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New method for localizing proteins in periodic structures: Fab fragment labeling combined with image processing of electron micrographs

(antigenic sites/electron microscopy/protein conformation/bacteriophage T-even)

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ABSTRACT **Fab fragments prepared from antisera directed against purified bacteriophage T4 structural proteins and head-related structures were used to label proteins on the surface of T-even giant phage capsids. Optically filtered electron micrographs of the Fab-labeled capsids reveal both the location of specific proteins within the capsomeres and differing conformational states of the protein subunits. We describe parameters affecting the utility of this technique for the study of molecular organization and protein conformation in periodic biological structures.**

Many biological structures, including virus shells, contractile filaments, microtubules, and parts of bacterial cell walls, are built up as ordered arrays of one or several species of protein subunits. Electron microscopy of negatively stained specimens followed by image processing of the electron micrographs has proved to be a useful tool for establishing the supramolecular organization of these assemblies (1). The most common way of localizing individual protein subunits within their repeating unit has been to compare the filtered images of related structures that differ in their protein composition. These are usually obtained either from mutants that cannot synthesize one or more of the constituents (2, 3), by differential dissociation of the structures (4), or by *in vitro* complementation of the deficient structures with the proteins they are lacking (2, 4–6). However, this approach cannot unambiguously identify the various stain-excluding regions of the repeating unit with particular constituent proteins, because binding of a protein to a periodic assembly may alter the unit cell morphology indirectly by inducing a change in tertiary or quaternary structure. This change may mask the direct change caused by the added protein.

One way to localize the individual proteins or protein domains within a supramolecular structure is to label them with specific markers such as antibodies (7–10). However, the utility of this approach is limited both by the size of the antibody molecules [molecular weight (M_r) 150,000] and the dimensions and surface topography of the structure in which the protein is to be localized. To improve the precision of localizing antigenic sites on a structure, Craig and Offer (11) have used monovalent Fab fragments (12) (M_r 50,000) rather than whole antibodies. We have extended this method for analyzing the molecular organization and conformation of periodic structures by applying image enhancement techniques to electron micrographs of specimens that have been specifically labeled with Fab fragments. By using Fab fragments prepared from specific antisera directed against the constituent proteins of bacteriophage T4 capsids we have been able to confirm the localization (2, 4) of these proteins within the capsomere. Furthermore, this technique has allowed us to demonstrate an induced conformational change on the binding of one of these proteins to the basic capsid matrix.

MATERIALS AND METHODS

Antigens and Antisera. The T4 outer capsid proteins hoc and soc were purified as described by Ishii and Yanagida (2). T4 coarse polyheads (13) composed only of the product of gene 23 (P23) obtained from a mutant in gene *20* were purified by differential centrifugation. Gene 23ts aberrant preheads containing most of the T4 head proteins including hoc and soc (L. Onorato, unpublished data) were purified by two successive sucrose gradients (14). A 200- to 500-μg sample of each antigen was injected in Freund's complete adjuvant into the hind footpads of rabbits. Animals were bled twice weekly, starting 4 weeks after injection, and serum from several bleedings was pooled for the preparation of IgG and Fab fragments. The production of high-titer soc antiserum required bimonthly intravenous boosting with 50 μg of the purified antigen. The sera were characterized on Ouchterlony plates and by immunoreplicate electrophoresis (15). The anti-hoc serum gave no reaction with T2 phage proteins, but reacted strongly with hoc and with another T4 protein, possibly derived from hoc. Anti-soc serum reacted only with soc protein. Antiserum to 23ts aberrant preheads reacted strongly with hoc, weakly with P23 and P24, and gave no detectable precipitin reaction with soc or the proteolytically processed form of P23 found in the mature phage head, P23* (16). Anti-polyhead serum reacted with both P23 and P23*.

IgG and Fab Fragments. IgG was purified from crude serum by precipitation three times with a saturated ammonium sulfate solution [33% (wt/vol) final concentration] followed by DEAE-cellulose chromatography (17). Monovalent Fab fragments were prepared from purified IgG by cleavage with mercuri-papain as described by Porter (12). Where the fragmentation was incomplete, Fab fragments were separated from undigested IgG by chromatography on Sephadex G-100.

Immune Electron Microscopy. Samples containing 1 to 5 $\times 10^7$ T2L (18) or T4 (19) giant phage particles were incubated with Fab fragments (0.5–1000 μg) in 20–200 μl of 10 mM sodium phosphate buffer, pH 7.0, for 1 hr at 37°, 4 hr at 20°, or

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Abbreviations: M_r, molecular weight; P23, product of bacteriophage T4 gene 23 (M_r 58,000); P23*, proteolytically processed form of P23 found in the mature phage head (M_r 47,700).

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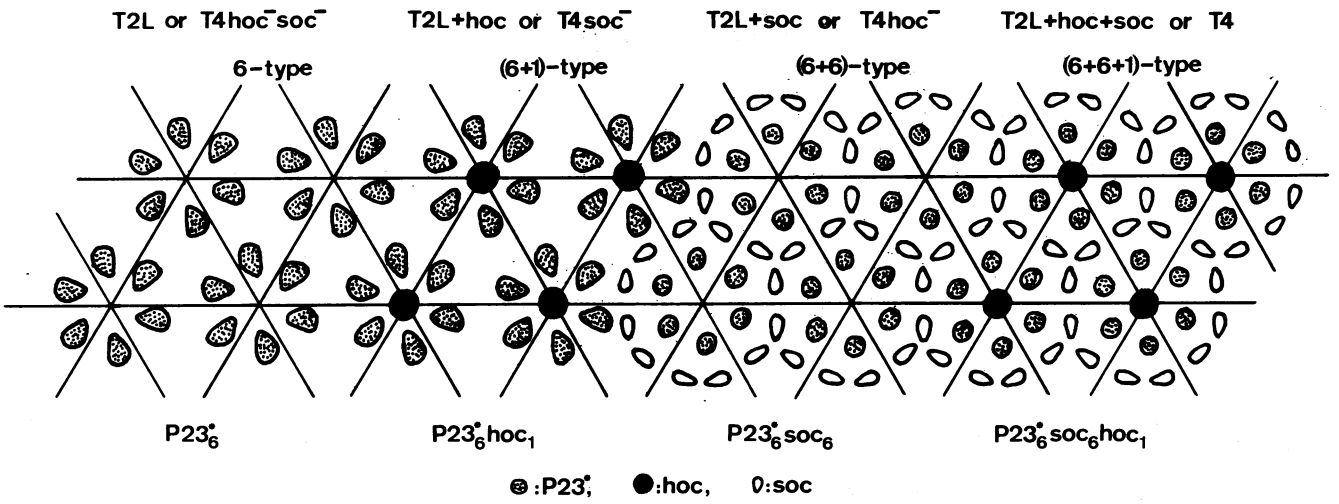

FIG. 1. Schematic representation of bacteriophage T-even capsomere morphologies. The top line indicates the bacteriophage capsid and purified proteins which produce the capsomere types shown below. Each capsomere type is also labeled with its morphological type and protein composition.

12 hr at 4°. The phage particles were freed of excess label by two cycles of centrifugation and resuspended in 50 mM TrisCl, pH 7.2, at 5×10^9 particles per ml. The conditions for antibody excess were established by showing that the supernatant remaining after the first centrifugation of the giant phage was able to label a second aliquot of phages to the same extent as the first.

Both labeled and unlabeled samples were prepared for electron microscopy by either negatively staining them with 1% uranyl acetate, pH 4.5 (19), or by freeze-drying and shadowing them (20). Electron micrographs were recorded at a magnification of approximately 35,000 times as described previously (19).

Image Processing of Electron Micrographs. Optical diffraction and subsequent filtering of electron micrograph areas containing approximately 200 well-ordered unit cells of the tubular part of giant phage capsids were carried out as described previously (19).

RESULTS

The capsomere morphologies of bacteriophages T2 and T4 are shown schematically at the extreme left and right of Fig. 1. They were obtained from optically filtered electron micrographs of negatively stained giant phage capsids (2, 4, 21). These giant phages are viable polymorphic variants of normal T-even phage that are characterized by an abnormally elongated tubular part of the wild-type capsid (22). They can be induced by either genetic (19, 23) or chemical (18) means.

The hexagonal T2 lattice is built exclusively from P23* molecules, which are clustered into 6-type capsomeres (21). Bacteriophage T4 is built from the same P23* lattice, but in addition contains the two outer capsid proteins hoc and soc in the ratio $P23^*_6soc_6hoc_1$ (2).

The localization of hoc and soc in the (6 + 6 + 1)-type T4 capsomeres was inferred from lattices whose capsomeres are shown between those of T2 and T4 in Fig. 1. These intermediate forms were obtained (as indicated in Fig. 1) from T4 mutants defective in production of hoc, soc, or both (2), by chemically extracting these proteins from the T4 lattice (4) and by the addition of hoc and soc *in vitro* to a T2 6-type lattice (2, 4, 24). The experiments we describe below were designed to test whether the new stain-excluding regions that appear in the capsomeres of (6 + 1)-, (6 + 6)- and (6 + 6 + 1)-types actually localize hoc and soc, or result from the rearrangement of portions of the P23* molecules upon binding of hoc and soc.

Localization of hoc within the P23* lattice

Fig. 2*A* illustrates the change in surface morphology that is obtained when (6 + 1)-type giant phage capsids (*top*) are reacted with an excess of anti-hoc Fab fragments (*bottom*). Fig. 2*B* shows areas of optically filtered micrographs of the unlabeled (*top*) and labeled (*middle*: nonsaturated; *bottom*: saturated) capsids. Although the labeling does not look very regular on unprocessed micrographs, it is sufficiently so to enhance significantly the central stain-excluding region in the filtered images. When (6 + 6 + 1)-type giant phage capsids are reacted with the same Fab fragments, a similar enhancement of the central stain-excluding region is obtained. Preincubation of the Fab fragments with purified hoc protein completely inhibits this reaction. Therefore, the central stain-excluding region of (6 + 1)- and (6 + 6 + 1)-type capsomeres actually localizes the hoc molecule in the capsomere, as previously suggested (4, 24).

Localization of soc within the P23* lattice

Reaction of (6 + 6 + 1)-type giant phage capsids with Fab fragments prepared from a serum directed against purified soc protein did not give specific and regular labeling of the hexagonal surface lattice, even when the Fab fragments were added in great excess. However, when these capsids (Fig. 2*C* *top*) were reacted with an excess of Fab fragments prepared from a serum raised against soc-containing 23ts aberrant preheads (14) absorbed with both T2L phage and purified hoc protein prior to the reaction, specific and regular labeling of the hexagonal surface lattice was obtained (Fig. 2*C* *bottom*). As can be seen on optically filtered electron micrographs (Fig. 2*D*), saturation of the (6 + 6 + 1)-type lattice (*top*) with Fab fragments results in a unit cell morphology that is characterized by six radially elongated stain-excluding regions surrounding a central one (Fig. 2*D* *bottom*). From superposition of the two stain-excluding patterns (shown schematically in the *middle* of Fig. 2*D*), we conclude that each of the radially elongated stain-excluding regions localizes a specifically bound Fab fragment. The only feature of the (6 + 6 + 1)-type capsomere that can still be visualized in the labeled pattern is the hoc molecule, which gives rise to a reduced central stain-excluding

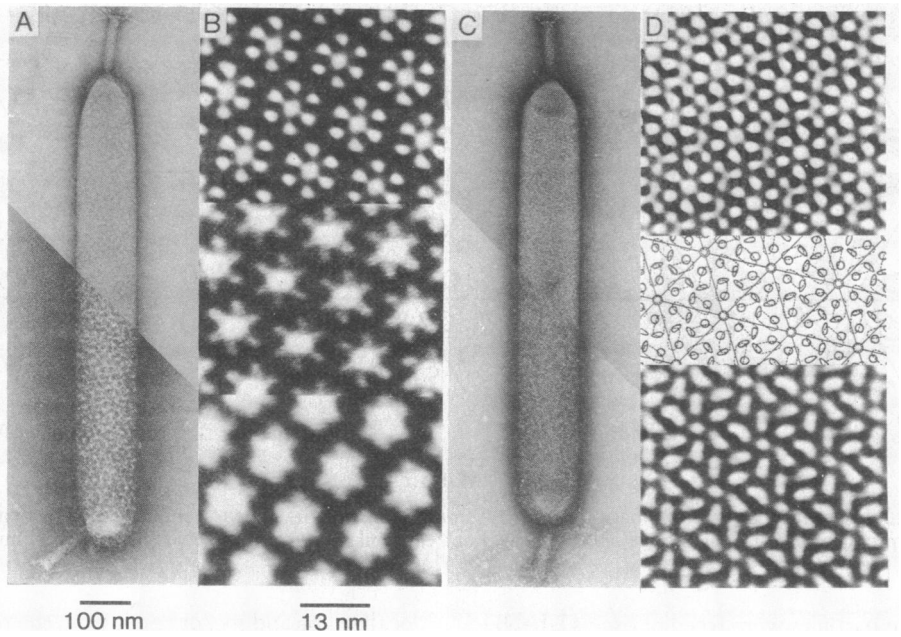

FIG. 2. (*A*) Montage of electron micrographs of negatively stained (6 + 1)-type giant phage capsids (*Top*) that have been labeled with an excess of anti-hoc Fab fragments (*Bottom*). (*B*) Optically filtered areas of the micrographs to the left in *A*. The *Middle* panel is a filtration of a capsid labeled with an undersaturating amount of Fab fragments. (*C*) Montage of electron micrographs of negatively stained (6 + 6 + 1)-type giant phage capsids (*Top*) that have been labeled with an excess of anti-soc Fab fragments (*Bottom*) prepared from a serum raised against soc-containing *23*ts aberrant preheads. (*D*) Optically filtered areas of the micrographs shown to the left in *C*. In the *Middle*, the unlabeled (solid lines) and the labeled (broken lines) stain-excluding patterns are schematically superimposed.

region. In order to find out to which stain-excluding regions of the (6 + 6 + 1)-type capsomere the Fab fragments actually bind, we reacted giant phage capsids with gradually increasing but nonsaturating amounts of Fab fragments. As the amount was increased, filtered micrographs showed a gradually increasing "blurring" of the stain-excluding trimers without significantly affecting the remainder of the capsomeres.

We conclude that, under saturating conditions as shown in Fig. 2*C*, each trimer binds three Fab fragments, each of which "bridges" identically to the closest (6 + 1)-mere to give rise to one of the six radially elongated arms. This specific labeling is completely inhibited when the Fab fragments are incubated with purified soc protein prior to reacting them with (6 + 6 + 1)-type giant phage capsids. Therefore, it must be caused by anti-soc-specific Fab fragments. These results confirm (2, 4, 24) that at least a part of each stain-excluding trimer of the (6 + 6 + 1)-type capsomeres localizes the binding of three soc molecules. The finding that purified soc protein is able to absorb the anti-soc antibodies without giving a precipitin reaction with the serum suggests that the anti-soc antibodies are probably all directed against a single soc antigenic determinant.

Labeling of the hexagonal P23* lattice

Fab fragments were prepared from antisera raised against purified soluble P23 and against P23* prepared from soluble P23 by cleavage with T4 prehead proteinase (16). Sera from two rabbits were pooled in each case. Although high-titer sera were obtained, as evidenced by their strong reactions both structurally on T-even phage capsids and in immunoreplicate electrophoresis, these Fab fragments did not label giant T-even phage capsids regularly enough so that the labeling patterns could be enhanced by optical filtering. We then tested Fab fragments obtained from sera raised against T4 coarse polyhead shells, which are tubular head-related structures composed only of P23. These did give regular labeling of each of the T-even phage capsid types shown in Fig. 1 except the (6 + 1). As an example, Fig. 3*A* shows 6-type capsids (*top*) reacted with these Fab fragments (*bottom*). Optically filtered micrographs (Fig. 3*B*) show that on Fab binding, the 6-type P23* hexamers (*top*) surround a strong central stain-excluding region (*bottom*). The absence of this reaction on (6 + 1)-type lattices indicates that the P23* antigenic site responsible for it is blocked on binding hoc to the 6-type P23* lattice.

When 6-type capsids were reacted with nonsaturating but gradually increasing amounts of Fab fragments, filtered images showed a gradual exclusion of stain from the central depression of the 6-type capsomeres. However, when the labeled preparations were examined directly by freeze-drying and shadowing, it was found that this increase was due to increasing numbers of capsomeres that had bound Fab fragments to their centers. There was no apparent increase in the amount of material bound per capsomere. These anti-polyhead Fab fragments must be directed against one or more antigenic determinants that are localized in or around the central mass depression (~50-Å diameter) of the 6-type capsomeres. The binding of one Fab fragment [dimension 40 × 50 × 80 Å (25)] into this depression probably blocks the binding of further fragments to the same capsomere.

Specific labeling of a conformation-dependent site in (6 + 6 + 1)-type capsomeres

A phage T-even capsid composed only of P23* (6-type) can be converted to a (6 + 6 + 1)-type by the addition of the outer capsid proteins hoc and soc (4, 24). We expect that this conversion might entail conformational changes in the P23* that would be detectable as a change in its antigenicity. The anti-polyhead (i.e., anti-P23) serum appears to detect just such a change.

As described above, the anti-polyhead serum labels 6-type capsids only in the center of the P23* hexamer, at a site that is blocked by hoc binding in that position. The hoc-containing (6 + 1)-type capsids show no regular labeling. However, when this

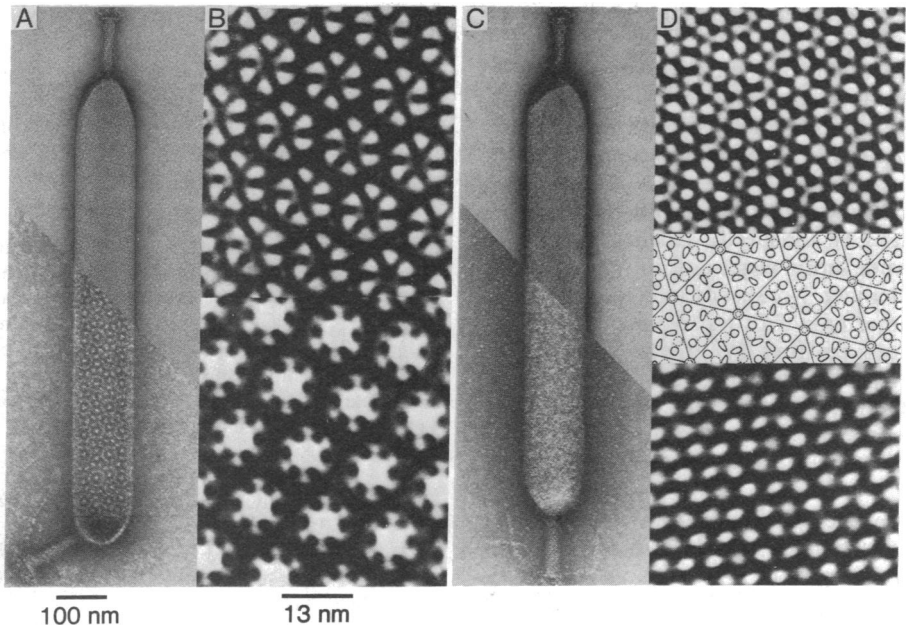

FIG. 3. (*A*) Montage of electron micrographs of negatively stained 6-type giant phage capsids (*Top*) that have been labeled with an excess of anti-P23 Fab fragments (*Bottom*) prepared from a serum raised against P23-containing coarse polyheads. (*B*) Optically filtered areas of the micrographs shown to the left in *A*. (*C*) Montage of electron micrographs of negatively stained (6 + 6 + 1)-type giant phage capsids (*Top*) that have been labeled with an excess of anti-P23 Fab fragments (*Bottom*) prepared from a serum raised against P23-containing coarse polyheads. (*D*) Optically filtered areas of the micrographs shown to the left in *C*. In the *Middle*, the unlabeled (solid lines) and the labeled (broken lines) stain-excluding patterns are schematically superimposed.

serum is tested on P23* lattices that contain soc [(6 + 6)- or (6 + 6 + 1)-types], strong and regular labeling is found (Fig. 3*C bottom*). On optical filtration of the labeled capsids, both the P23* hexamer and the soc trimers have disappeared, replaced by six new stain-excluding regions per unit cell (Fig. 3*D bottom*). Superposition of the labeled and unlabeled patterns (Fig. 3*D middle*), shows that Fab fragments now bind at an antigenic site localized between the P23* hexamers and the soc trimers.

The new labeling pattern is unaffected by pre-incubation of the Fab fragments with 6-type capsids, soluble hoc, or soluble soc alone. This shows that the antigenic site is exposed only on soc binding to the P23* lattice. But how can we distinguish between an antigenic site induced on the P23* lattice on binding soc and one induced on soc by binding to the P23* lattice? We favor the former interpretation for the following reasons. First, the polyhead antigen could have contained soc only as a minor contaminant. Second, immunodiffusion tests of the serum showed a high titer of anti-P23* but no detectable anti-soc. Third, the soc-dependent binding shown in Fig. 3*D* is as strong as the soc-independent binding to P23* (6-type) capsomeres. And, finally, the labeling pattern of the anti-polyhead Fab fragments is completely different from that found using *bona fide* anti-soc Fab fragments (cf. Fig. 2*D*). At nonsaturating concentrations the anti-polyhead Fab fragments did not cause blurring of the soc trimers. We therefore suggest that the Fab fragments used in this experiment are detecting a conformational change in the P23* hexamers brought about by the binding of soc to the 6-type or the (6 + 1)-type lattice.

DISCUSSION

We have shown that the subunits of a periodic structure can be specifically and regularly labeled with antibodies or monovalent Fab fragments directed against the constituent proteins. By comparing optically filtered electron micrographs of labeled and unlabeled structures, an average labeling pattern of the repeating unit can be deduced. Such specific labeling patterns can be used to localize the constituent proteins or even specific parts of them within the repeating unit.

By applying this technique to bacteriophage T-even capsids, we were able to localize the two outer capsid proteins hoc and soc (2), whose positions within the P23* matrix had been only inferred on the basis of capsomere morphology (2, 4, 24). The hoc- and soc-specific labeling patterns provide assurance that the new stain-excluding regions that appear on their addition to the 6-type P23* lattice correspond at least in part to the newly bound proteins rather than only to conformationally altered states of the P23* matrix.

In addition, we have found that binding of soc to the P23* lattice induces the exposure of a new antigenic site not accessible on either 6- or (6 + 1)-type capsids or on soluble soc protein. We suggest that a conformational change in P23* occurs on soc binding. Aebi *et al.* (4) have observed that the binding of hoc to a 6-type P23* lattice alters its affinity for soc binding. They suggested that this alteration is mediated by a hoc-induced conformational change in the P23* molecules because a direct hoc–soc interaction seems excluded by capsomere geometry. Our results indicate an additional conformational change on soc binding, because only soc, and not hoc, binding exposes the new antigenic site detected by anti-polyhead serum. This site must be exposed on the immunogen (coarse polyheads) during processing in the immunized animal, because we cannot absorb antibodies to it with the coarse polyheads that were injected. We have also observed this phenomenon with sera raised against soluble P23, which still show extensive binding to both coarse and smooth polyheads even after exhaustive absorption with the antigen.

We have observed additional changes in antigenicity when comparing the 6-type P23 lattice of coarse polyheads with that of the P23* lattice of polyheads cleaved *in vitro* (26) by the T4 head maturation protease. The nature of these changes can be

investigated using the specific Fab fragment labeling techniques described in this paper.

Because specific labeling of periodic structures with Fab fragments appears to be a generally useful technique for solving structural problems, we mention briefly some of our observations that may be applicable to other periodic biological structures.

We have compared the use of both crude antisera and purified IgG with Fab fragments for specific labeling of giant T-even phage capsids. Crude serum was useful only to detect the presence of antibody activity. Nonspecific adhesion of serum proteins apparently interferes with the regular labeling of structures. In a few cases, purified IgG preparations gave labeling as regular as that found with Fab fragments derived from them, but this was not the rule. We find that the most satisfactory procedure is to prepare Fab fragments and to establish their specificity by preincubation with an excess of competing antigen.

We have found more specific labeling from antisera raised against ordered structures than from antisera directed against the purified structural components. Although antibodies directed against purified P23, P23*, and soc reacted strongly with phage T-even capsids, Fab fragments prepared from them did not show specific and regular labeling patterns that could be enhanced by optical filtration of micrographs of the labeled structures. It seems that when a structure is used as an antigen, antibodies are produced mainly against a limited number of the antigenic sites exposed on its surface. For example, we have found that hexagonal sheets assembled from a 14,000 M_r fragment of P23* (M_r 47,700) can completely inhibit the reaction of anti-polyhead serum on a 6-type lattice. If soluble structural components elicit a more heterogeneous class of antibodies, it might be expected that different repeating units of the structure would be labeled at different sites. This would account for the relative lack of specific labeling we usually observe with such antisera. It may be, however, that the specificity of an antiserum to solubilized components can be increased by appropriate depletions.

Regular labeling of a periodic structure depends not only on the specificity of the Fab fragments used but also on the topography of the specimen around the binding site. We can distinguish two extreme cases. In the first, the binding site is in or near a depression that can accommodate and thereby localize and stabilize the Fab fragment within the repeating unit (e.g., Fig. 3*B*). This gives a well-ordered labeling pattern, but if the label "bridges" two adjacent features of the repeating unit morphology (as in Fig. 2*D*), it may be difficult to determine which of the two represents the actual binding site. Use of nonsaturating labeling conditions may resolve the ambiguity, if one feature is affected at lower label concentration than the other. In the second case, the Fab fragment binds to an isolated elevation within the repeating unit of the structure (e.g., Fig. 2*B*). On specimen preparation, it may have different orientations in different repeating units. Under these conditions, averaging over many repeating units should only enhance the part of the Fab fragment that contains the combining site, because its location will be the most reproducible from one unit cell to the next. The localization may thus be even more precise than would be anticipated on the basis of the size of the Fab fragments [40 × 50 × 80 Å (25)].

The analysis of the T-even capsid structure is a problem that has many features in common with the analysis of other biological structures, such as actomyosin filaments, microtubules, ribosome crystals, cell walls, and cell membranes. We believe that specific Fab fragment labeling combined with image processing should be a useful tool for obtaining a better understanding of these structures.

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1. Crowther, R. A. & Klug, A. (1975) *Annu. Rev. Biochem.* **44,** 161–182.
2. Ishii, T. & Yanagida, M. (1975) *J. Mol. Biol.* **97,** 655–660.
3. Crowther, R. A., Lenk, E. V., Kikuchi, Y. & King, J. (1977) *J. Mol. Biol.*, **116,** 491–526.
4. Aebi, U., van Driel, R., Bijlenga, R. K. L., ten Heggeler, B., van den Broek, R., Steven, A. C. & Smith, P. R. (1977) *J. Mol. Biol.* **110,** 687–698.
5. Moore, P. B., Huxley, H. E. & DeRosier, D. J. (1970) *J. Mol. Biol.* **50,** 279–295.
6. Wurtz, M., Kistler, J. & Hohn, T. (1976) *J. Mol. Biol.* **101,** 39–56.
7. Yanagida, M. & Ahmad-Zadeh, C. (1970) *J. Mol. Biol.* **51,** 411–421.
8. Beckendorf, S. K. (1973) *J. Mol. Biol.* **73,** 37–53.
9. Tischendorf, G. W., Zeichhardt, H. & Stöffler, G. (1975) *Proc. Natl. Acad. Sci. USA* **72,** 4820–4824.
10. Lake, J. A. & Kahan, L. (1975) *J. Mol. Biol.* **99,** 631–644.
11. Craig, R. & Offer, G. (1976) *J. Mol. Biol.* **102,** 325–332.
12. Porter, R. R. (1959) *Biochem. J.* **73,** 119–127.
13. De Rosier, D. J. & Klug, A. (1972) *J. Mol. Biol.* **65,** 469–488.
14. Onorato, L. & Showe, M. K. (1975) *J. Mol. Biol.* **92,** 395–412.
15. Showe, M. K., Isobe, E. & Onorato, L. (1976) *J. Mol. Biol.* **107,** 55–69.
16. Tsugita, A., Black, L. W. & Showe, M. K. (1975) *J. Mol. Biol.* **98,** 271–275.
17. Weir, D. M., ed. (1974) *Handbook of Experimental Immunology* (Blackwell Scientific Publications, Oxford), 2nd Ed.
18. Cummings, D. J., Chapman, V. A., De Long, S. S. & Couse, N. L. (1973) *Virology* **54,** 245–261.
19. Bijlenga, R. K. L., Aebi, U. & Kellenberger, E. (1976) *J. Mol. Biol.* **103,** 469–498.
20. Kistler, J., Aebi, U. & Kellenberger, E. (1977) *J. Ultrastruct. Res.* **59,** 76–86.
21. Aebi, U., Bijlenga, R. K. L., ten Heggeler, B., Kistler, J., Steven, A. C. & Smith, P. R. (1976) *J. Supramol. Struct.* **5,** 475–495.
22. Aebi, U., Bijlenga, R. K. L., van den Broek, J., van den Broek, R., Eiserling, F., Kellenberger, C., Kellenberger, E., Mesyanzhinov, V., Müller, L., Showe, M., Smith, R. & Steven, A. (1974) *J. Supramol. Struct.* **2,** 239–254.
23. Doermann, A. H., Eiserling, F. A. & Boehner, L. (1973) *J. Virol.* **12,** 374–385.
24. Yanagida, M. (1977) *J. Mol. Biol.* **109,** 515–537.
25. Davies, D. R., Padlan, E. A. & Segal, D. M. (1975) *Annu. Rev. Biochem.* **44,** 639–667.
26. Steven, A. C., Couture, E., Aebi, U. & Showe, M. K. (1976) *J. Mol. Biol.* **106,** 187–221.