DENATURATION OF T4 DNA BY AN IN VITRO PROCESSED GENE 32-PROTEIN

Junko HOSODA*, Bela TAKACS and Christine BRACK
Department of Microbiology, Biozentrum University of Basel, Klingelbergstrasse 70, CH – 4056 Basel, Switzerland

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1. Introduction

The gene 32 product (P32) of bacteriophage T4 is a protein necessary for DNA replication and recombination [1,2]. Purified P32 cooperatively binds to single-stranded DNA and can denature certain double-stranded DNAs [3,4]. However, it has never been shown to denature T4 DNA. During the course of our electron microscopic investigations on the structure of complexes of P32 with various DNAs, we found a P32 derived product which denatures T4 DNA. Using the overproducing strain T4 55^- (amBL272) for the purification of P32 (mol. wt. 35 000), we observed a second component of mol. wt. 27 000 which copurifies with P32. Upon storage of the sample at 4°C, the second component increased, while the first one (P32) decreased. We tentatively conclude that the second protein is an in vitro processed product of P32 and call it P32*. Electron microscopy and analysis of the protein bound in T4 DNA complexes showed that it is only this P32* which can denature T4 DNA at various ionic conditions.

Material and methods

Purification of P32 by DNA-cellulose column and DEAE-cellulose column was carried out as described by Alberts and Frey [3], except that we used the overproducing strain T4 55^- (amBL272) (Krisch and Epstein, personal communication) and harvested the cells for protein extraction after a longer growth period, i.e. 90 min. at 37°C. The P32 fraction was further purified by Hydroxyapatite chromatography [4]. Purity of the fractions and the molecular weight of each protein component were checked by SDS-gel electrophoresis [5,6]. ^{35}S-labeled protein was prepared by growing *Escherichia coli* in M-9 medium with 2×10^{-5} M $NaSO_4$; ^{35}S-sulfate (56 μCi/ml) was added 7 min after infection. T4 DNA was phenol-extracted according to methods previously described [7]. Preparation and purification of the T4 DNA–protein complex for electron microscopy was done as described in the text and figure legends. Micrographs were taken with a Philips 300 electron microscope.

3. Results and discussion

During the purification of P32, we observed that the eluate of the DNA-cellulose column with 2 M NaCl contained, in addition to the usual single protein of mol. wt. 35 000 reported by Alberts and Frey [3], a second component of mol. wt. 27 000. This second protein copurified with P32 throughout the purification procedure.

The second component was present in several independently prepared protein samples at variable proportions of the otherwise pure P32 fraction. The relative composition of the protein sample changed with the age of the preparation: storage at 4°C resulted in an increase of the second component which was accompanied by a decrease of the first component (P32) (fig. 1). Antibodies raised against a pure fraction of P32 (prepared by two cycles of

* Present address: Space Sciences Laboratory, University of California, Berkeley, California 94720.

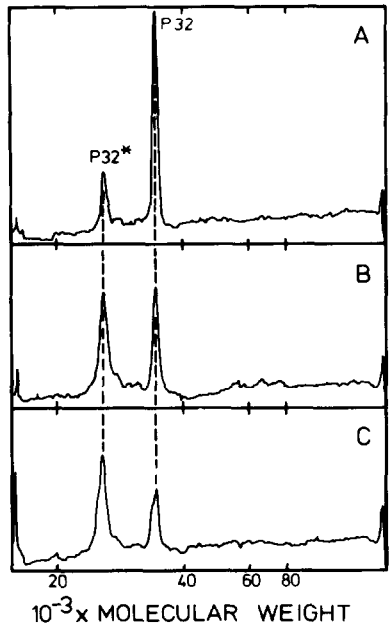


Fig. 1. In vitro conversion of P32 to P32*. Densitometer tracings of stained SDS-polyacrylamide (10%) slab gels [6,7]. The original purified protein sample was kept frozen at −70°C in a buffer containing 20 mM Tris–HCl pH 8.5, 1 mM Na_3EDTA, 1 mM mercaptoethanol, 10% glycerol. a) A freshly thawed sample; b) the same sample after 2 months' storage at 4°C; c) same sample after 4 months at 4°C. The same volume of sample was applied to each gel.

Sephadex G-100 chromatography) precipitated with both protein components. We conclude from all these data that the second component is a processed product of P32, and consequently call it P32* (the asterisk stands for a processed gene product, following the conventions of Laemmli [5]).

We examined DNA-binding properties of 'gene 32-proteins' (mixture of P32 and P32*) by electron microscopic examination of complexes formed between our proteins and various DNAs: λ, T4, PM_2 supercoils, and single-stranded fd DNA. Electron microscope observation of the various complexes showed that the protein mixture had the classical properties described earlier for P32 [3,4], i.e. they form a stable complex with single-stranded fd DNA, they partially denature λ DNA, and they open up the unpaired regions in PM_2 supercoils, showing 1–3 small coated loops (Brack, manuscript in preparation).

However, another unexpected feature showed up: under the conditions used here, the protein mixture also denatures T4 DNA. It has initially been reported that T4 DNA is not denatured by P32 under various conditions [3].

Under conditions of protein excess (weight ratio of P32:P32* : DNA = 20:20:1) about 30% of total protein was bound in the complex with T4 DNA. The complexes were purified from unbound protein by gel filtration [8], one sample was spread for electron microscope observation, and the rest of the purified complex was dissociated in SDS for analysis of the bound protein. SDS-gel electrophoresis of the sample before and after binding to T4 DNA showed that exclusively P32* was bound in the complex (fig. 2). Since the sample contained equal amounts of both components, about 60% of total P32* input was bound to T4 DNA. The protein:DNA ratio in the complex was calculated to be 12:1. Figs. 3 and 5 show electron micrographs of T4–P32* complexes with protein excess. Large stretches of DNA are denatured and covered by protein. Glutaraldehyde fixation results in a crosslinking of the two protein-coated strands and makes recognition of denatured regions more difficult (fig. 5).

Under conditions of DNA excess (P32:P32* : DNA = 5:5:1), about 40% of the total protein was retained in the purified complex, i.e. approximately 80% of

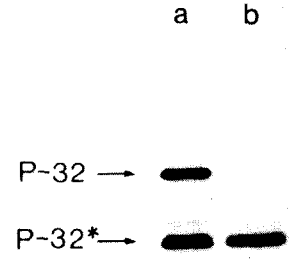
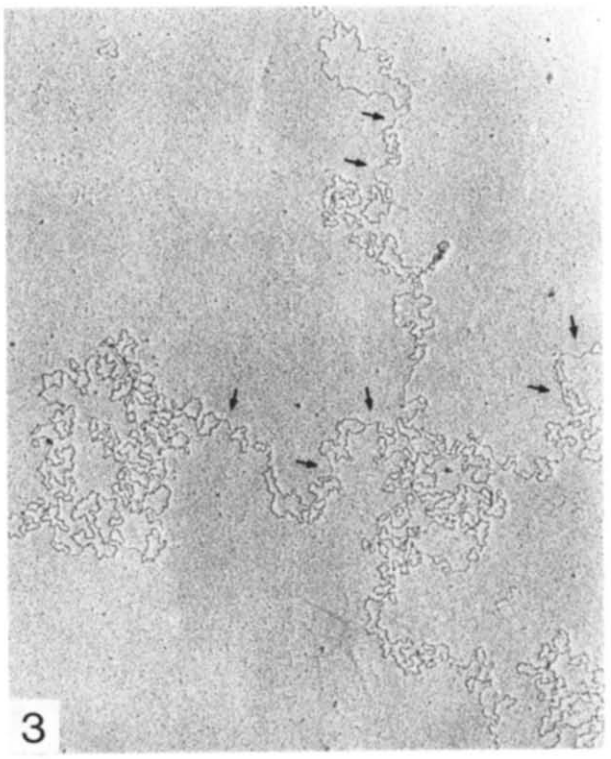


Fig. 2. Autoradiograms of an SDS-polyacrylamide gel of a protein sample containing a mixture of about 50:50% of ^{35}S-labeled P32 and P32*. a) before binding to T4 DNA; b) protein eluted from the purified complex.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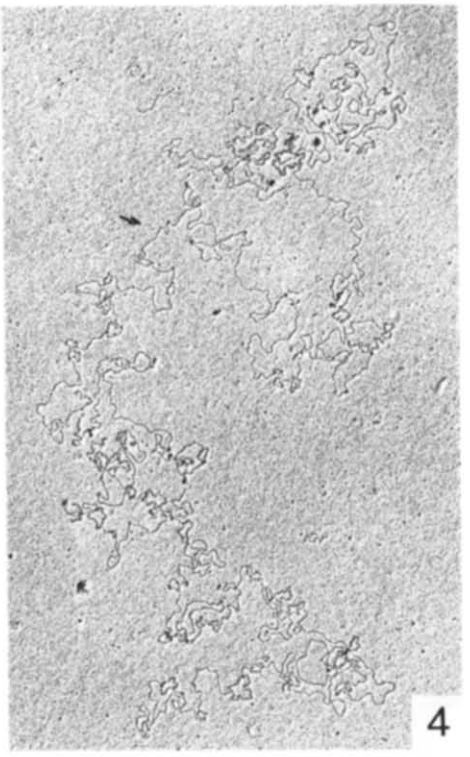
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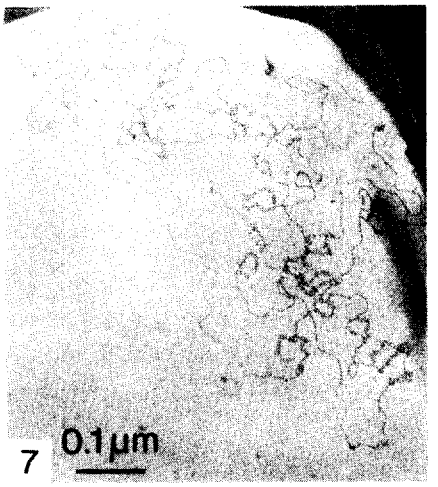

Fig. 7. Complex prepared as in fig. 4, protein:DNA = 100:10 µg/ml, glutaraldehyde fixed, stained with 0.5% uranyl formate for 1 min [10,11]. Magnification: × 120 000.

P32*. The electron micrographs in figs. 4 and 7 show that small protein-coated loops are rather equally distributed all along the DNA molecule. The spreading method without cytochrome *c* used here allows a better distinction between native double-stranded DNA (arrows in figs.) and protein-coated complex regions. The smallest denatured loop distinguishable in the stained preparation (fig. 7) is about 50 base pairs long. Delius et al. have shown that P32 preferentially denatures AT-rich regions in λ DNA [4]. As denaturation of T4 DNA by P32* starts in many places all over the molecule, we suggest that T4 DNA, in contrast to λ DNA, may have a rather homogeneous distribution of AT-rich regions.

In order to approach more closely the physiological conditions, complex formation at various ionic conditions has been studied. At 100 mM NaCl and with protein excess, P32* is capable of partially denaturing T4 DNA. Only a very small amount (2.5%) of total protein was retained in the DNA complex. Electron microscopy showed that denatured regions were much more localized (fig. 6). Denatured, protein covered regions are located in the middle of the molecule and very often also at the ends. As most of the DNA molecules are broken during the column chromatography, we cannot decide whether long protein-coated parts at the ends are broken denatured loops or whether some denaturation actually proceeds from the ends of the molecules. Protein binding at higher salt concentrations probably starts at very few sites only, from which further denaturation proceeds, rather than starting at many places as in low salt conditions.

We are presently investigating the mechanism by which the production of P32* can be controlled. Our preparations contain, in addition to the two main components, small amounts of some additional proteins (fig. 2), one of which might be an enzyme involved in the cleavage of P32 and P32*. Further experiments are needed to confirm the relationship between the two proteins. The present findings that P32* is able to denature native T4 DNA may help to a better understanding of the role gene 32-products play in DNA replication and recombination.

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Figs. 3–6. Electron microscopy of complexes of T4 DNA with 'gene 32-products'. T4 DNA was incubated with gene 32-protein (50:50% mixture of ^{35}S-labeled P32 and P32* with equal specific radioactivity) for 10 min at 37°C. Incubation buffer: 20 mM Tris–HCl, pH 8.5, 1 mM Na_3EDTA and varying NaCl concentrations. In some cases the complex was fixed with 0.1% glutaraldehyde for 10 min at 37°C. DNA–protein complexes were purified on a Sepharose-2B column [8]. The samples were spread by adsorption to positively charged carbon films [9], stained for 10 sec with uranyl acetate (2% aqueous solution), dried by blotting on filter paper, and rotary shadowed with Pt at an angle of 8-10°. fig. 3) protein: DNA = 160:4 µg/ml, 4 mM NaCl, complex unfixed. Fig. 4) protein:DNA = 100:10 µg/ml, 9 mM NaCl, glutaraldehyde fixed complex. Fig. 5) protein:DNA = 160:4 µg/ml, 4 mM NaCl, fixed complex. Fig. 6) protein:DNA = 160:4 µg/ml, 100 mM NaCl; in this case incubation was for 20 min at 37°C, glutaraldehyde fixed. Magnification 1–6: × 40 000. The → points to native DNA regions.

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