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Preliminary Molecular Replacement Results for a Crystalline Gene 5 Protein–Deoxyoligonucleotide Complex

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Complexes of the gene 5 protein from bacteriophage fd with a variety of oligodeoxynucleotides, ranging in length from two to eight and comprised of several different sequences, have been formed and crystallized for X-ray diffraction analysis. The crystallographic parameters of four different unit cells, all of which are based on hexagonal packing arrangements, indicate that the fundamental unit of the complex is composed of six gene 5 protein dimers. We believe this aggregate has 622 point group symmetry and is a ring formed by end-to-end closure of a linear array of six dimers. From our results we have proposed a double-helix model for the gene 5 protein–DNA complex in which the protein forms a spindle or core around which the DNA is spooled. Currently 5.0-Å X-ray diffraction data from one of the crystalline complexes is being analyzed by molecular replacement techniques to obtain a direct image of the protein–nucleic acid complex.

Key words: protein-nucleic acid interactions, X-ray diffraction, gene 5 protein, molecular replacement, DNA, fd bacteriophage

Determination of the structure of a complex between a DNA binding protein and fragments of nucleic acid by X-ray diffraction analysis promises substantial insight into the means by which these two important macromolecules interact. In addition to delineating the precise atomic interactions by which they recognize and bind to one another, knowledge of such a structure could potentially clarify many of the mechanisms by which the flow of genetic information is controlled. In the case of the DNA unwinding protein, which we discuss here, we believe considerable information may also be gained concerning the assembly and general architectural features of large protein-nucleic acid structures such as are found in chromosomal material and viruses.

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The gene 5 protein from fd bacteriophage has proved to be a particularly useful system for such studies since it can be crystallized as a monomer in the absence of nucleic acid [1]. Its complexes with oligonucleotides and native fd phage DNA can be studied by electron microscopy and a host of other physicochemical techniques. Finally, single crystals of complexes between the gene 5 protein and nucleic acid can be grown. The gene 5 protein is rather small (87 amino acids totaling 9,800 daltons [2]), and its structure in the uncomplexed state is presently known from the single-crystal X-ray diffraction analysis we describe in the accompanying paper. It is our intention to use the structure of the uncomplexed protein to determine the structure in the single crystals of protein-DNA complexes that we have grown and now have characterized by diffraction techniques.

The gene 5 protein–DNA complexes produced in vitro as visualized by electron microscopy are unique in that two protein-covered strands coalesce to yield a helical rod-like structure in which there are 12 gene 5 monomers per turn of the helix. The helix has a width of approximately 100 Å and a longitudinal repeat of about 80 Å [3]. The gene 5 protein–DNA complexes resemble mature filamentous bacteriophage virions though there are clear differences. The mature virus is formed by the displacement of the gene 5 protein at, or in, the host cell membrane by the coat protein, the product of gene 8 [4]. The gene 5 protein is never found in the virion but is returned to the cell for reuse.

In vitro complexes of the gene 5 protein with fd phage DNA have been reported to differ in structure from complexes isolated directly from infected cells. These in vivo complexes were observed to be composed of fibers 40 Å in width that were supercoiled to give an overall width of 160 Å and a longitudinal repeat of 160 Å [3]. More recent electron microscopy studies by Grey [5], however, find the in vivo and in vitro complexes to be identical and to resemble the helical rods described above. One difference has been noted between the two complexes: the stoichiometry of binding from presumably saturated in vitro complexes is one gene 5 monomer per four nucleotides while the in vivo complexes tend to give nonintegral values of approximately 4.6 nucleotides per gene 5 monomer [6].

The gene 5 protein–DNA complexes are considerably different from those of the helix-destabilizing protein from phage T4, the gene 32 protein, when visualized by electron microscopy [2]. These latter complexes, like those of the calf thymus DNA unwinding protein [7], form open ring structures under identical conditions.

There is evidence from cross-linking studies in solution that when gene 5 protein is combined with deoxyoligonucleotides from four to eight in length, high-molecular-weight aggregates containing up to about eight monomers are formed and can be seen on SDS-polyacrylamide gels. It was concluded in these studies that the oligomers gave rise to cross-linked aggregates very similar to those obtained with poly (dA-dT) and that the binding of short stretches of nucleic acid chain appears to induce the association of gene 5 monomers with one another [8].

We have formed complexes of the gene 5 protein with a number of different homogeneous deoxyoligonucleotides in solution, and have crystallized a variety of these complexes in a number of crystal forms. Four of these crystals have been characterized by X-ray diffraction and at least two have been found suitable for a high-resolution structure analysis. At present, we have collected 5.0-Å-resolution diffraction data on one of these crystals, and we describe here our progress in determining its structure using molecular replacement techniques with the monomeric gene 5 protein structure as model.

MATERIALS AND METHODS

The oligonucleotides used in the crystallization experiments were d-pGpC, d-pApT, d-$(Ap)_4$, and d-$(Ap)_8$ from Collaborative Research, Waltham, Massachusetts. The specific-sequence oligomers d-pCpTpTpC and d-$(Tp)_4$ were gifts of Dr. Robert Ratliff and Dr. Lloyd Williams of the University of California at Los Alamos, California; the homopolymers d-$(Cp)_3$ and d-$(Cp)_4$ were gifts of Dr. Gobind Khorana of MIT. d-GGTAAT and its complementary hexamer were supplied us by Dr. Jack Van Boom of the University of Leiden. Gene 5 protein from fd bacteriophage was made by a slight modification of the procedure of Alberts et al [2]. Infected cells were lysed in a French Press in 0.02 M tris (pH 7.6), 0.01 M $MgCl_2$ with no added DNA. The lysate was made 2 M in NaCl and nucleic acids were removed by polyethylene glycol precipitation. The supernatant was dialyzed to low salt by repeated changes of a dialysis buffer containing 0.02 M tris (pH 7.6), 0.05 M NaCl, 0.005 M EDTA, 0.001 M 2-mercaptoethanol and 10% (v/v) glycerol. The protein was isolated using DNA-cellulose and DEAE-cellulose chromatography. Occasionally the gene 5 protein had to be further purified by phosphocellulose chromatography to yield a homogeneous sample.

The complexes were crystallized by the vapor diffusion method in depression plates [9] using polyethylene glycol 6000 (PEG) from Fisher Scientific Co. as the precipitating agent. The methods and conditions employed were virtually the same as those described for crystallization of the native gene 5 protein [1]. The samples contained 5 μl of 14 mg/ml gene 5 protein, 5 μl of the oligonucleotide solution, both buffered at pH 7.5 by 0.01 M tris-HCl, and 10 μl of 12% PEG 6000 in H_2O. The sandwich box reservoirs contained 25 ml of 12% PEG 6000. All operations were carried out at room temperature. A variety of crystal forms were observed; the time required for crystal growth to occur varied from several days to several weeks with the mean at about 20 days. In general, the crystals were stable in their mother liquor for up to several months, although some apparently degraded in response to temperature fluctuations.

For X-ray diffraction analysis, the crystals were mounted by conventional means in sealed quartz X-ray capillaries along with a small aliquot of mother liquor. Precession photographs were recorded on Ilford Industrial G X-ray film, using a crystal-to-film distance of 90 mm and exposure times of about 18 h. Nickel-filtered CuK_α X-radiation was produced by an Elliot rotating anode generator operated at 40 kV and 40 mA with a focal spot size of 200 μm^2.

Three-dimensional X-ray diffraction data were collected to 5.0-Å resolution using the step scan mode [10] on a Picker FACS-1 diffractometer fitted with a 1,600-W Phillips fine-focus X-ray tube. The approximately 8,000 independent reflections were recorded from a single crystal. Standard deviations were estimated from counting statistics [11] and the reflections were merged and Lp-corrected using programs written by Dr. F.L. Suddath.

All computing operations were performed on a PDP 11/40 computer running under the RSX-11M operating system. The rotation function program was that of Crowther [12] as modified by Tanaka [13] for a spherical polar coordinate system and further modified by P.M.D.F. to run on the PDP 11/40 system. The translation function and structure factor calculation programs used were those of Lattmann [14] as modified by P.M.D.F. For rotation function calculations involving self vector searches within the native set, data between 10 Å and 6 Å in resolution and with intensity greater than 4 SD were employed. The maximum length of the vectors included in the search was varied from 20 to 35 Å. For the

searches of the complex using the uncomplexed gene 5 protein structure as the search model, we have used in succession only α-carbon atoms, all main-chain atoms, and all main-chain atoms plus tyrosines, phenylalanines, methionines, and cysteine. As above, data between 10 and 6 Å were used and the maximum vector length included was varied between 20 and 35 Å.

RESULTS

At least ten different crystal habits of the gene 5 protein complexed with deoxyoligonucleotides have been observed in our crystallization trials. The dominant forms are rhombic plates, though triangular and hexagonal prisms and plates as seen in Figure 1 are also frequently encountered. We have commonly observed polymorphism in single samples and transformations between different crystal forms as well. We find that at ratios of oligonucleotide to protein of less than 1.0 only native crystals are grown. At concentrations above this level only the complex crystals appear. We have not observed the coexistence of native crystals with complex crystals. Furthermore, we have never observed the complex crystals in the absence of added oligonucleotide. Most of the crystals grown were multiples, or twinned, or too small, or they presented some other problem to X-ray diffraction analysis. Several, however, grew large enough that we could determine their space groups and cell dimensions.

The unit-cell parameters and symmetry properties of four independent crystal forms of the gene 5 protein–DNA complex are shown in Table I. We noted that three of the crystals are based on hexagonal systems characterized by sixfold symmetry and the fourth, of space group $C222_1$, can be related to the unit cell of the $P6_3$ form if one assumes a pseudohexagonal packing arrangement. In fact, we frequently observe this orthorhombic crystal form growing as a twin or satellite crystal with a crystal of hexagonal habit.

Although we could not measure the density of any of the complex crystals directly, we assumed a volume-to-mass ratio for each that was near the center of the range of crystalline proteins compiled by Matthews [15] and was consistent as well with that measured for the uncomplexed gene 5 protein crystals, $V_m = 2.45$ [1]. Given this, we determined that the most reasonable number of gene 5 monomers in each asymmetric unit was consistently 12 (or six dimers), except for the $P3_1$ form, in which we judged that there are about 24.

The crystals of space group $P3_1$ were the best crystals we examined and X-ray diffraction data to 5.0-Å resolution were collected from this crystal. An example of the diffraction data from these crystals is shown in Figure 2. The volume of the asymmetric unit of this crystal form was twice that of the other forms, but the diffraction patterns of these trigonal crystals show very high 32 pseudosymmetry and this suggested the presence of a nearly crystallographic twofold axis along the 100 or 110 directions in the crystal. Thus the effective asymmetric unit contains 12 gene 5 monomers, the same number determined for the other crystal forms.

The rotation function search for local symmetry in the gene 5 protein–DNA complex crystals revealed three local symmetry axes. All of these can be seen in the section of the rotation function map at kappa = 180°, shown in Figure 3. (The angles psi and phi describe the orientation of a rotation axis; the angle kappa denotes the degree of rotation about that axis. Thus a peak in the map section at kappa = 180° indicates the orientation of a potential local twofold rotation axis.) There is a prominent peak in the kappa = 180° section at psi = 90°, phi = 55°, and an equivalent peak at psi = 90°, phi = 115°. These peaks have magnitude 0.66, expressed as a fraction of the magnitude of the peak corres-

ponding to the crystallographic threefold rotation along c. The position of the local peak indicates a twofold rotation axis nearly parallel to the crystallographic a^* or b^* axis and thus confirms our conclusion, based on the extra symmetry seen in the diffraction pattern, that the true space group of $P3_1$ contains molecules packed nearly, but not exactly, with the symmetry of space group $P3_121$, and that the pseudoasymmetric unit contains 12 gene 5 monomers.

A second peak occurs at psi = 0°, phi indeterminate; the magnitude of this peak is 0.71. The interaction of this local twofold parallel to c with the crystallographic threefold coincident with c will generate a local sixfold axis, and an equivalent peak is indeed found at psi = 0°, phi indeterminate in the section of the map at kappa = 60°. We believe that this twofold/sixfold axis is a manifestation of the packing of the 12-monomer aggregates into the unit cell, but we cannot rule out the possibility that this peak arises from symmetry internal to the 12-monomer aggregate.

The third prominent feature in the map occurs at psi = 90°, phi = 20°, with magnitude of 0.71. We can find no explanation for this local symmetry in the packing of aggregates within the unit cell and therefore we assume that this peak arises from a twofold-symmetry element, parallel to the crystallographic ab plane, that is internal to the 12-monomer aggregate.

The rotation function searches in which we seek to determine the 12 orientations of the gene 5 monomer in the pseudoasymmetric unit of the complex structure have so far given inconclusive results. We have found that searches using a model consisting of only main-chain atoms give very nearly the same results as searches using main-chain atoms plus large side chains, and we conclude that the former model is probably adequate for this type of search.

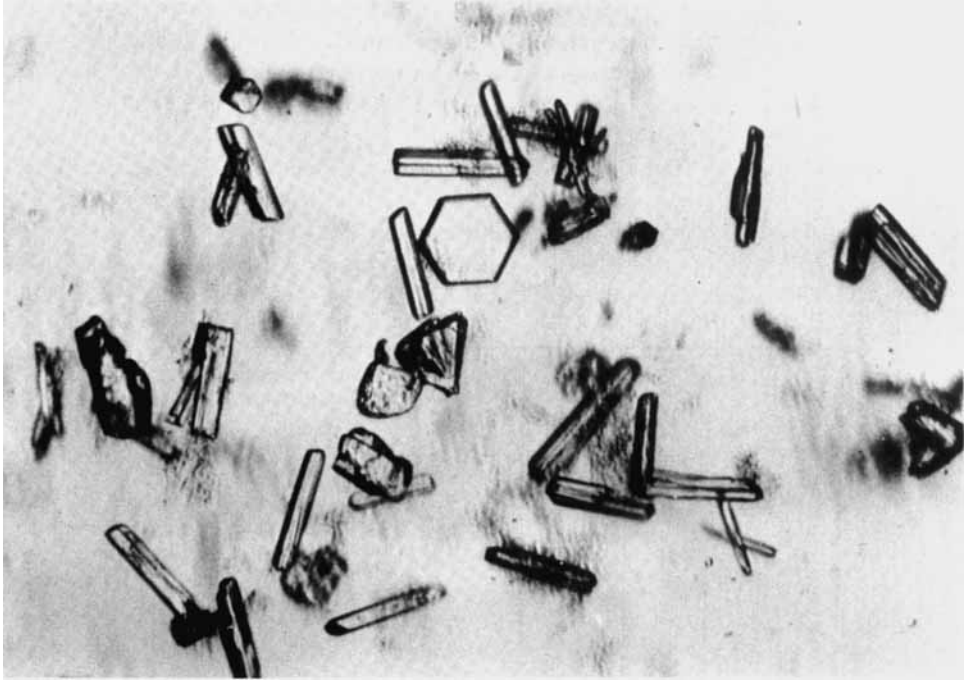

Fig. 1. Low-power light microscope photograph of the hexagonal plate habit of the complex between gene 5 protein and d-pCpTpTpC. These crystals have been observed to exist in the same sample with rhombic plates.

DISCUSSION

In the $P6_3$, $C222_1$, R32, and pseudo $P3_121$ crystals the number of gene 5 monomers per asymmetric unit is observed to be about 12. The repeated occurrence of this number of monomers as the asymmetric unit of the crystals suggests rather strongly a specific aggregate of 12 gene 5 monomers that is formed upon addition of oligonucleotides to the protein. The fact that these aggregates crystallize requires that they be a homogeneous population of identically structured complexes; they must represent some ordered mode of self assembly from the solution species.

There is evidence from solution studies that aggregation of gene 5 protein does occur in the presence of oligonucleotides as well as deoxyribonucleic acid. Rasched and Pohl [8] have found from suberimidate cross-linking and SDS gel electrophoresis of gene 5 protein combined with oligonucleotides that polymeric protein species up to "about eight" are formed. The lack of certainity in their upper limit is due, at least in part, to the anomalous electrophoretic mobility of cross-linked protein aggregates which would be expected to undergo more rapid migration since they are not completely extended polypeptide chains. Thus the size of these aggregates is not inconsistent with 12-monomer aggregate found in our asymmetric unit. In addition, the complex between gene 5 protein and fd phage DNA formed in solution and studied by electron microscopy shows "a helical rodlike structure" in which there are 12 gene 5 monomers per turn of the helix [2]. We believe we are observing crystallographically a structure similar to that observed in solution.

The gene 5 protein binds to DNA in a linear and highly cooperative manner, ie, successive gene 5 molecules tend to bind immediately adjacent to one already bound rather than to an isolated site. This apparently reflects the existence of strong protein-protein interactions between adjacent gene 5 molecules along the DNA strands, and may explain the powerful helix-destabilizing effect exerted by the protein. These strong protein-protein interactions do not occur between gene 5 molecules in solution in the absence of nucleic acid; if they did they would lead to aggregate formation of free molecules and this is not observed. It appears that the potential for forming such interactions is a consequence of conformational changes in the protein molecules induced by binding to DNA. The triggering of conformation change caused by DNA or oligonucleotide binding, the resulting cooperative interaction between protein molecules, and concomitant aggregation of the

TABLE I. Crystal Forms of fd Phage Gene 5 Protein Complexed With Oligodeoxynucleotides

Hexagonal plates	Diamond plates
$a = 107$	$a = 110$
	$b = 180$
$c = 206$	$c = 117$
$P6_3$	$C222_1$
12 * 9,800 daltons per asymmetric unit	12 * 9,800 daltons per asymmetric unit
Rhombohedra	Hexagonal prisms
$a = 140$	$a = 143$
	$c = 83$
$\alpha = 60$	
R32	$P3_1$
12–18 * 9,800 daltons per asymmetric unit	24 * 9,800 daltons per asymmetric unit

protein are likely responsible for the asymmetric unit of 12 monomers that we observe in our complex crystals.

Virtually all protein oligomers and large protein complexes studied so far by X-ray diffraction analysis have demonstrated symmetry relationships, or at least a high degree of quasisymmetry, between the units involved. This seems likely to be the case with the gene 5 protein–oligonucleotide complex as well. We know from crystallographic studies on the free protein that the gene 5 dimers contain perfect dyad axes relating monomers in pairs. The occurrence of six of these dimers in the asymmetric unit of the crystals suggests the likelihood of an aggregate having sixfold symmetry. This is reinforced by the finding that three of the four unit cells encountered are of hexagonal symmetry and that the fourth can be interpreted in terms of hexagonal packing. Although there is no required correlation, objects with hexagonal symmetry do tend to express such symmetry in the crystalline state and the number of hexagonal forms observed in this case argues for such a correlation.

The aggregate occupying the asymmetric unit of the complex crystals, which in all unit cells so far examined has contained 12 gene 5 protein monomers, is most likely a closed arrangement of fixed and determinate size which forms spontaneously in solution only when triggered by the binding of nucleic acid fragments. The simplest model for the asymmetric unit is that of a closed circle or disk having a sixfold axis along its center which is perpendicular to the twofold axes of the dimer units, ie, it possesses 622 point group symmetry.

The shape of the gene 5 protein dimer in the unliganded state is known from X-ray diffraction analysis (see accompanying paper) and has the gross features shown in the model in Figure 4. The structure created when one takes these dimers and arranges them in a

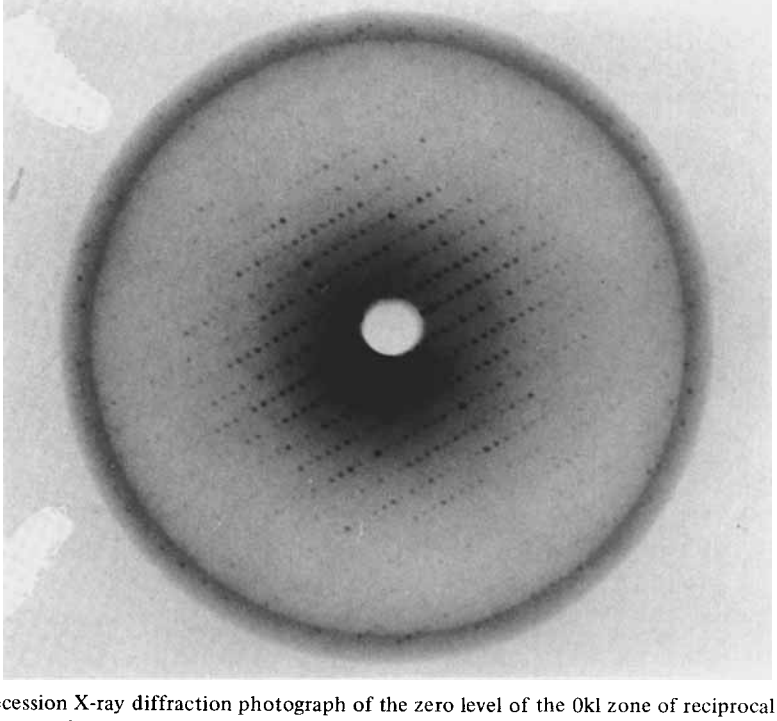

Fig. 2. Precession X-ray diffraction photograph of the zero level of the 0kl zone of reciprocal space of the $P3_1$ crystals of gene 5 protein complexed with d-(GGTAAT). These crystals grow to large size and diffract strongly to at least 3.0 Å in precession photographs.

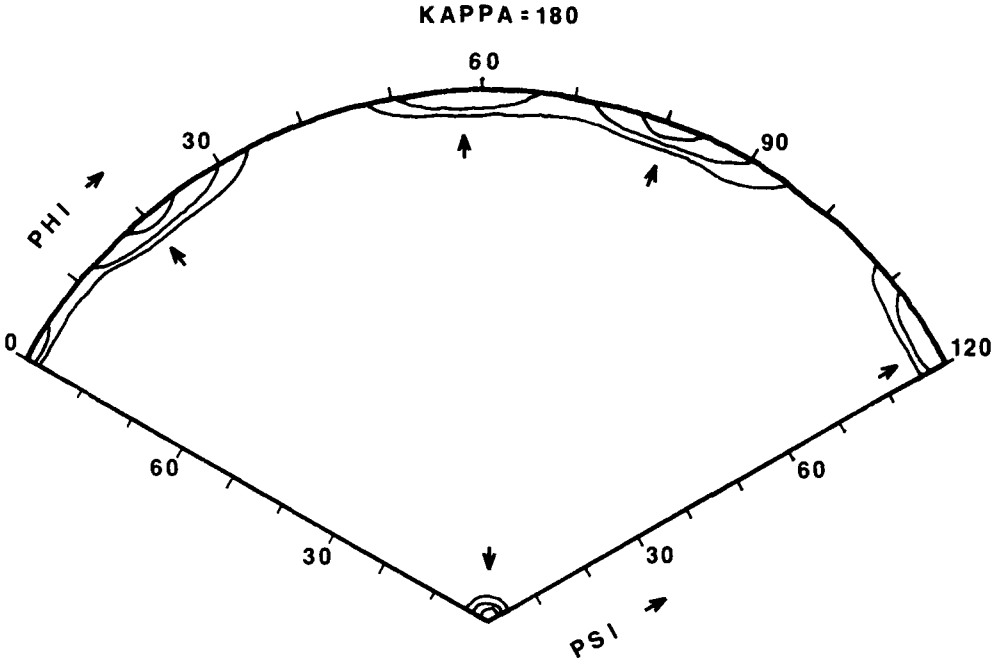

Fig. 3. The kappa = 180° section of the rotation function of the crystals of complexes between the gene 5 protein and d-(GGTAAT). All of the peaks along psi = 90° are of high magnitude with respect to the crystallographic symmetry peak, and all represent local, noncrystallographic symmetry elements present in the crystals.

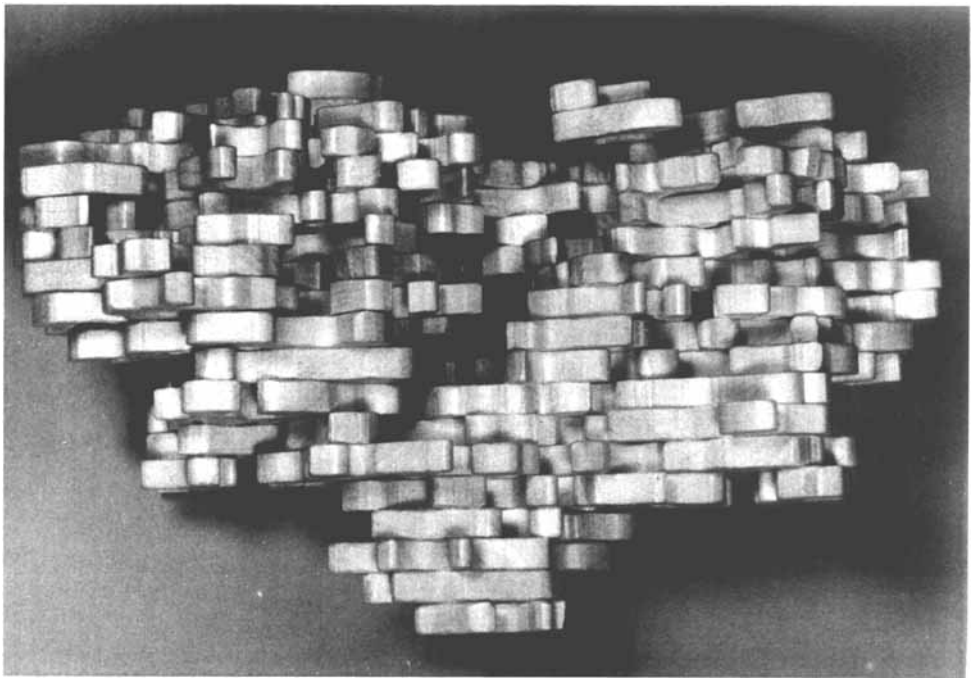

Fig. 4. A photograph of a three-dimensional model representation of the gene 5 dimer based on the structure derived from X-ray diffraction analysis. The postulated binding regions for the DNA single strands lie beneath the thick mass of density and are separated in the dimer by about 25 Å.

circle such that the dyad axes are perpendicular to a central sixfold axis is shown in Figure 5. Because of the double-wing character of the gene 5 dimer, the aggregate would have a twofold crown shape with a diameter of roughly 100 Å and a thickness of approximately 80 Å. Thus it is not a flat disk shape but that of a squat cylinder. The aggregate we are proposing can be packed without difficulty in each of the unit cells we have characterized.

The aggregation phenomenon we seem to be observing with the gene 5 protein–oligonucleotide complexes is not unprecedented. The tobacco mosaic virus disk [16] is an obvious analogy. Here again nucleic acid binding proteins are stimulated upon nucleotide binding to form a helical rod. Unlike the aggregation seen with the fd gene 5 protein, aggregation of the TMV coat protein can be induced in the absence of nucleic acid by careful selection of the environment. Under these conditions the TMV protein molecules organize into a closed circle or disk having a 17-fold symmetry axis [17].

Another example is the octameric aggregate of histones which forms the core of the chromosomal body [18]. This is clearly different in gross structure since it acts as a spool for superhelically coiled double-stranded DNA, but it may, with regard to the functional organization of the proteins in its center, bear some similarities to the gene 5 nucleoprotein complex.

The aggregate of 12 gene 5 monomers observed in the crystal is not identical in structure to the helical aggregates of the gene 5 protein and DNA observed in the electron microscope. This unit we postulate in Figure 5 is completely closed and does not allow an extended helix to be built up simply by translation along the direction of the sixfold axis. However, the relationship between the two structures may be somewhat analogous to the relationship that exists between the 17-fold TMV closed-disk structure and the TMV helix, which has a 17-fold screw axis. The latter structure arises from the first simply by opening

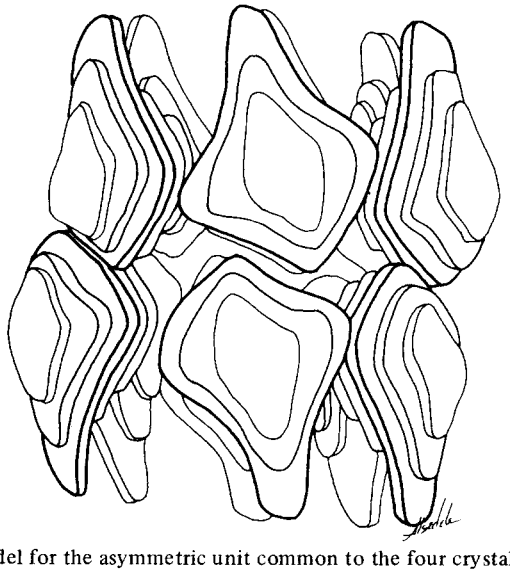

Fig. 5. A proposed model for the asymmetric unit common to the four crystal forms we have so far analyzed. The arrangement is a circle, or disk, having 622 point group symmetry formed by joining the two ends of a linear array of six gene 5 protein dimers, each of which possesses an inherent dyad, to produce closure. The upper hexagon of monomers will bind a single strand of DNA running in one direction and the bottom level a strand running in the opposite direction. The disk has a diameter of about 100 Å and a height of about 80 Å. We believe the DNA binding region of each monomer to be directed toward the outside of the circle.

the disk and displacing the two ends along the direction of the unique axis to produce a "lockwasher" unit. The free ends of these "lockwasher" units are joined as the units are stacked to produce the helix. A model of a helical structure that might be produced by the gene 5 protein binding to two strands of DNA running in opposite directions is shown in Figure 6. This helical structure would contain essentially the same lateral interactions between adjacent protein monomers as occur in the closed disk. This structure is a gene 5 double helix, one chain of which binds a DNA strand running 3′ to 5′ and the other a strand running 5′ to 3′. It has a sixfold screw axis with a linear repeat of about 80–90 Å and a diameter of approximately 100 Å.

The gene 5 protein–DNA aggregate could in principle form with the DNA binding interface to the outside of the ring or to the inside. Based on our analysis of the tertiary structure of the monomer, however, we conclude that the DNA must be to the outside. This suggests that the two DNA single strands are coiled around the gene 5 protein core with a radius of 30–40 Å in the complex. This radius is stereochemically reasonable and is about the same as the radius at which RNA is found in the TMV helical structure. Were the strands of nucleic acid on the inside of the gene 5 protein complex, they would need to be coiled with an unacceptably tight radius. Thus we believe that the DNA strands wind around a core of gene 5 protein which acts as a spool rather than a sheath.

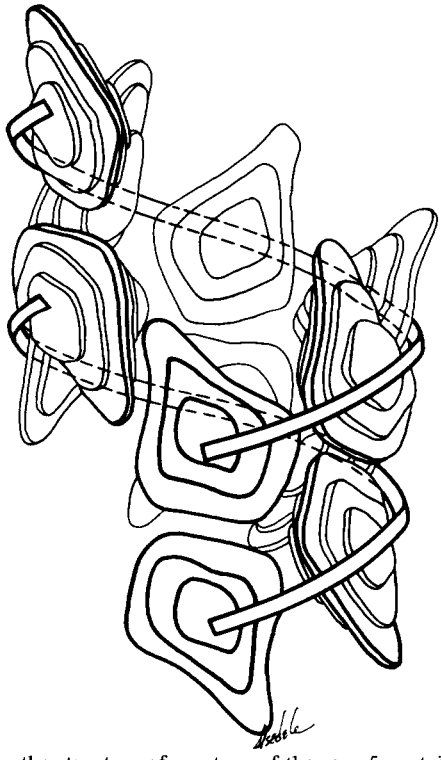

Fig. 6. A proposed model for the structure of one turn of the gene 5 protein–DNA double helix. This arrangement arises by opening the disk structure seen in Figure 5 between any two adjacent dimers and displacing the free ends along the unique axis direction. The stacking of these "lockwasher" units results in a double helix structure having a sixfold screw axis with perpendicular dyads, 12 gene 5 monomers per turn, and dimensions consistent with the helices observed by electron microscopy. The two DNA single strands are spooled around this spindle of gene 5 protein.

A mechanistic advantage of this model is that it provides a simple means for the displacement of the gene 5 protein by the gene 8 coat protein. Since the DNA strands are on the exterior of the gene 5 complex, they are exposed to the approach of the coat protein which also binds to DNA cooperatively but much more tenaciously than the gene 5 protein. Hence, when the gene 5 protein–DNA complex reaches the cell membrane the coat proteins binds very strongly to the DNA strands on the outside of the complex. Since it is of a much smaller size, the coat protein undoubtedly forms a helix with different parameters. This change in the conformation of the DNA produced by coat protein binding would disrupt the gene 5 protein–DNA bonds as well as the gene 5 monomer-monomer interactions. Thus, the gene 5 protein which forms a spindle on the interior of the nucleic acid is exchanged for a sheath of coat protein which must protect the virion after extrusion into the media.

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REFERENCES

1. McPherson A, Molineux I, Rich A: J Mol Biol 106:1077–1081, 1976.
2. Alberts B, Frey L, Delius H: J Mol Biol 68:139–152, 1972.
3. Pratt D, Laws P, Griffith J: J Mol Biol 82:425–439, 1974.
4. Henry TJ, Pratt D: Proc Natl Acad Sci USA 62:800–807, 1969.
5. Grey C: Paper presented at the ICN-UCLA Symposium on Recognition and Assembly in Biological Systems, Keystone, Colorado, 1979.
6. Pretorius HT, Klein M, Day LA: J Biol Chem 250:9262–9269, 1975.
7. Herrick G, Alberts BM: J Biol Chem 251:2124–2146, 1976.
8. Rasched I, Pohl FM: FEBS Lett 46:115–118, 1974.
9. McPherson A: In Glick D (ed): "Methods of Biochemical Analysis." New York: Wiley and Sons, 1976, vol 23, pp 249–345.
10. Wyckoff HW, Doscher M, Tsernoglou D, Inagami T, Johnson LN, Hardman KD, Allewell NM, Kelly DM, Richards FM: J Mol Biol 27:563–578, 1967.
11. Arndt UW, Willis BTM: "Single Crystal Diffractometry." Cambridge, UK: University Printing House, 1966, Chapter 10.
12. Crowther RA: In Rossmann, MG (ed): "The Molecular Replacement Method." New York: Gordon & Breach, 1972, pp 173–178.
13. Tanaka N: Acta Crystallogr A33:191–193, 1977.
14. Lattman EE: PhD thesis, Johns Hopkins University, Baltimore, Maryland, 1969.
15. Matthews BW: J Mol Biol 33:491–497, 1968.
16. Butler PJG: Cold Spring Harbor Symp Quant Biol 36:461–468, 1971.
17. Champness JN, Bloomer AC, Bricogne G, Butler PJG, Klug A: Nature 259:20–24, 1976.
18. Finch JT, Lutter LC, Rhodes D, Brown RS, Rushton B, Levitt M, Klug A: Nature 269:29–36, 1977.