JOURNAL OF STRUCTURAL BIOLOGY **116,** 390–398 (1996)
ARTICLE NO. 0056

The Interaction of DNA with Bacteriophage ϕ29 Connector: A Study by AFM and TEM

MIKEL VALLE, JOSE MARIA VALPUESTA, AND JOSE L. CARRASCOSA[1]

Centro Nacional de Biotecnología, CSIC, Universidad Autónoma de Madrid, Cantoblanco, 28049 Madrid, Spain

AND

JAVIER TAMAYO AND RICARDO GARCIA

Centro Nacional de Microelectrónica, CSIC, Serrano 144, 28006 Madrid, Spain

Received January 9, 1996, and in revised form February 19, 1996

The connector of bacteriophage ϕ29 is involved in DNA packaging during viral morphogenesis and we have studied its *in vitro* binding to DNA using either linear or circular DNA. The protein–DNA complexes have been analyzed by transmission electron microscopy (TEM) and by atomic force microscopy (AFM) of samples directly deposited on mica. TEM showed the presence of a specific binding due to the interaction of the protein with the free ends of the DNA. The study of these samples by AFM showed two major types of morphologies: The interaction of the connector with circular DNA revealed that the strands of DNA that enter and exit the protein complex form an angle with a mean value of 132°. Nevertheless, when the connector was incubated with linear DNA (and later circularized), there was an additional bend angle of about 168°. Further morphological analysis of the latter samples by AFM revealed a structure of the protein–DNA complex consistent with the DNA traversing the connector, probably through the inner channel. On the other hand, images from the samples obtained by incubation of the connector with circular DNA were consistent with an interaction of the DNA with the outer side of the connector.

INTRODUCTION

The packaging of DNA into viral heads is a complex process that has been studied extensively over recent years, not only because it is pivotal for understanding the morphogenesis of complex double-stranded DNA bacteriophages, but as a model system to study DNA translocation by a protein complex (for recent reviews, see Casjens and Hendrix, 1988; Black, 1989; Becker and Murialdo, 1990).

Among the different aspects that have emerged from these studies, it has become quite clear that the connector (or portal protein) plays a key role in the bacteriophage packaging process, as no DNA packaging has ever been detected in proheads without a connector. There is strong evidence that supports the involvement of the connector in both the selection and translocation of DNA (reviewed by Valpuesta and Carrascosa, 1994) as well as in the mechanism that controls the length of the DNA to be packaged (Casjens *et al.,* 1992; Tavares *et al.,* 1992). In particular, in the case of phage ϕ29 there is genetic evidence that the connector protein (p10) is involved in the determination of the prolate head size as well as in the DNA packaging (Camacho *et al.,* 1977; Guo *et al.,* 1992). More direct, biochemical data were provided by chemical modification of the connectors assembled into proheads that made them unable to package DNA (Herranz *et al.,* 1990) and by the use of hybryd λ/ϕ29 proheads, in which the connectors were modified either chemically (Donate and Carrascosa, 1991) or by mutagenesis (Donate *et al.,* 1992) with a concomitant loss of DNA binding and packaging.

The structure of connectors from different bacteriophages is quite similar (for a review see Valpuesta and Carrascosa, 1994): They are multimeric aggregates that are built up by several copies (12 or 13) of a single protein. The connector is located in the head-to-tail connecting region of the virion and possesses a rotational symmetry with two clearly defined domains: a wider one (around 14 nm in diameter), with a lobulated aspect, while the other is narrower and cylindrical (9 nm in diameter).

[1] To whom correspondence should be addressed. Fax: 34-1-5854506; E-mail: jlcarrascosa@samba.cnb.uam.es.

1047-8477/96 $18.00

Three-dimensional reconstructions of the connectors assembled from isolated proteins from phages ϕ29 and T3 have shown that there is a channel that runs along the longitudinal axis of the particle, with a diameter around 4 nm. On the other hand, the connectors extracted from viral particles that had packaged their DNA showed the channel closed. Based on these data, it has been proposed that the channel is the actual place where the linear DNA is translocated into the viral head during the packaging process.

The basic steps in the interaction of the DNA with the connector have been studied using defined *in vitro* systems with purified components. In this way, the region of the ϕ29 connector that is involved in DNA binding has been located in the first eight amino acids of the amino-terminal region of the protein by proteolysis experiments and site-directed mutagenesis (Donate *et al.,* 1992). Additionally, this region has been located in the tip of the narrow cylindrical region of the connector aggregate using low-angle X-ray diffraction experiments (Herranz *et al.,* 1990). This region is located opposite to the side where the prohead attaches to the connector and which presumably should interact first with the incoming DNA.

There are several hypothesis on how the DNA interacts with ϕ29 connectors. Based on biochemical experiments of prevention of degradation of either protein or DNA upon interaction, Valpuesta *et al.* (1992b) have proposed that there is an interaction of the DNA in the outer part of the connector structure, together with an inner binding that would take place inside the channel of the connector. The different interaction of either linear or circular DNA with the connector was supported by results obtained by fluorescence studies (Urbaneja *et al.,* 1994). A clear decrease of the intrinsic tryptophan fluorescence was associated to the specific binding of linear DNA to the connector. An important consequence of these studies was the evidence that this interaction induces structural changes both in the connector (also reflected in the destabilization of the connector structure) and in the DNA (restraining negative supercoiling, Valpuesta *et al.,* 1992b), thus making unlikely packaging models based on the existence of a rigid structural match between the DNA and the connector oligomer (Dube *et al.,* 1993).

Taken together, the experimental results obtained so far strongly suggest that the binding of linear DNA by the connector has distinctive features from those promoted by circular DNA, namely, the induction of a conformational change in the ϕ29 connector upon binding of linear DNA. Nevertheless, there is no direct structural proof that the DNA is actually interacting with the inner channel of the connector. On the other hand, because circular DNA binds *in vitro* to the ϕ29 connector, it has been suggested that the substrate for DNA packaging is supercoiled DNA, whose interaction with the outer surface of the connector would lead to the wrapping of this DNA around the connector dodecamer (Turnquist *et al.,* 1992).

In an effort to further analyze the structural basis of the interaction between ϕ29 connector and linear or circular DNA, we have studied complexes formed *in vitro* by transmission electron microscopy (TEM) and atomic force microscopy (AFM). The combination of the well-established TEM for the quantification of the complex formation and the higher resolution given by scanning probe microscopies has been previously used to study DNA/protein complexes (Müller *et al.,* 1994). We have chosen AFM, instead of tunneling mycroscopy, due to its higher resolution for imaging DNA, as no coating of the sample is needed (Hansma *et al.,* 1992; Rees *et al.,* 1993). Direct visualization of the complexes by AFM has allowed us to obtain new structural information that supports two clear specific interaction modes between the DNA and the connector: one takes place in the outside, while the other requires linear DNA and probably takes place in the channel inside the connector.

MATERIALS AND METHODS

Complex Formation

Phage connectors were obtained from *Escherichia coli* transformed with a plasmid harboring the gene coding for the bacteriophage ϕ29 connector protein p10 and purified following previously published procedures (Ibáñez *et al.,* 1984: Carrascosa *et al.,* 1985). The complexes of the ϕ29 connector with linear DNA were obtained by incubation of 350 μg/ml connector protein with 100 μg/ml DNA (plasmid pSGT41, 4500 base pairs (bp), previously linearized by treatment with *Bam*HI endonuclease), at a 10 to 1 approximate molar ratio. The incubation was carried out for 15 min at room temperature, in a buffer containing 50 m*M* Tris–HCl, pH 7.5, 30 m*M* $MgCl_2$, 60 m*M* NaCl, 10 m*M* ATP, and 50 μg/ml bovine serum albumin. Reannealing of the DNA was obtained by treatment with 50 units of T4 ligase for 6 hr at room temperature. Complexes of connectors with circular DNA were obtained following an identical procedure, except that the plasmid DNA was previously circularized before interaction with the connectors.

Proteolysis of the Connectors Complexed with DNA

After the formation of the connector/DNA complex, the DNA was relaxed by treatment with 10 units of Topoisomerase I, in the presence of 50 m*M* KCl. Endoproteinase Glu-C from *S. aureus* V8 (V8 protease) was added up to 6 μg/ml and incubated at 37°C. Samples were subjected to polyacrylamide gel electrophoresis to check for the characterized pattern of V8-treated p10 (Donate *et al.,* 1992). The reaction was then stopped with 100 μ*M* dichloroisocumarin.

Purification of the Protein/DNA Complexes

Samples containing the proteolyzed connector/DNA complexes were further purified to remove the unbound connectors and the by-products of the different reactions. As the isolated connectors

interact strongly with phophocellulose (Carrascosa *et al.,* 1985), ion exchange columns in pipette tips were run in 50 m*M* Tris, pH 7.7, and 5% glycerol to separate the complexes with DNA (eluting at 0–0.2 *M* NaCl) from the free connectors (eluting at 0.6 *M* NaCl).

Sample Preparation for Transmission Electron Microscopy (TEM)

The purified connector/DNA complexes were diluted in 50 m*M* Tris, pH 7.5, and 20 m*M* $MgCl_2$ up to a DNA concentration around 2 μg/ml. Then the samples were adsorbed to mica during 1 min and then thoroughly washed with milliQ water, using three washes, 1 hr each. After ethanol dehydration and air drying, the mica adsorbed samples were shadowed at a 4° angle with a layer of platinum/carbon (7 nm thick) and further stabilized by a 10-nm-thick carbon layer evaporated on top, at 90° with respect to the plane of the sample, using a Balzers 400T unit. The replicas were then released from the mica and collected with grids to allow their analysis in a JEOL 1200 EX-II electron microscope. The images used for statistical analysis were obtained from three different experiments, and from each one different grids were used to assure the representativity of the sample. From each experiment, a confidence interval test was applied using 95 and 99% values. In every case, the results for complexes with linear and circular DNA, respectively, showed that there was no interval overlapping, indicating that the preparations under study were different with a confidence, at least, of 99%.

Sample Preparation for Atomic Force Microscopy (AFM)

The purified connector/DNA complexes were diluted in the same buffer as for TEM, but in this case up to a concentration of DNA around 5 μg/ml. A 10-μl drop of the solution was deposited onto a freshly cleaved mica substrate. The complexes were allowed to adsorb for 1 min, rinsed with milliQ water, and then dried in a stream of N_2 gas. Imaging was performed by operating the AFM in the tapping mode with a Nanoscope III (Digital Instruments, Inc.). This mode minimizes the lateral forces between the biomolecule and the probe, and it also reduces the contact time (100–500 nsec in these experiments). All images were taken in air with silicon cantilevers of resonance frequencies about 300 kHz and using free amplitudes of about 100 nm. To measure the angular distribution of DNA strands in the complexes, we used two different approaches: (a) determination of the angle built up by the incoming DNA strand and the exiting strand ($\alpha \leq 180°$) and (b) measurement of the angle determined by the lines joining the center of the complex with those points where the DNA strands exit the outside of the complex. In both cases, the profile of the distributions and their main characteristics were identical.

RESULTS

TEM Studies on the Connector/DNA Complex

The interaction of ϕ29 connectors with either linear or circular DNA was studied using purified connectors assembled from overexpressed p10 and a pSGT41 plasmid DNA. The DNA was linearized before incubation with the protein in the experiment, while the control to study the interaction of the connector with circular DNA was done by circularization of the linear DNA before the incubation with the protein, to ensure that both DNAs had gone through the same experimental handling. Furthermore, as the analysis of the DNA/protein complexes by electrophoresis showed a heterogeneous pattern of supercoiling after circularization (not shown), the DNA was relaxed with Topoisomerase I under conditions that rendered an identical band pattern after electrophoresis of control and experimental samples, to avoid differences in the behavior of the complexes due to this factor.

Samples were treated with V8 protease after the formation of the complexes to avoid further contacts of the connector with the DNA that could eventually lead to secondary (nongenuine) complexes. The results in Fig. 1 show that most of the connector protein is fully cleaved, giving rise to a pattern similar to that described previously that involves the removal of the amino termini of p10, in which the DNA binding domain of this protein is included (Donate *et al.,* 1992). In this way, only those interactions that are protected by stable contacts (either by threading of the DNA or by a long loop interaction) could be permanently maintained.

To further avoid nonspecific contacts between proteolyzed connectors and DNA, samples were extensively purified by ion exchange chromatography that retained free connectors, thus rendering clean protein/DNA complexes without free contaminants. These samples were then shadowed and visualized by TEM, showing images such as those found in Fig. 2. Under these purification conditions, most of the DNAs had no protein, and those containing protein did not show more than one oligomer in each molecule, probably due to the cleavage of the connectors and the extensive cleaning of the samples. In the case of the incubation of connectors with linear DNA, of a total of 6000 circularized molecules coming from four different experiments, only 166 were found to contain a protein oligomer, building a well-defined complex. In the control experiment, in which the connectors were incubated with circular DNA, only 41 of 6000 molecules were found to contain protein. The difference found between the frequencies

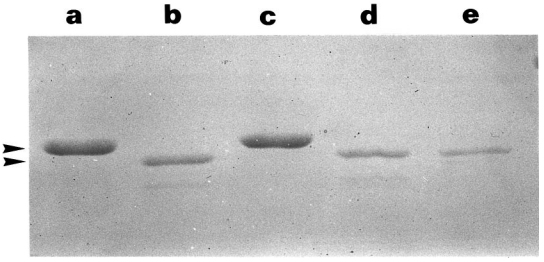

FIG. 1. Proteolysis of ϕ29 connector protein (p10) by V8 protease. Complexes of connectors with either circular DNA (a,b) or linear DNA that was subsequently circularized (c,d) were treated with V8 protease. Samples before (a,c) and after (b,d) the protease incubation were subjected to SDS–polyacrylamide gel electrophoresis and stained with Coomassie blue. (e) A control of free connectors treated with the V8 protease under the same conditions. Arrowheads mark the position of native and digested p10. No traces of nondigested protein were present under the conditions used in these experiments.

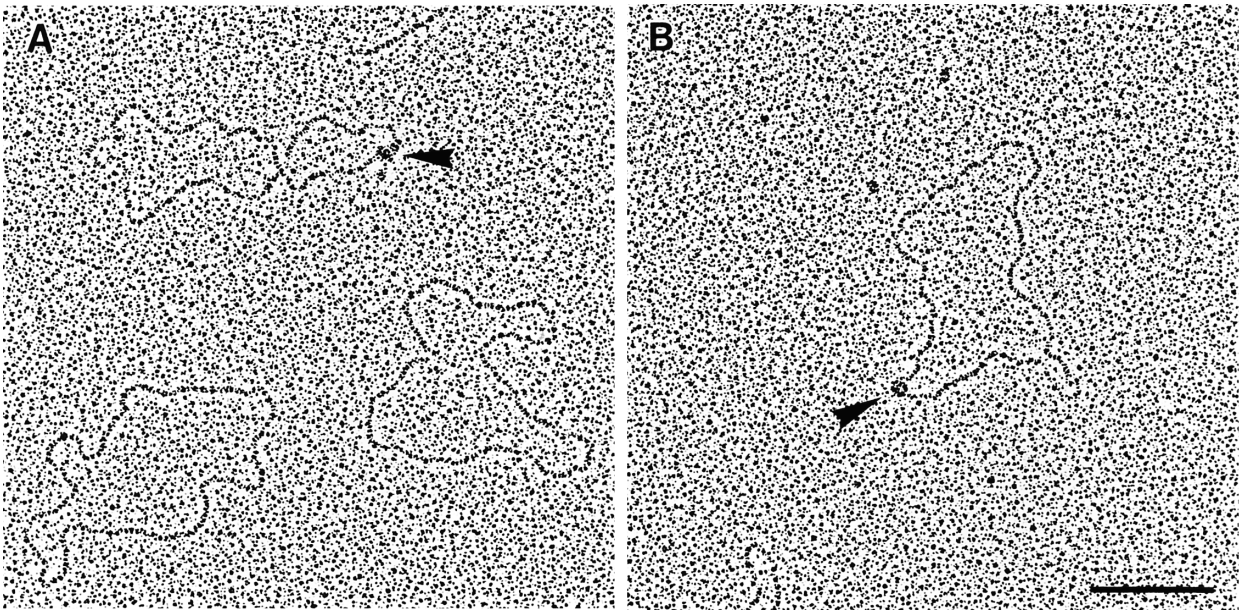

FIG. 2. TEM images of ϕ29 connector/DNA complexes adsorbed to mica and shadowed with platinum/carbon. (A) Complex formed with circular DNA. (B) Complex formed with linear DNA that was later circularized. Arrowheads point to connector complexed with DNA. Bar, 200 nm.

of the complexes respectively formed with linear and circular DNA is significant up to a 99% interval, thus suggesting that the interaction of the linear DNA with the connector forms complexes that are not generated by interaction with circular DNA.

AFM Studies on the Connector/DNA Complex

Two representative images from complexes formed by interaction of protein with either linear or circular DNA are shown in Fig. 3. The DNA was

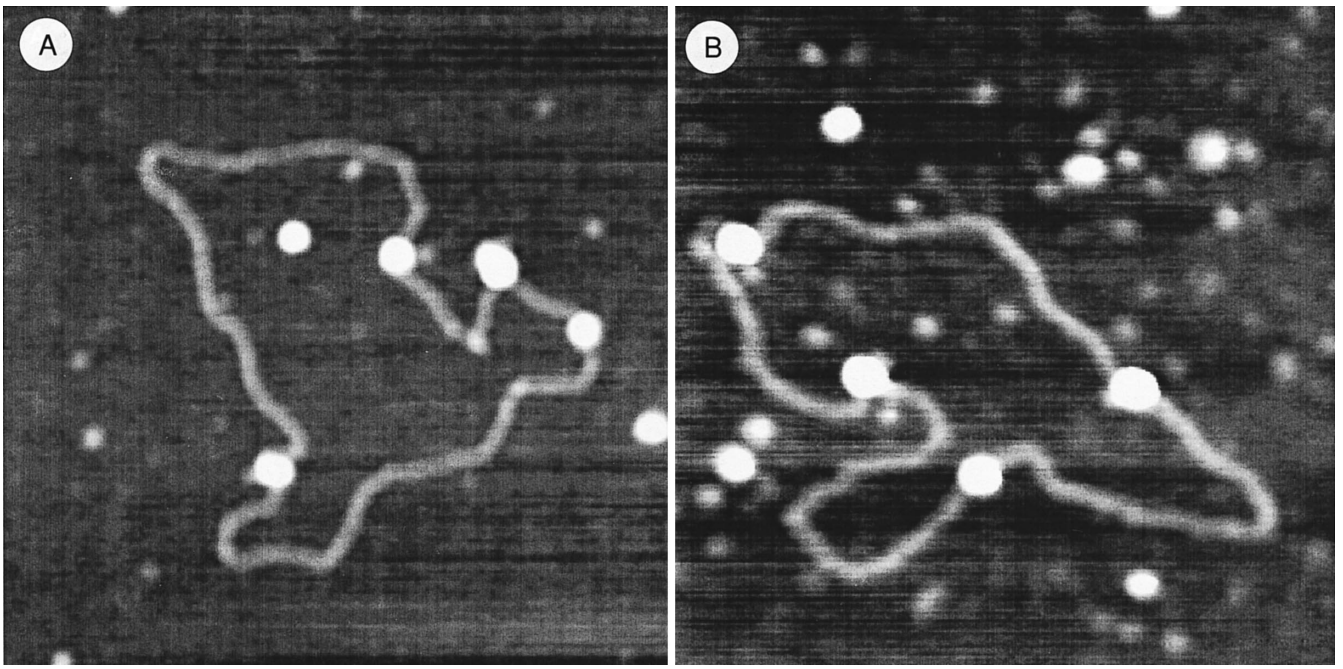

FIG. 3. AFM images (top view representation) of DNA/connector complexes. The connector appears as rounded aggregates. In each image, four connectors are interacting with the DNA. The differences in height between the connectors and the DNA makes it difficult to present simultaneously both molecules with a similar contrast. (A) Complexes formed with circular DNA; scansize, 606 × 606 nm^2. (B) Complexes formed with linear DNA (and later circularized); scansize, 595 × 595 nm^2.

visualized as a well-contrasted continuous ribbon, while the connectors appeared as rounded aggregates. The differences in height between the DNA and the connector makes it difficult to present both in the same image with similar contrast. The lateral dimensions of the biomolecules obtained from AFM images tend to be a factor 3 to 6 times larger than the nominal one depending essentially of two factors: (a) An AFM image has always to be interpreted as a convolution between the geometry of the object and that of the tip (Keller and Franke, 1993), thus strongly depending on the size and shape of the tip. (b) The intrinsic elastic properties of different materials in one sample should be reflected in different signals in the corrugation map. However, these limitations do not affect the identification of biomolecules or the determination of bend angles in protein/DNA complexes (Rees *et al.,* 1993).

The high signal to noise ratio of AFM images made possible to perform a qualitative analysis of the morphology of these complexes. In the case of the complexes made with circular DNA, the DNA strands seem to build a bend around the connector complex. The images suggest that the DNA seems to partially embrace the connector with a geometry compatible with an outer contact (Figs. 4A–4C). On the other hand, the histogram representing the complexes made with linear DNA and later circularized shows more complex features. In addition to a morphology similar to the one described above, there is a new type, where DNA strands seem to traverse the connector complex (fig. 4D–4F).

To investigate the relevance of the morphologies associated to these complexes, we performed a statistical analysis on the angles formed between the strand entering into the protein domain and the DNA strand exiting the complex. The observed angles for the case of the complexes formed with circular DNA (Fig. 5A) can be fitted into a Gaussian distribution with a mean value of 132°. This distribution could be interpreted as DNA bent around the protein domain (Figs. 4A–4C). Nevertheless, when complexes are formed with the linear DNA and then circularized, the angle distribution is more complex and can be best fitted using a bimodal distribution (Fig. 5B): There is a broad peak centered around 106°, which is consistent with a DNA bent around the protein, while the other peak is centered around 168°. This peak is consistent with the DNA traversing the connector structure (Figs. 4D–4F).

DISCUSSION

The interaction of phage connectors with DNA has been studied *in vitro* using different approaches (Herranz *et al.,* 1986; Feiss and Anderson, 1991). In the case of phage ϕ29 connector, there is biochemical

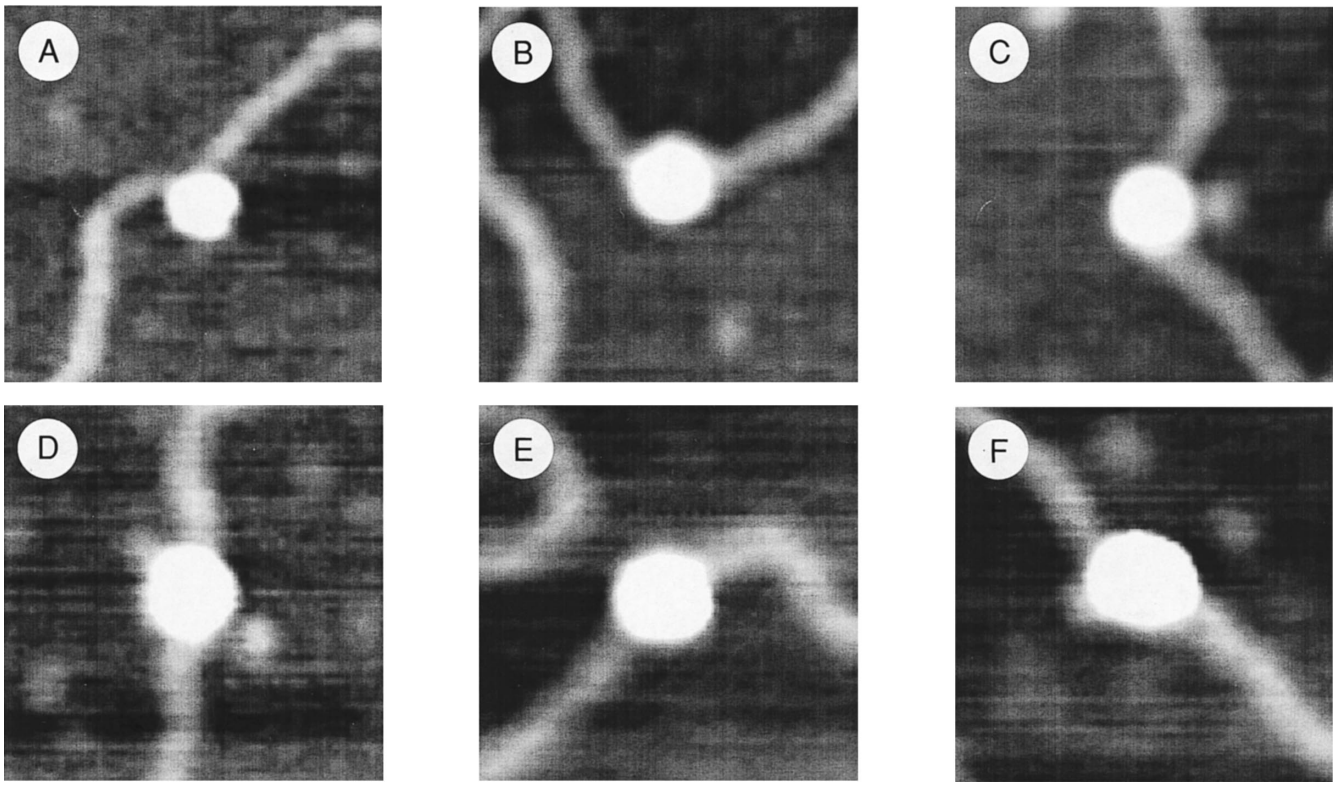

FIG. 4. Representative AFM images of the different morphologies shown by the DNA/connector complexes. (A–C) Complexes formed with circular DNA. (D–F) Complexes formed with linear DNA (and later circularized). Scansize, 150 nm.

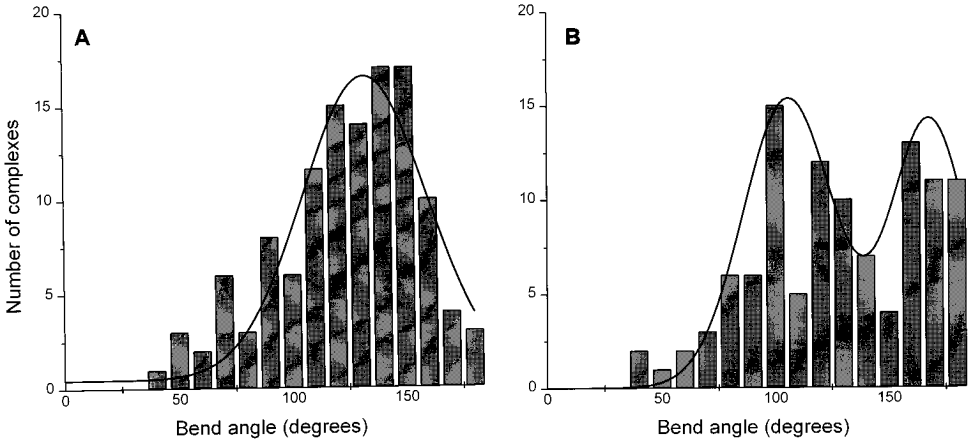

FIG. 5. Statistical analysis of the distribution of bend angles. The solid line represents a Gaussian fitting. (A) Histogram obtained from images of the complexes formed by the interaction of circular DNA with the connector. The mean bend angle is 132°. (B) Histogram of the interaction of linear DNA and connectors. Two Gaussian distributions are used to fit the data revealing mean bend angles of 106° and 168°, respectively. The comparison of both histograms suggests that there is a specific complex where the DNA goes through the connector channel (bend angle of 170°). This morphology only appears when the connector interacts with linear DNA.

evidence that the connector is better protected from proteolysis by incubation with double-stranded DNA than with other types of DNA (single-stranded or circular). The connector also protects a fragment around 60 bp of DNA from DNase digestion when it is incubated with linear, double-stranded DNA, and this binding restrains negative supercoiling as measured by circularization assays (Valpuesta *et al.,* 1992b). These data have been interpreted as indicative of an interaction of the DNA with the inner channel of the connector. On the other hand, an alternative model based on STEM measurements has been suggested (Turnquist *et al.,* 1992), in which supercoiled DNA interacts with the outer surface of the connector. To try to further clarify these conflicting models, we have studied the interaction of the ϕ29 connector with either linear or circular DNA, using two imaging techniques: the well-established TEM, which allows a quantification of the complexes formed with each type of DNA, and the new AFM, which provides direct images of the protein/DNA complexes with a high signal to noise ratio, thus allowing us to draw their low-resolution morphology.

To analyze the diferential interaction of the connector with either linear or circular DNA, an experiment was designed as shown in Fig. 6 (left side): Plasmid DNA was linearized by a restriction endonuclease and then allowed to interact with purified connectors. Several interacting possibilities are suggested in the figure: (a) Interaction involving the outer surface of the DNA binding domain (outer binding in Valpuesta *et al.,* 1992); (b) wrapping around the outer surface (similar to the coiling in the connector surface proposed by Turnquist *et al.,* 1992), and (c) threading along the inner channel (inner binding in Valpuesta *et al.,* 1992). After circularization of the DNA with ligase, those weak interactions (represented by single, nonspecific contacts or those that are only transient) were removed using extensive washing of the samples and the removal of the DNA binding domain of the connector by prote-

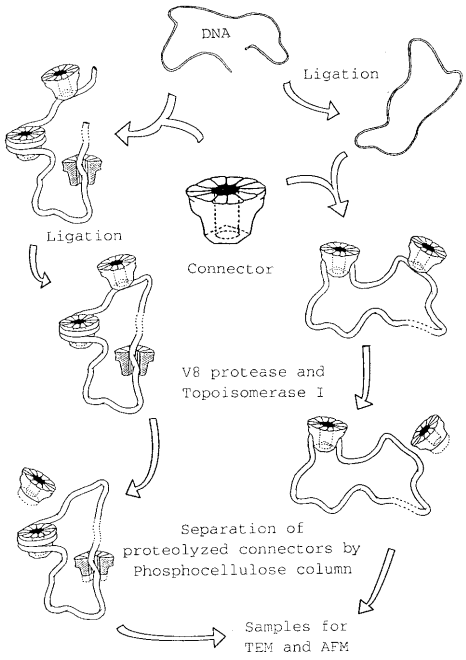

FIG. 6. Outline of the experiments carried out to produce complexes of ϕ29 connectors with either linear DNA (left) or circular DNA (right). An interpretation of the results is also drawn in the figure. Dashed areas in the connector represent the proposed location of the amino-terminal domain of p10 that is released upon V8 proteolysis (Herranz *et al.,* 1990 ; Donate *et al.,* 1992). Dashed lines in the DNA represent out of scale length.

olysis. The removal of the DNA binding domain of the connector inhibits completely the packaging of the DNA into proheads, thus preventing the binding of DNA *in vitro* by the proteolyzed connectors (Donate *et al.,* 1992). Therefore, after this treatment, only those stable interactions involving strong contacting areas (derived from the wrapped DNA) and those derived from the threading of the DNA along the internal channel would survive as a protein/DNA complex. As a control (Fig. 6, right side), the linear DNA was circularized first and then allowed to interact with the purified connectors. The rest of the experiment was performed in parallel with the other sample, including a relaxation of the circular DNA to avoid any interference due to differential supercoiling in the two types of samples. This way, the difference between the two samples should be related to the specific interaction of the connector with the free ends in the linear DNA (i.e., the threading of the DNA along the inner channel in Fig. 4). The results obtained by TEM showed four times more complexes in the case of the connectors interacting with linear DNA than with circular DNA, thus indicating that the presence of open ends in the DNA allows a type of interaction with the connector that is not present in the case of circular DNA.

As the images from TEM of platinum/carbon-shadowed DNA/protein complexes are not clear enough to define their structure (even to a moderate resolution), mostly due to the uneven corrugation given by the layer of platinum/carbon needed to contrast the sample, we studied the connector/DNA complexes by AFM. This technique has been used to produce images of nucleic acids and their complexes with proteins with a high signal to noise ratio and without any coating material (Hansma *et al.,* 1992; Rees *et al.,* 1993). AFM images from connector/DNA complexes allow to define the overall geometry of the DNA interacting with the protein domain. Complexes prepared with circular DNA show morphologies that support the bending of the DNA around the connector. The mean bend angle of 132° could be derived from either a half-turn interaction (as in Figs. 4B and 4C) or one turn and a half (Fig. 4A). On the other hand, the complexes prepared with linear DNA show a more complex aspect with, at least, two different morphologies: One has a broad distribution with a mean bend angle around 106°, with an aspect very similar to that found for the complexes obtained with circular DNA. The difference between this mean bend angle and that found for complexes obtained with circular DNA (132°) could thus probably be related to the different bending behavior of circular closed DNA with respect to linear DNA. The second morphology shows an angle between the strands around 168°. In fact, this value is probably an underestimation, as values over 180° have been accumulated under this figure in the representation. Then, an almost straight DNA path could be characteristic of this type of complex, consistent with the DNA traversing the connector (Figs. 4D–4F). As the complexes were treated with proteases to remove the DNA binding domain of the connector, this interaction should be mainly due to a threading of the DNA inside the channel of the connector that was later stabilized by the circularization of the DNA.

The results obtained so far by TEM and AFM of the complexes built up by ϕ29 connectors and DNA are consistent with two types of stable interactions: One is probably due to an outer contact of the DNA with the surface of the connector. The other suggests a threading of the DNA along the inner channel of the connector, and it only appears with linear DNA. This latter structure is probably quite relevant *in vivo* because mature ϕ29 DNA is linear and double-stranded. Furthermore, it has been described that the source of DNA to be packaged both *in vivo* and *in vitro* in the case of ϕ29 is the linear DNA. Circular DNA has never been successfully used as a substrate for DNA packaging in this system (Bjornsti *et al.,* 1983; Guo *et al.,* 1991; Donate and Carrascosa, 1991).

Our data support a model in which the interaction between the connector and a linear DNA (similar to the one used as a substrate for viral DNA packaging) gives rise to a specific complex with the DNA traversing the channel that runs along the connector structure. This interpretation is consistent with the protection of DNA fragments around 60 bp from digestion by nucleases in the DNA/ϕ29 connector complexes (Valpuesta *et al.,* 1992), as well as in the acidic hydrolysis of T4, T7, and ϕ29 viral particles, in which a DNA fragment around 40 bp is also protected (Zachary and Black, 1992). Additional information comes from data showing that the interaction of linear DNA with the connector promotes a conformational change in the DNA that restrains negative supercoiling (Valpuesta *et al.,* 1992b), thus making unlikely those packaging models based on a rigid geometry fit between DNA and the connector aggregate (Dube *et al.,* 1993). Also supporting this model are the results obtained by fluorescence studies of the connector/DNA interaction (Urbaneja *et al.,* 1994). The interaction of linear DNA with the ϕ29 connector leads to a decrease in its intrinsic tryptophan fluorescence. This decrease is not observed either when the DNA is incubated with connectors lacking the DNA binding domain or when the DNA is circular. In addition, interaction of the connectors with linear DNA (but not with circular DNA) lowers the denaturant agent concentration re-

quired to denature the connector structure. Taken together, these results strongly suggest that the binding of linear DNA by the connector has distinctive features than those promoted by circular DNA, namely, the induction of a conformational change both in the DNA and in the ϕ29 connector upon binding of linear DNA.

As stated above, the DNA that is a substrate for the packaging reaction in ϕ29, both *in vivo* and *in vitro,* has been shown to be linear and double-stranded (Bjornsti *et al.,* 1983; Guo *et al.,* 1991; Donate and Carrascosa, 1991). Thus, besides those features described above, the interaction of this type of DNA with the connector channel would also account for the main structural change that has been observed in this particle: while the channel is fully open in the connector before assembly in the phage head (Carazo *et al.,* 1986; Donate *et al.,* 1988; Valpuesta *et al.,* 1992a), it is closed in the connectors already assembled that have packaged DNA inside the viral heads (Carazo *et al.,* 1985; Donate *et al.,* 1988). The opening and closing of the channel would be coupled in some way to the possibility of DNA translocation (open state) and the stabilization of the DNA inside the viral head (closed state) (Carrascosa *et al.,* 1990).

The complete structural characterization of the DNA/connector complex would demand higher-resolution studies, either by improved AFM methods (even in an aqueous environment) or by TEM. To this end, production of two-dimensional aggregates of connector/DNA complexes are under way that would allow us to use image processing techniques to obtain much higher structural details relevant to the protein/DNA interaction.

This work was partially supported by Grants PB91-0109 to J.L.C. and PB94-0016 to R.G. from the Dirección General de Investigación Científica y Técnica and by Specific Action on Protein Structure grants from the CSIC (1994, 1995) to J.L.C. and R.G. M.V. is a recipient of Fellowship BF193 110 from the Gobierno Vasco.

REFERENCES

Becker, A., and Murialdo, H. (1990) Bacteriophage lambda DNA: The beginning of the end, *J. Bacteriol.* **172,** 2819–2824.

Bjornsti, M. A., Reilly, B. E., and Anderson, D. (1983) Morphogenesis of bacteriophage ϕ29 of *Bacillus subtilis:* Oriented and quantized *in vitro* packaging of DNA protein gp3, *J. Virol.* **45,** 383–396.

Black, L. W. (1989) DNA packaging in dsDNA bacteriophages, *Annu. Rev. Microbiol.* **43,** 267–292.

Camacho, A., Jimenez, F., de la Torre, J., Carrascosa, J. L., Mellado, R. P., Vásquez, C., Viñuela, E., and Salas, M. (1977) Assembly of *Bacillus subtilis* phage ϕ29. I. Mutants in the cistrons coding for the structural proteins, *Eur. J. Biochem.* **73,** 39–55.

Carazo, J. M., Santisteban, A., and Carrascosa, J. L. (1985) Three-dimensional reconstruction of bacteriophage ϕ29 neck particles at 2.2 nm resolution, *J. Mol. Biol.* **183,** 79–88.

Carazo, J. M., Donate, L. E., Herranz, L., Secilla, J. P., and Carrascosa, J. L. (1986) Three-dimensional reconstruction of the bacteriophage ϕ29 at 1.8 nm resolution, *J. Mol. Biol.* **192,** 853–867.

Carrascosa, J. L., Carazo, J. M., Ibáñez, C., and Santisteban, A. (1985) Structure of phage ϕ29 connector protein assembled *in vivo, Virology* **141,** 190–200.

Carrascosa, J. L., Carazo, J. M., Herranz, L., Donate, L. E., and Secilla, J. P. (1990) Study of two related configurations of the neck of bacteriophage ϕ29, *Computers Math. Appl.* **20,** 57–69.

Casjens, S., and Hendrix, R. W. (1988) Control mechanisms in dsDNA bacteriophage assembly, *in* Calendar, R. (Ed.), The Bacteriophages, Vol. 1 pp. 15–90, Plenum; New York.

Casjens, S., Wyckoff, E., Hayden, M., Sampson, L., Eppler, K., Randall, S., Moreno, E. T., and Serwer, P. (1992) Bacteriophage P22 portal protein is part of the gauge that regulates packaging density of intravirion DNA, *J. Mol. Biol.* **224,** 1055–1074.

Donate, L. E., Herranz, L., Secilla, J. P., Carazo, J. M., Fujisawa, H., and Carrascosa, J. L. (1988) Bacteriophage T3 connector: Three-dimensional structure and comparison with other viral head-tail connecting regions, *J. Mol. Biol.* **201,** 91–100.

Donate, L. E., and Carrascosa, J. L. (1991) Characterization of a versatile *in vitro* DNA-packaging system based on hybrid lambda/ϕ29 proheads, *Virology* **182,** 534–544.

Donate, L. E., Valpuesta, J. M., Rocher, A., Méndez, E., Rojo, F., Salas, M., and Carrascosa, J. L. (1992) Role of the amino-terminal domain of bacteriophage ϕ29 connector in DNA binding and packaging, *J. Biol. Chem.* **267,** 10919–10924.

Dube, P., Tavares, P., Lurz, R., and van Heel, M. (1993) The portal protein of bacteriophage SPP1: a DNA pump with 13-fold symmetry, *EMBO J.* **12,** 1303–1309.

Feiss, M., and Anderson, D. (1991) Capsid caper in Cable (meeting review), *New Biol.* **3,** 1048–1054.

Guo, P., Erickson, S., Xu, W., Olson, N., Backer, T., and Anderson, D. L. (1991) Regulation of the phage ϕ29 prohead shape and size by the portal vertex, *Virology* **183,** 366–373.

Hansma, H. G., Vesenka, Y., Siegerist, C., Kelderman, G., Horret, H., Sinsheimer, R. L., Elings, V., Bustamante, C., and Hansma, P. K. (1992) Reproducible imaging and dissection of plasmid DNA under liquid with the Atomic Force Microscope, *Science* **256,** 1180–1184.

Herranz, L., Salas, M., and Carrascosa, J. L. (1986) Interaction of the bacteriophage connector protein with the viral DNA, *Virology* **155,** 289–292.

Herranz, L., Bordas, J., Towns-Andrews, E., Méndez, E., Usobiaga, P., and Carrascosa, J. L. (1990) Conformational changes in bacteriophage ϕ29 connector prevents DNA binding activity, *J. Mol. Biol.* **213,** 263–273.

Ibáñez, C., García, J. A., Carrascosa, J. L., and Salas, M. (1984) Overproduction and purification of the connector protein of *Bacillus subtilis* phage ϕ29, *Nucleic Acids Res.* **12,** 2351–2365.

Keller, D., and Franke, F. (1993) Envelope reconstruction of probe microscope images, *Surface Sci.* **294,** 409–419.

Müller, T., Gilson, E., Schmidt, R., Giraldo, R., Sogo, J., Gross, H., and Gasser, S. M. (1994) Imaging the asymmetrical DNA bend induced by Repressor Activator Protein 1 with Scanning Tunneling Microscopy, *J. Struct. Biol.* **113,** 1–12.

Rees, W. A., Keller, R. W., Vesenka, J. P., Guoliang, Y., and Bustamante, C. (1993) Evidence of a DNA bending in transcription complexes imaged by Scanning Force Microscopy, *Science* **260,** 1646–1649.

Tavares, P., Santos, M. A., Lurz, R., Morelli, G., de Lencastre, H., and Trautner, T. A. (1992) Identification of a gene in *Bacillus subtilis* bacteriophage SPP1 determining the amount of packaged DNA, *J. Mol. Biol.* **225,** 81–92.

Turnquist, S., Simon, M., Egelman, E., and Anderson, D. A. (1992) Supercoiled DNA wraps around the bacteriophage ϕ29 head-tail connector, *Proc. Natl. Acad. Sci. USA* **89,** 10479–10483.

Valpuesta, J. M., Fujisawa, H., Marco, S., Carazo, J. M., and Carrascosa, J. L. (1992a) Three-dimensional structure of T3 connector purified from overexpressing bacteria, *J. Mol. Biol.* **224,** 103–112.

Valpuesta, J. M., Serrano, M., Donate, L. E., Herranz, L., and Carrascosa, J. L. (1992b) DNA conformational change induced by the bacteriophage ϕ29 connector, *Nucleic Acids Res.* **20,** 5549–5554.

Valpuesta, J. M., and Carrascosa, J. L. (1994) Structure of viral connectors and their function in bacteriophage assembly and DNA packaging, *Q. Rev. Biophys.* **27,** 107–155.

Zachary, A., and Black, L. W. (1992) Isolation and characterization of a portal protein-DNA complex from dsDNA bacteriophage, *Intervirology* **3,** 6–16.