Novel crystal forms of a proteolytic core of the single-stranded DNA-binding protein (SSB) from *E. coli*

R. Hilgenfeld, W. Saenger*, U. Schomburg+ and G. Krauss+

Institut für Kristallographie, Freie Universität Berlin, Takustr. 6, D-1000 Berlin 33 and *+Zentrum Biochemie, Abt. Biophysikalische Chemie, Med. Hochschule Hannover, D-3000 Hannover 61, FRG*

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A proteolytic core of the *Escherichia coli* single-stranded DNA-binding protein (SSB) has been crystallized from phosphate buffer. Crystals suitable for X-ray data collection display monoclinic space group C2 with $a = 106.8$, $b = 62.3$, $c = 100.2$ Å, $\beta = 112°$ and contain one tetramer of proteolysis product SSB*-A per asymmetric unit. Two other crystal forms have been obtained in the presence of the inhibitor diisopropylfluorophosphate.

Single-strand binding protein *Proteolysis product* *Protein crystal* *X-ray diffraction*

1. INTRODUCTION

The single-stranded DNA-binding protein from *E. coli* (SSB) is essential for replication, recombination, and repair of *E. coli* DNA and is an important factor of the SOS response system that is induced upon DNA damage (review [1,2]). Furthermore, SSB influences the activity of DNA-specific proteins such as DNA polymerase II [1,2] and of the *recA* gene product [1,3].

In spite of the central role of the SSB protein, nothing is known about its three-dimensional structure. In solution, SSB exists as a tetramer of identical 18.9 kDa subunits [4], each of which contains a binding site for an octanucleotide [5]. From ^{1}H-NMR measurements it has been proposed that cooperative SSB binding to poly(dT) is accompanied by fast movements of the protein on the polynucleotide lattice [6]. Electron microscopic investigations have shown that SSB binds single-stranded DNA in a manner similar to the binding of dsDNA by histones; i.e., the DNA–SSB complex displays a nucleosome-like structure [7].

Here we describe the crystallization and preliminary X-ray investigation of SSB. We have obtained several crystal forms, some of which are clearly distinct from those reported recently as the result of an independent study [8].

2. MATERIALS AND METHODS

2.1. *Protein isolation*

SSB was purified as in [5].

2.2. *Protein crystallization*

The protein was crystallized by the 'sitting-drop' vapour diffusion method [9]. Seven to 9 mg/ml solutions of SSB in 0.5–0.7 M NaCl, 20 mM phosphate, 3 mM NaN_3 (pH 7.0) were equilibrated at 4°C against 20–60 mM phosphate, thus slowly decreasing the salt concentration. This approach was chosen on the basis of the observation that SSB shows a pronounced tendency to precipitate at low ionic strengths [5].

In some instances, 10 mM DFP (Serva) or

* To whom correspondence should be addressed

Abbreviations: SSB, single-stranded DNA-binding protein from *E. coli*; dsDNA, double-stranded DNA; ssDNA, single-stranded DNA; DFP, diisopropylfluorophosphate; PMSF, phenylmethylsulfonylfluoride; HPLC, high performance liquid chromatography

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0.5 mM PMSF (Merck) in ethanol were added to the protein solution in order to prevent proteolysis. X-ray photographs of crystals were taken using an Elliot GX6 rotating anode generator, operated at 1.6 kW, 200 μm focus.

2.3. *SDS-gel electrophoresis*

Ten percent SDS–PAGE (w/v) was carried out in the usual way. Crystals were washed extremely carefully before being redissolved and subjected to gel electrophoresis.

2.4. *HPLC*

SSB and its proteolytic cleavage products were also analyzed by HPLC using a C-18 reversed-phase column (LiChrosorb, Merck) and Knauer pumps. Before injection, the protein was denatured by boiling with SDS in order to avoid artifacts due to partial denaturation on the reversed-phase support.

3. RESULTS AND DISCUSSION

Under the conditions described above, two crystal forms appeared almost simultaneously after 6–8 weeks in the same drops. Needle-like crystals were too tiny to be used for X-ray diffraction experiments. Prismatic crystals grew up to $0.6 \times 0.3 \times 0.3$ mm and are suitable for X-ray analysis (fig.1).

By SDS-gel electrophoresis (fig.2) and HPLC we were able to show that both crystal forms grew only after proteolytic cleavage of SSB had occurred. This proteolytic activity is probably due to traces of an unidentified protease that has been

Fig.1. Prismatic crystals (SSB*-A) and needles grown as described in the text.

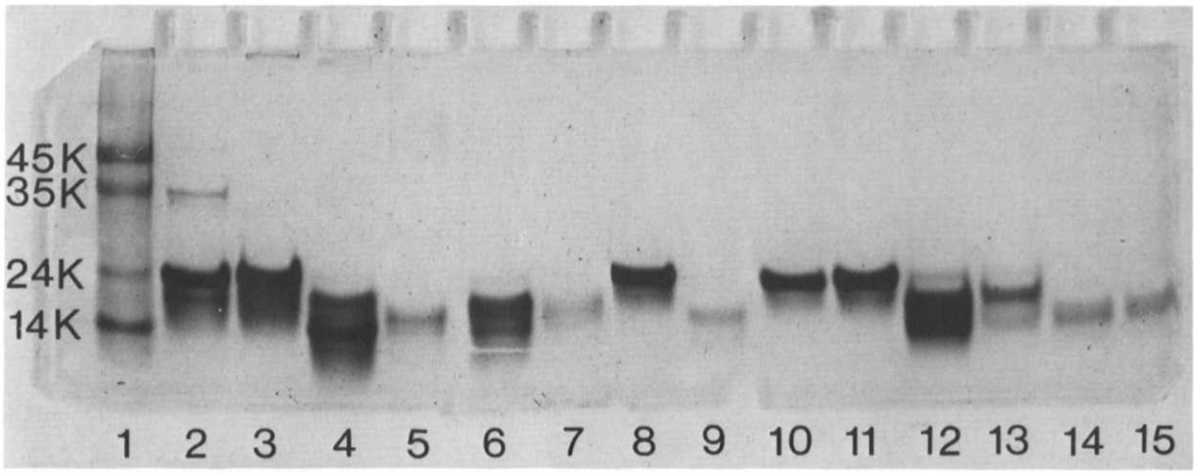

Fig.2. A 10% (w/v) SDS–PAGE of SSB and its proteolytic fragments: Lane 1, marker (14.3, 24.0, 35.0, 45.0 kDa); 2, old preparation of SSB (4 mg/ml); 3, 6 mg/ml SSB in the presence of glycerol; 4 and 6, redissolved precipitates; 5 and 12, old preparations, 8 mg/ml SSB; 7 and 13, redissolved 3-dimensional crystals; 8, 10 and 11, freshly prepared SSB, 10 mg/ml; 9, chymotryptic cleavage product SSB^{*}_{c}; 14 and 15, redissolved needle-shaped crystals.

copurified with SSB, or due to autoproteolysis of SSB.

In freshly prepared SSB solutions, no proteolytic cleavage products could be observed (fig.2, lanes 8,10,11), whereas after several weeks a second and, later, a third band appeared on the SDS polyacrylamide gel, corresponding to apparent M_r values of 13800 (SSB*-A) and 10500 (SSB*-B) (fig.2, lane 12). These fragments show an even more pronounced tendency to precipitation than native SSB. Thus precipitates very often consisted mainly of fragment SSB*-B (fig.2, lane 4). Proteolysis could not be observed at low SSB concentrations (below 2 mg/ml) or in the presence of glycerol (fig.2, lane 3). In these solutions, only needle-shaped crystals appeared which probably represent intact SSB. Prismatic crystals were unambiguously shown by gel electrophoresis to consist of SSB*-A (fig.2, lanes 7,13). These latter crystals are monoclinic C2 with a = 106.8, b = 62.3, c = 100.2 Å, β = 112°.

As far as its dimensions are concerned, this unit cell is very similar, if not identical, to that reported recently for SSB crystals obtained by microdialysis against 0.2 M NaCl, 50 mM Tris–HCl at pH 9 or against 40–60 mM spermidine in the pH range 6.8–8.2 [8]. However, there is an important difference in the unit cell content. Whereas our crystals contain exclusively proteolytic fragments SSB*-A, the latter ones [8] are composed of two native and two proteolytically degraded subunits of SSB per asymmetric unit. Our crystals diffract to at least 2.5 Å on 'stills' and have a packing density parameter V_m = 2.80 Å^3/Da [10] assuming one tetramer of SSB*-A (M_r 55200) per asymmetric unit.

Attempts to prevent proteolysis by addition of DFP or PMSF (which are potent inhibitors of serine proteases) to the crystallization probes failed. However, DFP obviously changed crystallization conditions so as to result in two other crystal forms, plates of hexagonal shape and thick needles. The former are apparently also monoclinic and still under investigation, whereas the latter do not diffract well despite their appreciable size. These needles were found to be crystals of proteolytic fragment SSB*-B (fig.2, lanes 14,15).

Recently, authors in [11] have shown that limited degradation of SSB by chymotrypsin or trypsin yields stable core tetramers, SSB^{*}_{c} (M_r 14500) or SSB^{*}_{t} (M_r 12800) lacking the acidic C-terminal region of SSB. These proteolytic core proteins bind at least as tightly to fd ssDNA as does intact SSB [11]. Crystals of SSB^{*}_{c} suitable for X-ray analysis have been obtained in [8]. Our cleavage product SSB*-A is similar in M_r to the chymotryptic core, SSB^{*}_{c} (fig.2, cf. lanes 5,9,13).

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