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Preliminary Crystallographic Analysis of a Proteolytically Modified Form of *E. coli* Single Stranded DNA Binding Protein

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Abstract

A proteolytically modified form of the *Escherichia coli* single-stranded DNA-Binding protein (SSB) has been crystallized from 15% saturated sodium citrate. Crystals as large as 1.0mm × 0.3mm × 0.2mm were obtained and these diffract beyond 3Å resolution. X-ray photographic analysis demonstrated a rhombohedral unit cell of space group R3 with an equivalent triple centered hexagonal unit cell having dimensions of a = b = 62.9Å and c = 264.3Å. These crystals were judged to be adequate for a three dimensional structure determination.

Introduction

The Single Strand DNA Binding protein (SSB) from *Escherichia coli* is involved in the destabilization of DNA duplexes (1) and plays a vital role in DNA replication, recombination and repair (2-6). The protein is a tetramer of identical 18.9kDa subunits (7) which binds non-sequence specifically to single-stranded DNA in a cooperative manner (8). In addition, there is evidence that SSB may interact specifically with other nucleic acid binding proteins including DNA polymerase II (9), Exonuclease (10) and recA (11). The exact role of SSB and its mode of action in these biological processes is however, still not clear.

The binding of SSB to single strands of DNA is a complex process that may proceed by a number of different modes. These may be characterized by discrete nucleic acid base to protein subunit ratios of n = 35, 40, 56 or 65, where n denotes the average site size. The mode of binding apparently depends on the concentration and species of the ions present (12,13,14).

An interesting feature of SSB is that while it exhibits a high degree of positive cooperativity in its binding to single stranded DNA, it demonstrates negative cooperativity among its four identical subunits (15). This implies that certain events, such as initial DNA binding, may induce asymmetry into the tetrameric molecule.

Because of its importance in cellular processes and its extensive study using a wide variety of physical-chemical techniques (15,16), SSB is a particularly inviting subject

for structural studies. With the objective of providing a means for carrying out the X-ray diffraction analyses essential for a precise structural determination, we undertook the crystallization of the protein and, subsequently, the characterization of a new crystal form of SSB.

Materials and Methods

SSB Protein was purified, according to Lohman (17) to greater than 95% homogeneity as determined by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The protein was crystallized by the hanging drop vapor diffusion method as described by McPherson (18). The initial volume of the hanging drop was 10 µl. It

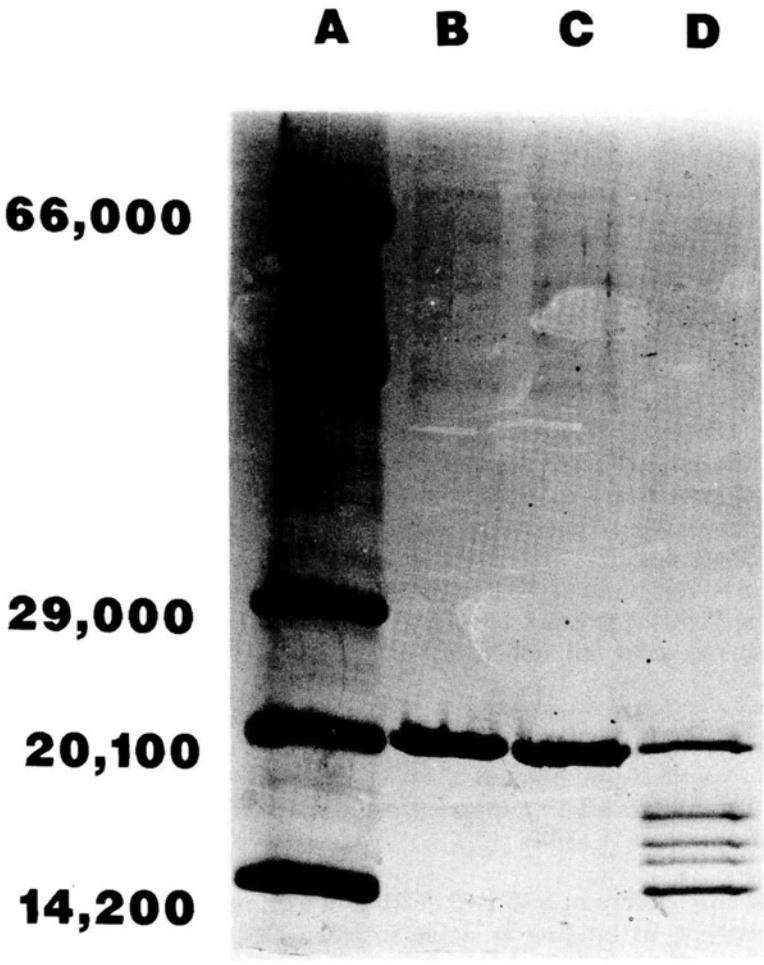

Figure 1: SDS-PAGE of redissolved SSB crystals grown from 15% saturated sodium citrate at pH 7.0. The gel demonstrates the crystals to be composed of five prominent fragments of the SSB and these are seen in lane D.

consisted of 5 µl of the protein solution containing 10 mg/ml SSB in 20 mM Tris buffer (pH 7.0) with 300 mM NaCl, and 5 µl of a reservoir solution containing 15% saturated sodium citrate in 20 mM Tris at pH 7.0. All crystals were grown at 23°C. Diffraction photographs were recorded on a Buerger precession camera using an Enraf-Nonius generator fitted with a conventional broad focus X-ray tube operated at 1.4 kW. Nickel filtered CuK_α radiation was employed and the exposure times were generally 12 to 18 hours with a crystal to film distance of 75 mm.

After X-ray analysis the SSB crystals were carefully washed with 20 mM Tris (pH 7.0), redissolved in the same buffer by addition of NaCl to 400 mM and then subjected to SDS-gel electrophoresis. The resulting protein crystal products, as seen in Figure 1, appeared as 5 main bands corresponding to molecular weights of approximately 20,000, 16,800, 15,900, 15,000 and 14,200 (Lane D). The sizes of the SSB fragments were estimated by comparing the electrophoretic mobilities of the redissolved crystal products to those of molecular weight marker proteins. Lanes B and C contained 20 µg and 25 µg respectively of the purified SSB. Lane A contained α-lactalbumin (14,200 M.W.), egg albumin (45,000 M.W.) and bovine albumin (66,000 M.W.). The samples were subjected to electrophoresis on a 5-20% polyacrylamide gel in the presence of sodium dodecyl sulfate. The gel was stained in a solution containing 25% (v/v) methanol, 10% (v/v) acetic acid and 300 µg/ml of Coomassie brilliant blue for 3 hours at 25°C. The gel was destained in 10% (v/v) methanol and 10% (v/v) acetic acid for 5 hours at 25°C.

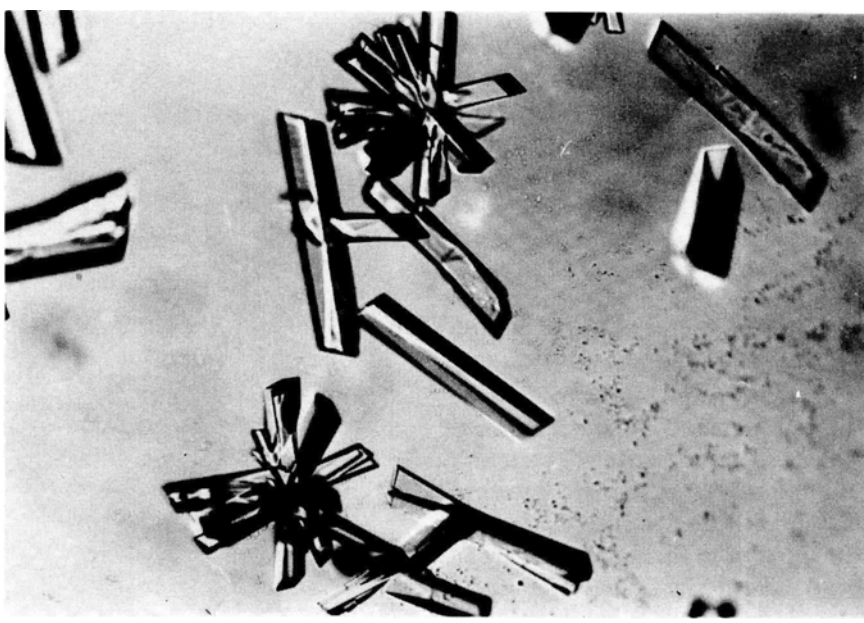

Figure 2: Crystals of SSB grown by vapor diffusion versus sodium citrate as seen under a low power light microscope. In this photgraph the largest crystal was measured to be 0.8 mm × 0.2 mm × 0.2 mm.

Results

A prominant prismatic form of SSB crystal appeared after 2 days equilibration in the hanging drop under the conditions described above. The crystals ranged in size with the largest being approximately 1.0 mm × 0.3 mm × 0.2 mm. Figure 2 shows a typical droplet containing crystals of SSB. Success in producing large crystals under these conditions depends strongly on temperature. We observed significant effects on crystal growth with temperature variance of as little as 3° from 23°C.

As described above, SSB crystals were shown to contain proteolytically modified products of native SSB as well as intact subunits. We suspect this may probably be due to undetectable traces of a protease copurified with SSB. While redissolved crystals yielded 5 major bands on the SDS-PAGE corresponding to molecular weights of approximately 20.0 kDa, 16.8 kDa, 15.9 kDa, 15.0 kD and 14.2 kD, freshly prepared SSB samples yielded only a single band corresponding to a molecular weight value of about 20 kDa. This band was homogeneous to greater than 95% as determined by densitometric spectroscopy. We observed that pure SSB in solution was also cleaved simply by standing at room temperature. This was shown by loading freshly prepared SSB samples that had been maintained at 22°C temperature for more than 2 days on SDS-polyacrylamide gel. The proteolyically cleaved products had band patterns similar to the crystals that were run on the SDS-PAGE.

A prismatic crystal was mounted with its long axis parallel with the direction of the capillary thereby serving as the axis of rotation when placed on the precession camera. A major zone of the reciprocal lattice having $\bar{1}$ symmetry was located by recording a series of photographs at incremental angles about the rotation axis. Identical photographs were obtained at ±120° rotations from the initial zone. At +30° rotations from any one of these equivalent zones, additional zones also having $\bar{1}$ symmetry were recorded. The spacings of the reflections along reciprocal lattice rows perpendicular to the rotation axis in the second set of zones were related by $\sqrt{3}/2$ to the spacings along these rows in the first set of planes. Thus the reciprocal lattice was demonstrated to have a unit cell characterized by a single threefold axis, but with no symmetry elements perpendicular to the unique axis. The two zones could be indexed on a hexagonal lattice which exhibited systematic absences of $-h+k+l \neq 3n$ to the maximum resolution of the photographs. The crystallographic space group

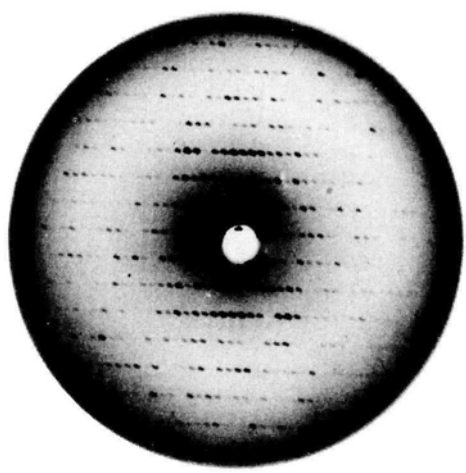

Figure 3: A 12° precession X-ray diffraction photograph of the hhl zone of the reciprocal lattice of a rhombohedral SSB crystal. Exposure time was 20 hours.

is therefore R3. A photograph of the hhl zone of reciprocal space of the trigonal crystals in hexagonal indexing is shown in Figure 3.

Discussion

Assumption of an entire intact protein tetramer of 75.6 kDa as the asymmetric unit of the crystals would yield a volume to mass ratio of 1.38 Å/dalton which is very low for most protein crytals (19), though not impossible. If however, the asymmetric unit contains proteolytically cleaved protein, or mixtures of native and cleaved subunits, as seems to be indicated by the SDS-PAGE results, then a higher and more reasonable value may be assumed. Indeed reports by two other groups of investigators of SSB crystals, having unit cells different from that reported here, described crystals composed of such proteolytically modified molecules (20,21).

We have compared the R3 unit cell with the unit cells reported by others and it appears that the packing of the protein tetramers in the various unit cells may be related. In particular, the similarity of the cell described here with the $P6_{2(4)}22$ cell having dimensions a = b = 61.4 Å and c = 264.3 Å (20) seems striking and suggests a possible orientation for local symmetry axes in the R3 cell. Dimensions found in these two unit cells appear as well in the monoclinic crystals of space group C2 with a = 106.8 Å, b = 62.3 Å, c = 100.2 Å and $\beta = 112°$ (21).

The results we present here imply that the SSB tetramers, composed of four identical subunits in the native form, can exist in proteolytically modified forms containing two intact and two cleaved subunits. Thus molecules that form the crystals represent a structurally asymmetric form of the tetramer which is otherwise likely to be of higher symmetry. This is somewhat surprising since one might expect the four subunits to be approached identically by whatever protease is responsible for cleaving SSB.

On the other hand, it has been demonstrated that asymmetry can arise in the tetramer during the course of normal cellular events (15). Perhaps then, the asymmetry that results from proteolytic action is another manifestation of the cooperativity function inherent to the structure of the protein. Just as DNA binding to two subunits of SSB influences the mode of binding to the third and forth, cleavage of two subunits may discourage attack on the remaining pair.

The crytals we have grown diffract well to at least 3.0 Å resolution and are quite stable in the X-ray beam. They show little decay until more than 40 hours of exposure have elapsed. Thus we judge the crystals to be adequate for a three dimensional structure determination.

Acknowlegments

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