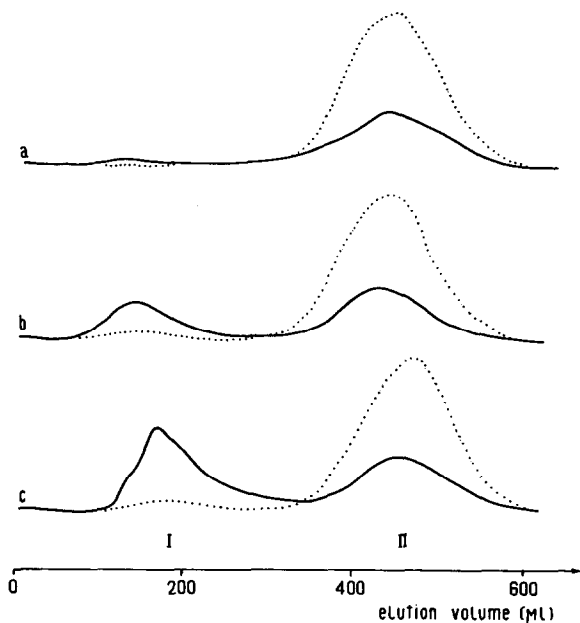


Fig. 3. Gel filtration of the  $\lambda$  DNA complex with the recA protein on Sephadex G-75. The duration of complex formation is 120 min, the DNA concentration is 14  $\mu\text{g}/\text{ml}$ , the sample volume is 50  $\mu\text{l}$ . The recA:DNA weight ratio: 30 (a), 40 (b), 60 (c). Optical density at wavelength 230 nm (—) and at 260 nm (.....).

tion of the complexes was performed for different recA:DNA weight ratios. Aggregates which formed during the recA protein–DNA interaction did not pass through Sephadex G-75 while the free protein and ATP- $\gamma$ -S were eluted.

Shown in fig. 3 are the elution profiles of DNA complexes with the recA protein for different recA:DNA weight ratios. At a weight ratio of  $\sim 30$  all of the DNA and almost all of the protein are retained on the column. Only ATP- $\gamma$ -S is eluted (peak II, fig. 3).

With an increase in the weight ratio a peak appears in the elution profile which corresponds to the free recA protein (peak I; fig. 3). Fig. 4 depicts the relationship between the amount of protein eluted from the column and the recA:DNA weight ratio. Thus free recA protein appears in the solution at recA:DNA weight ratios  $> 30$ . The data on the micrococcal nuclease sensitivity of DNA in a complex with the recA protein and on the gel filtration of such complexes imply that the recA protein can almost completely cover the DNA when their weight ratio is  $30 \pm 6$  i.e. each bound monomer of the recA protein covers  $2 \pm 0.4$  base pairs (the  $M_r$  of the monomer was taken to be 38 000 [9,10]).

It was suggested in [6] that changes in the DNA conformation on its binding to the recA protein may be due to an intercalation of protein groups or ATP- $\gamma$ -S between the DNA bases. In that case there should be one intercalated element to every

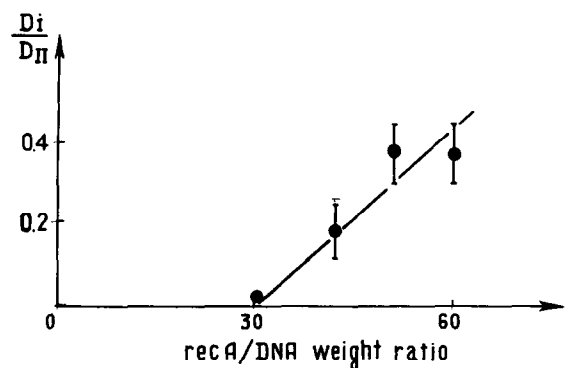


Fig. 4. Relationship between the amount of free protein in solution and the recA:DNA weight ratio. The ordinate is the amount of protein eluted from the column (see fig. 3), normalized to the amount of eluted ATP- $\gamma$ -S. (Ratio of peak I to peak II intensity in elution profile. See fig. 3.)

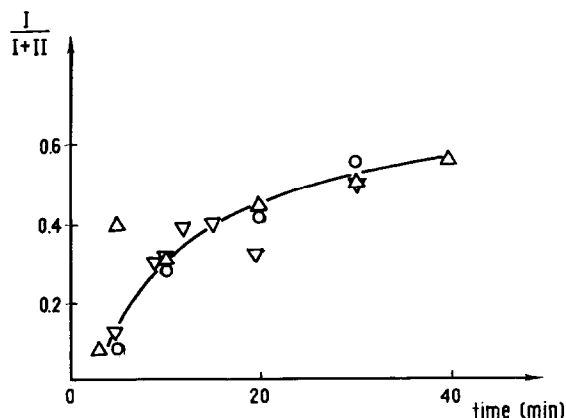


Fig.5. Kinetics of complex formation between the recA protein and  $\lambda$  phage DNA at different concentrations of the recA protein in solution. The DNA concentration is 9  $\mu\text{g}/\text{ml}$ , the recA:DNA weight ratio is 20 ( $\circ$ ), 40 ( $\Delta$ ), 80 ( $\nabla$ ). The ordinate is fraction of nuclease-resistant DNA.

two DNA base pairs. If the assumption is correct, then in accordance with our data each recA protein monomer should account for one intercalant.

The micrococcal nuclease technique of evaluating the fraction of DNA covered with the recA protein allows the kinetics of the recA protein–DNA binding to be studied under different conditions. Shown in fig.5 are the time dependencies of the micrococcal nuclease-resistant DNA fraction for different concentrations of protein in solution. The figure shows that the recA protein–DNA complexing does not depend on the protein concentration within the tested concentration range. This suggests that the complexing rate is being limited by some kind of conformational rearrangement in DNA and/or protein. It may be, for instance, the unwinding of DNA during complex formation (cf the mechanism discussed in [6]). In our case the DNA molecules are linear, but in complexes with the recA protein they form elongated three-dimensional nets. That arrangement may strongly impede their rotation. The unwinding of the DNA double helix, if it occurs during complex formation, might be the limiting stage of the process. So far there are no direct data indicating that the recA protein should unwind the DNA double helix. Therefore our explanation of the fact that the complexing rate does not depend on the recA protein concentration may be regarded as a working hypothesis.

#### 4. CONCLUSIONS

- (i) Treatment with a micrococcal nuclease was shown to be an effective method in studying the recA protein–DNA binding.
- (ii) This method plus elution on Sephadex offered a means of determining that each DNA-bound monomer of the recA protein covered  $2 \pm 0.4$  DNA base pairs.
- (iii) The kinetics of the recA protein binding to  $\lambda$  phage DNA was investigated. It took 30 min to make DNA half-saturated with the recA protein independently of the recA protein concentration in solution. It was hypothesized that the unwinding of the DNA double helix is the limiting stage of the recA protein–DNA binding process under the experimental conditions.

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