Electron microscopic study of DNA complexes with proteins from the Archaeobacterium *Sulfolobus acidocaldarius*

R.Lurz, M.Grote, J.Dijk, R.Reinhardt and B.Dobrinski

Max-Planck-Institut für molekulare Genetik, Ihnestr. 73, D-1000 Berlin 33, FRG

Communicated by W.Zillig

DNA–protein complexes formed *in vitro* with isolated DNA-binding proteins from the thermoacidophilic archaeobacterium *Sulfolobus acidocaldarius* were analyzed by electron microscopy. Two of the proteins (10a and 10b) formed specific structures after incubation with DNA. Protein 10a, at low protein concentrations, showed individual small spots on the DNA and at high concentrations evenly covered double-stranded DNA. Protein 10b showed three different types of regular structures: one with single-stranded and two with double-stranded DNA. Using double-stranded DNA, 10b first bound cooperatively to two strands forming long, plait-like structures only slightly shorter than respective free DNA. The complex consists of two right-handed, interwound fibers, with a helical pitch of 26 nm and a diameter of ~10–11 nm. At higher protein concentration than needed to package all DNA into the complex with two double-stranded DNAs, the two DNAs were separated again and a new structure was formed evenly covering only one DNA strand. Both structures showed no significant contraction of the length of the DNA covered in the complex. Nucleoprotein formed with single-stranded ϕ X174 DNA had a diameter of ~11 nm and could form circles with a contour length of 0.5 μ m.

Key words: archaeobacteria/DNA-binding protein/DNA packaging/helix/nucleoprotein

Introduction

The divergence between eubacteria, archaeobacteria and eukaryotes is reflected in the mechanisms for the packaging of their DNAs. The chromatin structure of the latter is now established in reasonable detail. The basic structure is that of the nucleosome in which two turns of DNA are wound around an octamer composed of four histones (Richmond *et al.*, 1984).

A search for histone-like proteins in bacteria has revealed several candidates. However, their role in DNA folding is not yet understood, largely because details of the structure of bacterial chromatin remain so far unclear (Pettijohn, 1982).

In *Escherichia coli* three proteins have been identified as potential DNA-binding proteins, their mol. wts were determined to be 10 000, 17 000 and 30 000, respectively (Pettijohn, 1982). One of them, protein HU or DNA-binding protein II, has been investigated in considerable detail, and homologous proteins from other eubacteria have also been characterised (Laine *et al.*, 1983). The three-dimensional structure of the respective protein from the thermophile *Bacillus stearothermophilus* has been solved (Tanaka *et al.*, 1984). A model for DNA binding has been proposed in which DNA coils around a protein core resembling one half nucleosome in eukaryotes (Broyles and Pettijohn, 1986). In electron micrographs of complexes of the protein with viral DNA

nucleosome-like structures have been observed (Rouvière-Yaniv *et al.*, 1979).

Little is known about DNA-binding proteins of archaeobacterial DNA. In the thermoacidophile *Thermoplasma acidophilum* a single protein has been found which is homologous to the *E. coli* DNA-binding protein II (DeLange *et al.*, 1981). From a methanogen, *Methanosarcina barkeri*, a DNA-binding protein of unknown relationship with a mol. wt of 14 000 has been isolated (Chartier *et al.*, 1985). The possible diversity between the different archaeobacteria is demonstrated by the results of an analysis of bacterial chromatin (Thomm *et al.*, 1981).

The thermoacidophile *Sulfolobus acidocaldarius* has several DNA-binding proteins of similar size, with mol. wts of 7000 (7a–e), 8000 (8a and 8b) and 10 000 (10a and 10b), respectively (Grote *et al.*, 1986). We have analyzed, by electron microscopy, the nucleoprotein complexes formed *in vitro*. The fine structure of the complexes involving protein 10b and two double strands of DNA has been investigated in detail.

Results

DNA-binding properties of 7- and 8-kd proteins

After incubation with double-stranded (ds) plasmid DNA and preparation for electron microscopy by adsorption to mica, the 7-kd and 8-kd proteins showed very similar structures. At low protein:DNA ratios (3:1, w/w), clusters of bound protein on the DNA could be observed (Figure 1a). With increasing protein:DNA ratios, the single clumps on the DNA were compacted more and more into one central protein–DNA cluster surrounded by loops of free DNA (Figure 1b). At protein:DNA ratios of 10:1 or higher, free DNA was no longer visible, and aggregation of the single clusters occurred, resulting in conglomerates of >200 nm diameter. In mixed preparations of single-stranded (ss) and dsDNA the proteins bound to both DNAs. As with dsDNA only clumped structures were found using ssDNA.

Binding of protein 10a

At low protein:DNA ratios (1:1) with ds-DNA, small protein spots of distinct size could be detected on circular plasmid molecules (Figure 1c, arrows). Increasing protein:DNA ratios (up to 5:1) first showed only unspecific clumping of the nucleoprotein (Figure 1c,d). The DNA was saturated with protein around ratios of 6:1. Under these conditions, the irregular clumping pattern had changed and the plasmids were evenly covered by the protein (Figure 1f). Binding to ss-DNA occurred unspecifically distributed on the whole molecule, and at higher protein concentrations compact clumps were formed (Figure 1e).

Covalently closed circular (ccc) DNA molecules were heavily supertwisted after binding of protein 10a. Mica-adsorbed samples showed the two interwound strands as one compact structure (Figure 1f). After negative staining the two strands could be resolved better (Figure 1h). No fine structure details could be resolved within the nucleoprotein, indicating that protein 10a molecules may not be arranged in a higher ordered structure around the DNA.

Fig. 1. Electron micrographs of nucleoprotein formed with *Sulfolobus* proteins 7 and 10a. Preparation of the complexes for electron microscopy by adsorption to mice (**a–g**). (**a**) Protein 7c incubated with plasmid DNA (ratio 3:1, Sepharose 4B). (**b**) At higher protein concentration bigger clumps are formed. Some of the complexes are already aggregated. One complexed plasmid does not show free DNA any more (protein 7d, ratio 6:1, Sepharose 4B). (**c**) Protein 10a incubated with plasmid DNA (ratio 1:1). The arrows indicate small protein dots on the DNA. (**d**) Nucleoprotein formed at a protein:DNA ratio of 3:1. (**e**) Incubation of ssDNA with protein 10a (ratio 3:1). The insert shows two compact complexes at a ratio of 6:1. (**f**) Two supertwisted and one relaxed plasmid molecule, evenly covered with protein 10a (ratio 6:1). (**g**) Same preparation as in (**f**) but the complexes were not fixed. Most of the bound protein is lost. The typical high twisting of cccDNA and lateral aggregation of parts of ocDNA within the complexes is still preserved. (**h**) Negative staining (1% uranyl acetate) of one ccc plasmid molecule covered with protein 10a (ratio 6:1). Magnifications: **a–g**: 50 000 $\times$; **h**: 160 000 $\times$.

Within relaxed open circular (oc) molecules some tendency to lateral aggregation of the protein-covered DNA strands could be observed (Figure 1f). Length measurement of 51 oc molecules gave a mean contour length of $2.897 \pm 0.043 \mu\text{m}$; plasmids prepared in parallel without bound protein were $2.887 \pm 0.031 \mu\text{m}$ ($n=53$). Unfixed complexes prepared by adsorption to mice lost most of their proteins, but the typical structure of the complexes – extreme twisting of cccDNA and lateral aggregation within ocDNA – was preserved (Figure 1g).

Nucleoprotein structures formed with protein 10b

Protein 10b in solution is a dimer (Grote *et al.*, 1986). In negatively stained preparations of the protein without DNA added, we could find no indications for aggregation into larger struc-

tures. After incubation with DNA, protein 10b formed three different specific structures: two with ds- and one with ss-DNA.

Protein 10b interaction with two DNA double strands

Using plasmid DNA, protein 10b first formed its most unique structure by enveloping two strands of dsDNA into a linear, flexible structure (Figure 2a,b). Binding seemed to be initiated at one or a few positions on plasmid DNA. Once such a complex was initiated, it was elongated by addition of protein 10b to already bound protein rather than by random binding to the DNA. Outside these structures no binding of the proteins to the DNA was visible. This type of complex formation is typical for cooperative interaction of the proteins. We could also find plasmid molecules without bound protein in samples having most of the

Fig. 2. Electron microscopy of protein 10b-DNA complexes. Samples in **a-f** and **o-r** were prepared by adsorption to mica. **(a)** Protein 10b complexes formed with plasmid DNA (ratio 2:1). Two strands of DNA are covered by the protein. At the ends of some of the complexed regions (arrows), no loops of free DNA are visible because the incubation buffer (buffer A) did not contain magnesium ions. Free DNA still has the typical shape of ccc- and ocDNA prepared with the mica adsorption technique (adsorption buffer: buffer B without KCl). **(b)** Most of the 10b-2 × ds complexes formed in the presence of magnesium (buffer B, ratio 3.5:1) show loops of free DNA at their ends. A diagonal striation of the complexed regions is already visible in this preparation. **(c)** 10b-DNA complexes formed at a protein:DNA ratio of 7:1. Protein 10b forms complexes with one DNA double strand. One supertwisted and two open circular molecules can be easily distinguished. One oc molecule still shows a region of the 10b-2 × ds complex (arrows). **(d)** Preparation as in **c** but the complexes were not fixed with glutaraldehyde. The complexes had lost much of the bound proteins. Arrowheads indicate one region of 10b-2 × ds. **(e)** Unfixed complexes at a protein:DNA ratio of 50:1. **(f)** Treatment with DNase I (ratio 7:1), 10b-1 × ds complexes are cut into a few fragments. A region showing 10b-2 × ds is indicated by arrowheads. **(g)** Part of a negatively stained oc 10b-1 × ds complex (ratio 7:1, fixed, 1% uranyl acetate). **(h)** Prepared as in **(g)**, but not fixed. The DNA has lost part of the bound proteins which are visible as dots in the background. **(i)** Negatively stained (2% uranyl acetate) 10b-2 × dsDNA complex (ratio 3.5:1). Arrowheads indicate the two protein helices. **(k)** Negative staining as in **(i)** with 2% uranyl formate. The stain layer is very thin; therefore, the complex structure was not fully stabilized after adsorption. **(l)** Negative staining (1% uranyl acetate) of complexes formed with ssDNA (ratio 5:1). **(m)** Unidirectionally and **(n)** bidirectionally tungsten-shadowed freeze-dried samples of 10b-2 × ds complexes (ratio 3.5:1). **(o)** Typical preparation of ϕ X174 ssDNA in buffer B after adsorption to mica. The DNA is collapsed into spider-like structures. **(p)** Protein 10b bound at a ratio of 3.5:1 to ssDNA. The protein bound irregularly to the DNA. **(q)** Partially regular structures formed at a ratio of 6:1. **(r)** One ϕ X174 DNA molecule shows a perfect circle. At this high protein concentration the complexes tend to aggregate (ratio 8:1). Magnifications: **a-f**, **o-r**: 40 000×; **g-l**: 128 000×; **m**, **n**: 96 000×.

Fig. 3. Length measurements of free DNA and partially complexed molecules. Free DNA and plasmids showing the $10b-2 \times ds$ complexes were measured within one preparation to minimize systematic errors. In partially complexed molecules between 50 and 60% of the DNA length were covered by protein 10b. These regions contained two DNA strands; therefore, their length was measured twice. The mean length of free DNA molecules was $2.83 (\pm 0.048) \mu m$, that of the molecules partially covered with protein was $2.70 (\pm 0.047) \mu m$.

DNA complexed in this $10b-2 \times ds$ complex. However, cooperativity seems to be not as pronounced as for T4 gene 32 protein (Delius *et al.*, 1972) or fd protein 5 (Alberts *et al.*, 1972). An indication for an initiation complex as reported for PM2 protein IV (Marcoli *et al.*, 1979) could not be detected in our samples.

These $10b-2 \times ds$ complexes were formed identically using ccc, oc or linear DNA as substrates. Discrimination between ccc and ocDNA was only possible when less than $\sim 50\%$ of the DNA was covered with the protein. When more DNA has formed this complex, free DNA loops of ccc molecules no longer exhibited typically the superhelical cross-overs in the electron microscope (Figure 2a,b). Incubation of covalently closed relaxed DNA with protein 10b induced no supertwists after formation of the nucleoprotein (data not shown). The observation that complex formation, using either ccc or relaxed covalently closed DNA, did not introduce supertwists to the DNA outside the complexed regions, suggests that supertwists are not induced and this complex may even compensate superhelical turns within its structure. The energy needed to form supertwists in free DNA should be higher than to compensate this superhelical stress within the $10d-2 \times ds$ structure, if the complexed region of a plasmid is not too short.

DNA at the ends of the complexed regions could be bent in such a way that no loops of free DNA were visible anymore (Figure 2a, arrows). When magnesium ions were present in the incubation buffer, more structures showed a loop of free DNA at their ends (Figure 2b).

Length measurements of plasmid molecules in which 50–60% of the DNA was covered by the protein revealed a small but significant reduction of the mean length by 4.5%, compared with free DNA in the same preparation (Figure 3). This value corresponds to a contraction of $\sim 8\%$ in complexed regions.

Protein 10b complexes with one DNA double strand

When more protein was added than needed to package all the DNA into the $10b-2 \times ds$ complex (ratio $> 5:1$), the two strands were gradually opened again and a new type of nucleoprotein was formed containing only one dsDNA ($10b-1 \times ds$; Figure 2c). This happened only after DNA was fully complexed in the $10b-2 \times ds$ structure. Complexes formed with ocDNA could be completely unfolded, while cccDNA remained super-twisted and could be again easily distinguished from ocDNA. This also demonstrates that the protein preparation did not nick the DNA. When more protein was added up to a maximum ratio of 50:1, the structures formed did not change significantly (Figure 2e). The contour length of complexed ocDNA ($2.781 \pm 0.028 \mu m$, $n = 66$) was identical within the error of measurement to that of plasmids prepared in parallel ($2.815 \pm 0.023 \mu m$, $n = 60$).

Binding of protein 10b to ssDNA

Protein 10b, when incubated with ds- and ssDNA, bound to both DNAs, like protein 10a. However, in contrast to the other *Sulfolobus* DNA-binding proteins which showed only irregular clumping using ssDNA, 10b was able to form well defined regular structures. While dsDNA exhibited a cooperative formation of the complex, with protein bound only within the regular structures, ssDNA, at low protein:DNA ratios, showed random binding with many small irregular protein clusters to $\phi X174$ DNA (Figure 2 o,p). With increasing protein:DNA ratios, more and longer structures of a regular type was present (Figure 2q). At protein:DNA ratios of $> 6:1$ partially circular nucleoprotein structures were formed, but the single complexes were more and more entangled into aggregates (Figure 2r). The contour length of full circles was measured to $0.493 \pm 0.007 \mu m$ ($n = 48$). This represents a 3.4-fold shortening of the complexes compared with the length of $\phi X174$ DNA (assuming the same length for ss- and dsDNA).

Stability of the 10b–DNA complexes

Nucleoprotein incubated at $75^\circ C$, the normal growth temperature of this *Sulfolobus* strain, showed the same three typical complexes with protein 10b as at $37^\circ C$. The lower standard temperature was chosen for easier handling of the samples and the quality of electron microscopical preparations were in general better when temperature differences were not too extreme. $10b$ –DNA complexes were formed at pH values between 3 and 9. They were stable up to 200 mM KCl in the incubation buffer. At salt concentrations of 300 mM and more only DNA without bound proteins was detected.

The $10b-2 \times ds$ complex showed identical structures after preparation for electron microscopy in fixed and unfixed preparations. $10b-1 \times ds$ complexes, like nucleoprotein formed with protein 10a, had lost part of their proteins after adsorption to mice or a carbon film (Figures 2d,h). Negatively stained samples showed free protein in the background (Figure 2h). Similar observations that adsorption to a carbon film had disrupted a nucleoprotein structure were reported for phage PM2 protein IV complexes (Marcoli *et al.*, 1979). Unfixed samples of protein 10b with dsDNA showed no aggregation of the complexes, even when highly oversaturated up to a ratio of 50:1. The complexes at this high protein concentration had more protein bound than at lower concentrations and looked more like fixed structures. Possibly adsorption of free protein partially saturated the binding capacity of the adsorption surface and the unfixed complexes remained better preserved.

We have tested the resistance of the protein–DNA complexes against nucleolytic attack in order to clarify whether or not the DNA is protected by the proteins. After 30 min incubation with DNase I, free DNA was completely degraded and could no longer be detected in the electron microscope. These drastic conditions cut all free DNA in partially complexed molecules, but the 10b–2 × ds structures were still present and had about the same size as in an untreated preparation. Nucleoprotein formed at higher 10b–DNA ratios showed few cuts per DNA molecule in 10b–1 × ds regions (Figure 2f). Very long incubation times (e.g. overnight) caused gradual degradation of both protein–DNA complexes.

Fine structure analysis of protein 10b–DNA complexes

Fine structure analysis was focused on 10b–2 × ds complexes, as the most characteristic nucleoprotein structure formed with this protein. Some indications for a diagonal striation could already be seen in some preparations of the complexes adsorbed to mica and shadowed with platinum–iridium (Figure 2b). To obtain more detailed information on the fine structure of 10b–2 × ds complexes, we used tungsten shadowing of freeze-dried samples, and negative staining with uranyl acetate or uranyl formate.

In negatively stained samples free DNA was invisible; however, the protein–DNA complex displayed structural details especially of the protein part, not visualized by shadowing techniques. Two interwound fibers formed plait-like structures which were more clearly visible when the structure of the complex was opened due to flattening caused by a very thin stain layer (Figure 2i,k).

Freeze-dried samples may be expected to warrant better preservation of the fine structure as compared with air-dried samples; tungsten shadowing using an electron gun is known to give higher resolution of details than adsorption of the particles to mica, combined with platinum–iridium shadowing. After freeze-drying and unidirectional or bidirectional shadowing, principal structural details of the complex and free DNA were imaged as with mica adsorption. However, as expected, the striation was clearly resolved (Figure 2m,n), indicating a right-handed helical structure. Each of the two DNA strands appeared to enter the complex and was found to be associated with one protein fiber (Figure 2n).

After negative staining the diameter of the complexes with dsDNA was measured to be between 10 and 11 nm. Freeze-dried samples bidirectionally shadowed with tungsten exhibited a diameter of the complex of 13 nm. The single protein helix fiber could only be measured in negatively stained preparations; it showed a diameter of ~4.5 nm. The repeating distance of the striation along the length axis of the complex was very constant, varying between 12.5 and 13.5 nm in negatively stained, freeze-dried or mica-adsorbed complexes. All these data indicate two right-handed interwound helices showing 180° phasing, with a helical pitch of 26 nm.

Negatively stained complexes with ssDNA (Figure 2l) had about the same diameter as observed with 10b–2 × ds. The 10b–1 × ds complexes appeared to be slightly thinner. Both structures showed the typical pattern of helically arranged subunits after negative staining (Figure 2g,i) as known from many biological structures like bacterial flagella, pili or phage tails. The structural details were finer than those of the two helices of the 10b–2 × ds complex and could not be visualized using shadowing techniques.

Fig. 4. Model drawing of one helical turn of the 10b–2 × ds complex.

Discussion

The 7-kd DNA-binding proteins from the thermoacidophilic archaeobacterium *S. acidocaldarius* are a very homogeneous group. The protein sequences differ in at most three amino acids, and they are the most abundant DNA-binding proteins isolated from this bacterium. Protein 8a and 8b show no sequence homologies to these proteins (Dijk *et al.*, 1986). All 7-kd and 8-kd DNA-binding proteins formed complexes with DNA which appeared as compact structures in the electron microscope.

With protein 10a at high ratios, structures are obtained that are similar to an unfixed structure found by Rouvière-Yaniv *et al.* (1979) with *E. coli* protein HU. However we could not demonstrate the formation of nucleosome-like structures in any of our preparations. We cannot exclude that this failure to visualize a higher ordered structure in the electron microscope may be due either to sub-optimal reconstitution conditions or to the fact that the preparation method was not suited to stabilize a – presumably – very labile structure as is known for eubacterial chromatin (Broyles and Pettijohn, 1986).

Protein 10b differs significantly from the other proteins that we have isolated from *S. acidocaldarius*. It is characterized by its ability to form three different well defined nucleoprotein structures. We have analyzed the interaction of 10b with two DNA double strands in greater detail and found that the complex is made by two right handed, interwound helices. The model drawn in Figure 4 shows one helical turn of this structure and summarizes the data obtained from electron microscope analysis.

The second structure formed with protein 10b covers only one DNA double strand and is only made after oversaturating the 10b–2 × ds structure. As in complexes with protein 10a, formation of 10b–1 × ds does not influence the contour length of the DNA. In contrast to protein 10a, where no fine structure could be detected in the nucleoprotein, protein 10b shows the typical

pattern of helically arranged subunits in complexes with one dsDNA and with ssDNA. The arrangement of ssDNA within the complex is still unknown, but it should be in a regular, possibly helical order because the length of the nucleoprotein is very constant 3.4 times shorter than that of corresponding ϕ X174 DNA.

Similar structures as those formed with 10b and dsDNA are found with phage PM2 protein IV (Marcoli *et al.*, 1979). Like protein 10b, dimers of this protein can form cooperatively linear complexes with two double strands of DNA, without influencing the superhelicity of protruding DNA loops. However, protein IV predominantly complexes four double strands in ccc molecules and complexes with two or three double strands were interpreted as intermediary stages of the complex formation. We never observed more than two DNA strands in 10b–DNA complexes. About a 5-fold contraction of the length of ds-DNA was found within regions complexed with PM2 protein IV, whereas the complexes with protein 10b showed no or <10% contraction of dsDNA.

One protein forming several different helically ordered structures with ss- and dsDNA, is RecA protein from *E. coli*. In the presence of adenosine 5'- γ -thiotriphosphate, RecA protein interacts with gapped dsDNA providing fibers ~ 10 nm thick (Stasiak *et al.*, 1981). RecA–ssDNA complexes have been described to form two different structures with helical pitches of 5.5 or 9.3 nm depending on the absence or presence of ATP (Williams and Spengler, 1986), respectively. Gene 5 protein from fd phage and a similar protein from *Pseudomonas* phage Pf1 show complexes containing two anti-parallel single strands in a single helical structure (Gray *et al.*, 1982). The diameters in these two examples, are ~ 10 and 11 nm, and the helical pitches are ~ 10 and 6 nm. Gene 5 protein is also reported to cover dsRNA uniformly forming an 8–9 nm thick filament (Gray *et al.*, 1984).

The path of the DNA within the 10b–2 $\times$ ds complex shown in Figure 4 could not be visualized directly in the electron microscope. From length measurements of the partially complexed plasmids we know that the complexed regions are only slightly shorter than expected for free DNA, and that the complexed DNA is protected against DNase attack. Any extensive wrapping of the DNA around a protein core, as known for nucleosomes (Finch *et al.*, 1977), the gyrase–DNA complex (Liu and Wang, 1978), or as suggested for eubacterial chromatin (Tanaka *et al.*, 1984; Broyles and Pettijohn, 1986) can, therefore, be excluded.

We might consider two possibilities for the configuration of the DNA within the complex.

(i) Assuming a configuration of the DNA in the complexed regions identical to that in solution, the DNA cannot follow the two protein helices. Because an interwinding of the two DNAs together with the two helices would produce topological stress to the DNA, the small measured reduction in length allows only slight curving of the DNA in the complexed region. In this model, the length reduction may also represent superhelical turns from ccc-plasmid molecules hidden in the complex structure which are, as we have shown, no longer present in the protein-free part of the plasmid DNAs. The DNAs in the complexed regions have to be two linear strands parallel to the long axis of the complex. DNA and protein helix can interact only within a limited region of one protein helix fiber. With each half turn (13 nm), every DNA strand has to be in contact with the alternating protein fiber.

(ii) The DNA can follow the two helices without changing the linking number, if the DNA configuration is changed in a way which elongates the DNA and introduces positive supertwists. The increase of length cannot be calculated, because we do not

have the exact localization of the DNA in the complex structure. The helical repeat of the DNA helix has to be changed from 10.4 bp in solution (Wang, 1979) to ~ 12 bp in order to compensate positive superhelical turns which are introduced forming the two interwound helices of the complex. Elongation of dsDNA from 0.34 to 0.52 nm distance per base pair was found after interaction of RecA protein with gapped plasmid DNA (Stasiak *et al.*, 1981; see above). In the RecA system, loops of free DNA, locked by complex formation, were superhelically twisted. In our system compensation of superhelical twist seems to be favoured between the two helices of the complex, because neither negatively supertwisted nor covalently closed relaxed plasmid DNAs showed supertwists within loops of free DNA.

Although our present data do not allow us to discriminate between the two models, we are favouring the second one. The two stable helices of the complex as seen after negative staining suggesting a strong continuous interaction of protein 10b with either of the DNA double strands, which is not possible in the first model. Also, compensation of superhelical turns in the complex structure can be easier achieved in the second model.

The information on the functional role of protein 10b in the cells is limited. Some preliminary experiments using plasmid RSF1010 DNA did not show any positive or negative effects in experiments of *in vitro* replication or of transformation with amounts of protein 10b which completely protected the DNA against restriction enzyme digestion (E.Scherzinger, personal communication).

Protein 10b is one of the most abundant DNA-binding proteins isolated from this strain, only the 7-kd proteins were found in higher quantities (Grote *et al.*, 1986). The specific structures formed with protein 10b do not support the idea that protein 10b is primarily a packaging protein in *Sulfolobus* chromatin. One main purpose of chromatin structures is to condense the long chromosomal DNA into a compact and ordered structure, e.g. by wrapping the DNA around the nucleosome, and the additional formation of higher ordered superstructures as in eukaryotic chromatin (Thoma *et al.*, 1979; Woodcock *et al.*, 1984). Under the conditions which we have used, the structures formed by 10b protein, do not compact the DNA in the way one would expect for chromatin. The nucleoprotein complexes formed with protein 10b show some relationship to complexes made by RecA. In addition to other structural functions, protein 10b might play a role similar to that of RecA in recombination/repair process of *Sulfolobus*.

Materials and methods

Double-stranded plasmid RSF1010 (8.7 kb; Haring *et al.*, 1985) and single-stranded ϕ X174 DNA (5386 bases; Sanger *et al.*, 1978) were a gift from E.Scherzinger. Growth conditions of *S. acidocaldarius* (DSM 1616) and isolation of the proteins were as described (Grote *et al.*, 1986).

Formation of the DNA–protein complexes

Unless otherwise outlined, the standard procedure was the following: 30 ng DNA were incubated with variable amounts of protein for 15 min at 37°C in 6 μ l buffer A [50 mM KCl, 2.5 mM dithiothreitol (DTT), 0.5 mM EDTA, 10 mM TEA-Cl, pH 7.5]. 1% glutaraldehyde in buffer A was added to a final concentration of 0.2% to fix the complexes for 15 min. In some experiments unbound protein was removed by gel filtration using a small Sepharose 4B–C1 column equilibrated with mica adsorption buffer B (5 mM MgCl₂, 50 mM KCl, 1 mM DTT, 10 mM TEA-Cl, pH 7.0).

For DNase I treatment the samples were incubated in buffer B for 15 min, DNase I (Boehringer, Mannheim) was added to a final concentration of 50 μ g/ml and incubated for another 30 min. Then glutaraldehyde was added and preparation for mica adsorption was done as described.

Electron microscopy

Preparation of the fixed DNA–protein complexes for electron microscopy by

adsorption to mica was done essentially as described by Portmann and Koller (1976). The fixed nucleoprotein samples were diluted 6-fold into buffer B. Adsorption time was 1 min, followed by 1 min staining in 2% aqueous uranyl acetate, washed twice on water and air-dried. Rotary shadowing was done with platinum-iridium (80:20) at an angle of $\sim 8^\circ$.

For freeze-drying followed by high resolution shadowing a carbon film was floated from mica onto the solution containing the complexes. After 1 min adsorption time the film was collected from the surface using a 600 mesh grid, and washed twice for 10 s on distilled water, blotted almost dry and rapidly frozen in melting nitrogen which was solidified using a vacuum pump. The grids were fixed on a holder and introduced into a modified EPA 100 (Leybold-Heraeus) via an air lock. The holder was left without touching the contact surface of the cooling ring for ~ 2 h until a temperature of -85°C and a vacuum of $\sim 10^{-4}$ Pa were reached. The holder was cooled again to -120°C , and the samples were shadowed uni- or bidirectionally with tungsten at an angle of 20° (Tesche and Schmiady, 1985). The amount of evaporated tungsten was monitored using a quartz oscillator.

Negative staining was performed with aqueous uranyl acetate or uranyl formate as described by Valentine *et al.* (1968).

Electron micrographs were made with a Philips EM 400 on Agfa Scientia 23D56 6.5×9 cm sheets or RA 710 P 35 mm film (Agfa-Gevaert, Belgium). Contour lengths were measured on 10 or 16 times enlarged original negatives using an electronic digitizer (LM4, Brühl, Nürnberg).

Acknowledgements

We thank Frank Mayer and Walter Messer for helpful discussions, and Laura Wiener for comments on the manuscript.

References

- Alberts, B., Frey, L. and Delius, H. (1972) *J. Mol. Biol.*, **68**, 139–152.
 Broyles, S.S. and Pettijohn, D.E. (1986) *J. Mol. Biol.*, **187**, 47–60.
 Chartier, F., Laine, B., Sautière, P., Toukel, J.-P. and Albagnac, G. (1985) *FEBS Lett.*, **183**, 119–123.
 DeLange, R.J., Greene, G.R. and Searcy, D.G. (1981) *J. Biol. Chem.*, **256**, 905–911.
 Delius, H., Mantell, N.J. and Alberts, B. (1972) *J. Mol. Biol.*, **67**, 341–350.
 Dijk, J., Davie, P., Grote, M., Ashman, K. and Reinhardt, R. (1986) *Eur. J. Biochem.* in press.
 Finch, J.D., Lutter, L.C., Rhodes, D., Brown, R.S., Rushton, B., Lewitt, M. and Klug, A. (1977) *Nature*, **269**, 29–36.
 Green, G.R., Searcy, D.G. and DeLange, R.J. (1983) *Biochim. Biophys. Acta*, **741**, 251–257.
 Gray, C.W., Kneale, G.G., Leonard, K.R., Siegrist, H. and Marvin, D.A. (1982) *Virology*, **116**, 40–52.
 Gray, C.W., Page, G.A. and Gray, D.M. (1984) *J. Mol. Biol.*, **175**, 553–559.
 Grote, M., Dijk, J. and Reinhardt, D. (1986) *Biochim. Biophys. Acta*, **873**, 405–413.
 Haring, V., Scholz, P., Scherzinger, E., Frey, J., Derbyshire, K., Hatfull, G., Willetts, N.S. and Bagdasarian, M. (1985) *Proc. Natl. Acad. Sci. USA*, **82**, 6090–6094.
 Laine, B., Bèlaiche, D., Khanaka, H. and Sautière, P. (1983) *Eur. J. Biochem.*, **131**, 325–331.
 Liu, L.F. and Wang, J.C. (1978) *Cell*, **15**, 979–984.
 Marcoli, R., Pirotta, V. and Franklin, R.M. (1979) *J. Mol. Biol.*, **131**, 107–131.
 Pettijohn, D.E. (1982) *Cell*, **30**, 667–669.
 Portmann, L. and Koller, B. (1976) In Ben-Shaul, Y. (ed.), *Sixth European Congress of Electron Microscopy Jerusalem*. Tal, Israel, Vol. 2, pp. 546–448.
 Richmond, T.J., Finch, J.T., Rushton, B., Rhodes, D. and Klug, A. (1984) *Nature*, **311**, 532–537.
 Rouvière-Yaniv, J. (1974) *Eur. J. Biol.*, **71**, 439–447.
 Rouvière-Yaniv, J., Yaniv, M. and Germond, J.F. (1979) *Cell*, **17**, 254–274.
 Sanger, F., Coulson, A.R., Friedmann, T., Air, G.M., Barell, B.G., Brown, N.L., Fiddes, J.C., Hutchison, C.A., III, Slocumbe, P.M. and Smith, M. (1978) *J. Mol. Biol.*, **125**, 225–246.
 Stasiak, A., DiCapua, E. and Koller, Th. (1981) *J. Mol. Biol.*, **151**, 557–564.
 Tesche, B. and Schmiady, H. (1985) *Ultramicroscopy*, **16**, 423–435.
 Thoma, F., Koller, Th. and Klug, A. (1979) *J. Cell. Biol.*, **83**, 403–427.
 Thomm, M., Stetter, K.O. and Zillig, W. (1982) *Zbl. Bakt. Hyg., I. Abt. Orig.*, **C3**, 123–139.
 Valentine, R.C., Shapiro, B.M. and Stadtman, E.R. (1968) *Biochemistry*, **7**, 2143–2152.
 Wang, J.C. (1979) *Proc. Natl. Acad. Sci. USA*, **76**, 200–203.
 Williams, R.C. and Spengler, S.J. (1986) *J. Mol. Biol.*, **187**, 109–118.
 Woodcock, C.L.F., Frado, L.-L.Y. and Rattner, J.B. (1984) *J. Cell Biol.*, **9**, 42–52.

Received on 18 April 1986; revised on 22 September 1986