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Structure of a bacterial photosynthetic membrane

(*Rhodopseudomonas viridis*/thylakoid membranes/optical transforms/freeze-fracture electron microscopy)

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ABSTRACT **The internal photosynthetic membranes of a photosynthetic bacterium, *Rhodopseudomonas viridis*, have been studied with a variety of electron microscope techniques. The membranes are composed of a sheet of apparently identical subunits arranged in a hexagonal fashion. The individual subunits repeat at a distance of 110 Å. Optical transforms have been used to enhance micrographs of this ordered membrane, and the images synthesized in this way show details of each subunit. The individual subunits are asymmetric, differing slightly in appearance at the outer and inner surfaces of the membrane, and these surface patterns seem to be combined in the image of the thylakoid membrane in negative stain. These studies fix a maximum size for the photosynthetic unit of *R. viridis* and suggest the suitability of this membrane for further diffraction analysis.**

The light reaction of photosynthesis takes place in specialized membranes found in photosynthetic bacteria and blue-green algae and in the chloroplasts of green plants and eukaryotic algae. The structural organization of these membranes in eukaryotic systems has been intensively studied, and several general models for the organization of the photosynthetic membrane have been suggested (see refs. 1–3 for reviews). However, no general pattern for the organization of the prokaryotic photosynthetic membrane has been reported, and there have been few attempts to study the architecture of either bacterial or algal prokaryotic photosynthetic membranes in a systematic fashion.

This report will deal with the architecture of the photosynthetic membranes of *Rhodopseudomonas viridis*, a photosynthetic bacterium. This organism has been chosen for study because of an earlier report by Giesbrecht and Drews (4) that the thylakoid membranes of this species displayed hexagonal symmetry. This, in principle, should allow the use of image analysis techniques (5) to enhance the results of electron microscopy and obtain detailed structural information about this photosynthetic membrane. In this study, several electron microscope techniques have been used to observe the *R. viridis* thylakoid membrane, and from these different techniques a combined model for the architecture of the membrane has been developed.

MATERIALS AND METHODS

Cultures of *R. viridis* were kindly provided by John Olson of Brookhaven National Laboratory. They were grown anaerobically at 30°C, illuminated by a 40-W light source. The growth medium consisted of 0.5% yeast extract (Difco), 0.05% $MgSO_4 \cdot 7H_2O$, 0.01 M potassium phosphate buffer at pH 7.0, and 0.01 M ammonium succinate. The final pH of the medium was adjusted to 7.0 before autoclaving.

Thylakoid membranes were isolated from cells washed in 5 mM phosphate buffer, pH 7.0. Washed cells were broken by grinding for 30 min in a glass bead homogenizer at 4°C. The cell homogenate was centrifuged at 5000 rpm for 5 min to remove cellular debris, and the supernatant from this spin was centrifuged for 30 min at 15,000 rpm to pellet the photosynthetic membranes. All centrifugation steps were done with a Sorvall SS-34 rotor.

For thin-sectioning, intact cells were fixed for 30 min in 3% (vol/vol) glutaraldehyde in 0.1 M phosphate buffer, pH 7.5. Cells were rinsed twice in the buffer and postfixed in 2% OsO_4 in the same buffer for 1 hr. The specimens were rinsed twice in distilled water, and the cells were dehydrated in a graded series of acetone solutions and embedded in Spurr's resin. Sections were cut with glass or diamond knives.

Intact cells were prepared for freeze-fracturing by gradual infiltration with glycerol to a concentration of 25%. Isolated membranes prepared in the 5 mM phosphate buffer were used for deep-etching experiments. Such samples were frozen in liquid Freon 22, and replicas were prepared in the usual manner (6) in a Balzers freeze-etching device. Freeze-fracture replicas were made at −100°C without etching, and deep-etch replicas were prepared at −100°C with 5 min of etching to expose membrane surfaces.

Isolated membranes were spread on carbon-coated Formvar films for negative staining, and uranyl acetate (2% aqueous) was applied directly to the samples. Stained samples were air dried.

All micrographs were made with a Philips 300 electron microscope operating at 80 kV, and magnifications were calibrated with the use of a grating replica.

Optical transforms were recorded and filtered images were prepared on an optical bench according to the methods of deRosier and Klug (5). Some filtered images were also prepared by digital techniques (7).

RESULTS

The thylakoid membranes of *R. viridis* are lamellar in nature. Fig. 1*A* shows the general disposition of the thylakoid membranes in the organism. *En bloc* staining techniques reveal the periodic nature of the membrane in grazing sections (Fig. 1*B*). This periodic character is not observed in thin section unless the cells have been extensively stained with uranyl acetate.

The freeze-fracturing technique has been shown in other systems to split biological membranes and reveal internal details of membrane organization (8). Replicas of freeze-fractured *R. viridis* show membrane fracture faces on which particles measuring approximately 130 Å in diameter are visible against

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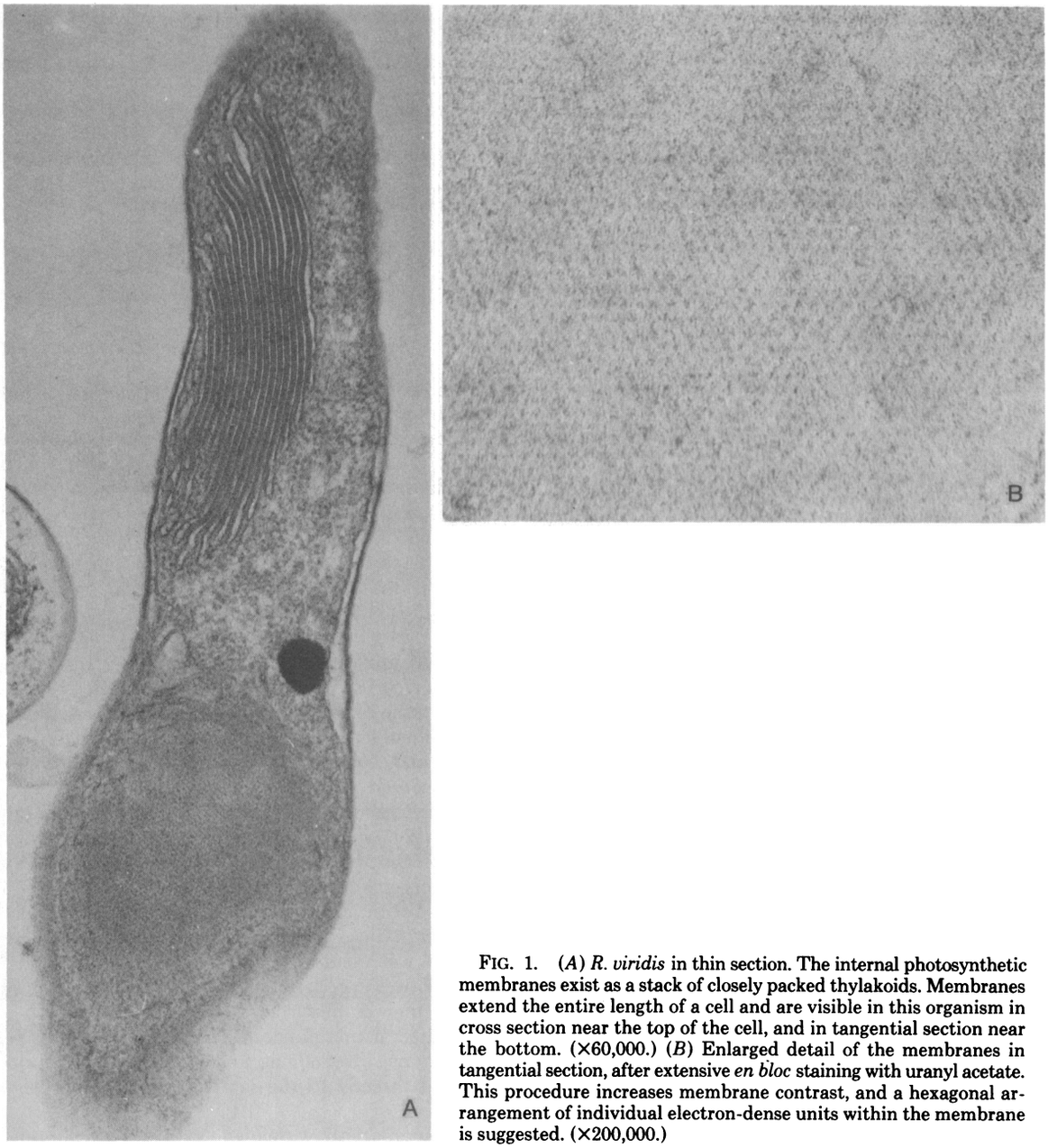

FIG. 1. (*A*) *R. viridis* in thin section. The internal photosynthetic membranes exist as a stack of closely packed thylakoids. Membranes extend the entire length of a cell and are visible in this organism in cross section near the top of the cell, and in tangential section near the bottom. (×60,000.) (*B*) Enlarged detail of the membranes in tangential section, after extensive *en bloc* staining with uranyl acetate. This procedure increases membrane contrast, and a hexagonal arrangement of individual electron-dense units within the membrane is suggested. (×200,000.)

a relatively smooth background matrix. However, under good shadowing conditions (Fig. 2*A*) a distinct hexagonal pattern is observable in this matrix, although the distribution of particles on the matrix is still apparently random.

When isolated biological membranes are frozen in dilute buffer solutions, ice can be sublimed from the frozen surface prior to the formation of a metal replica. In this manner the surfaces of the membrane can be exposed for study. Two slightly different surfaces were exposed in this way (Fig. 2 *B* and *C*), and these seem to correspond to the inner and outer surfaces of the *R. viridis* thylakoid membrane. The highly ordered nature of the membrane is evident at once in these micrographs, as well as the slight differences between the inner and outer surfaces of the membrane. Although the essential lattice pattern is the same on both surfaces, the particles within the hexagonal lattice are distinctly smaller on the inner membrane surface than those observed within the lattice on the outer membrane surface. Interestingly, the hexagonal membrane lattice is far more evident in these surface views of the membrane than in replicas made from freeze-fracture preparations (Fig. 2*A*).

Membranes examined by means of the negative-staining technique (Fig. 3) display the greatest amount of structural detail. The hexagonal lattice is clearly visible, and some substructure within each of the subunits can also be observed. An optical diffraction pattern obtained from this micrograph is shown in Fig. 3*B*. The regular nature of the membrane lattice makes it possible to form filtered images of the membrane according to the methods of deRosier and Klug (5), and such an image is shown (along with an unprocessed image in Fig. 3*C* for comparison) in Fig. 3*D*. Each unit contained in the hexagonal lattice contains a central region of low electron density. Due to the nature of the negative staining technique, it is quite likely that each of these regions represents surface structures on the membrane that are capable of excluding the electron-opaque stain.

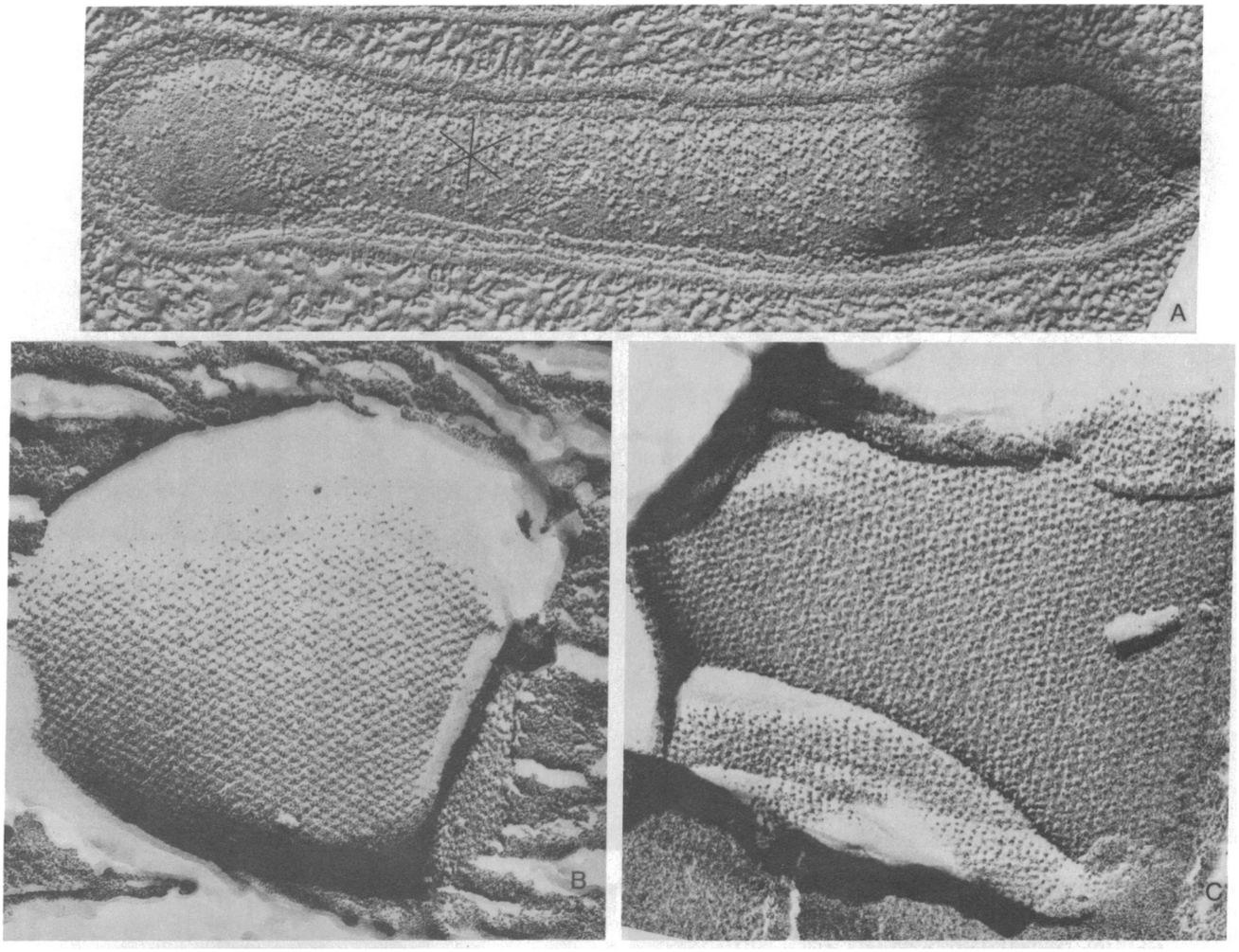

FIG. 2. (*A*) Freeze-fracture preparation of *R. viridis* cells. The fracture face of a thylakoid occupies most of the area of this cell, and small particles measuring approximately 130 Å in diameter are visible on the membrane fracture face. Although the particles are randomly distributed, the hexagonal lattice of the membrane is visible in the background, as marked on the micrograph. (×67,000.) (*B*) Outer surface of the thylakoid, exposed by a sublimination of frozen buffer during deep-etching. A regular lattice of protrusions from the outer surface of the membrane is evident. (×120,000.) (*C*) Internal surface of the membrane, exposed by deep-etching. The hexagonal lattice of subunits is similar to that on the outer surface (*B*), but the diameter of individual particles seems to be smaller (averaging 75 Å, whereas the particles on the outer surface measure the full 110 Å repeat distance). The inner and outer surfaces can be identified on the basis of their relationship to the surrounding buffer and other membranes. (×120,000.)

DISCUSSION

The crystalline nature of *R. viridis* thylakoid membrane is evident in all of the micrographs presented here. It is this repeating structure that makes the *R. viridis* thylakoid membrane such a promising subject for ultrastructural analysis. Nevertheless, it is clear that this regular pattern of organization is not typical of photosynthetic bacteria. My own search of the literature supports the observation of Drews (9) that the lattice structure of the *R. viridis* thylakoid is unique among photosynthetic bacteria that have been studied to date. Despite this fact, many spectroscopic and bioenergetic studies of the *R. viridis* thylakoid membrane support the contention that the photosynthetic characteristics of this thylakoid are typical of photosynthetic bacteria (10–12). It seems quite likely, therefore, that the *R. viridis* thylakoid will serve as a useful model for the structural analysis of the bacterial photosynthetic membrane.

In this study, I have presented micrographs of the *R. viridis* thylakoid membrane obtained from four very different electron microscope preparation techniques. The regular lattice of structural units in the membrane makes the comparison of images obtained in this way much easier, and also makes possible diffraction analysis of high-resolution micrographs. Thin sections of the membrane stained *en bloc* with uranyl acetate show periodic regions of high electron density in a regular hexagonal pattern (Fig. 1*B*). Several studies have implicated uranyl acetate in staining reactions with protein molecules (13–15), and it is possible that the differential pattern of staining that makes a hexagonal lattice visible is due to a high density of protein molecules in regular positions in the membrane lattice.

Metal replicas of both surfaces of the membrane show protrusions from the membrane surface in line with the hexagonal lattice (Fig. 2 *B* and *C*). These seem to be of slightly different shapes, with the particles visible on the outer surface of the membrane being somewhat wider than those on the inner surface. This difference in surface structure seems to be reflected in the image of the membrane obtained from negative staining. The filtered image of the membrane (Fig. 3*C*) shows two regions of low electron density in each unit of the membranes. Because the mechanism of negative staining superimposes the surface structure of each side of the membrane in the

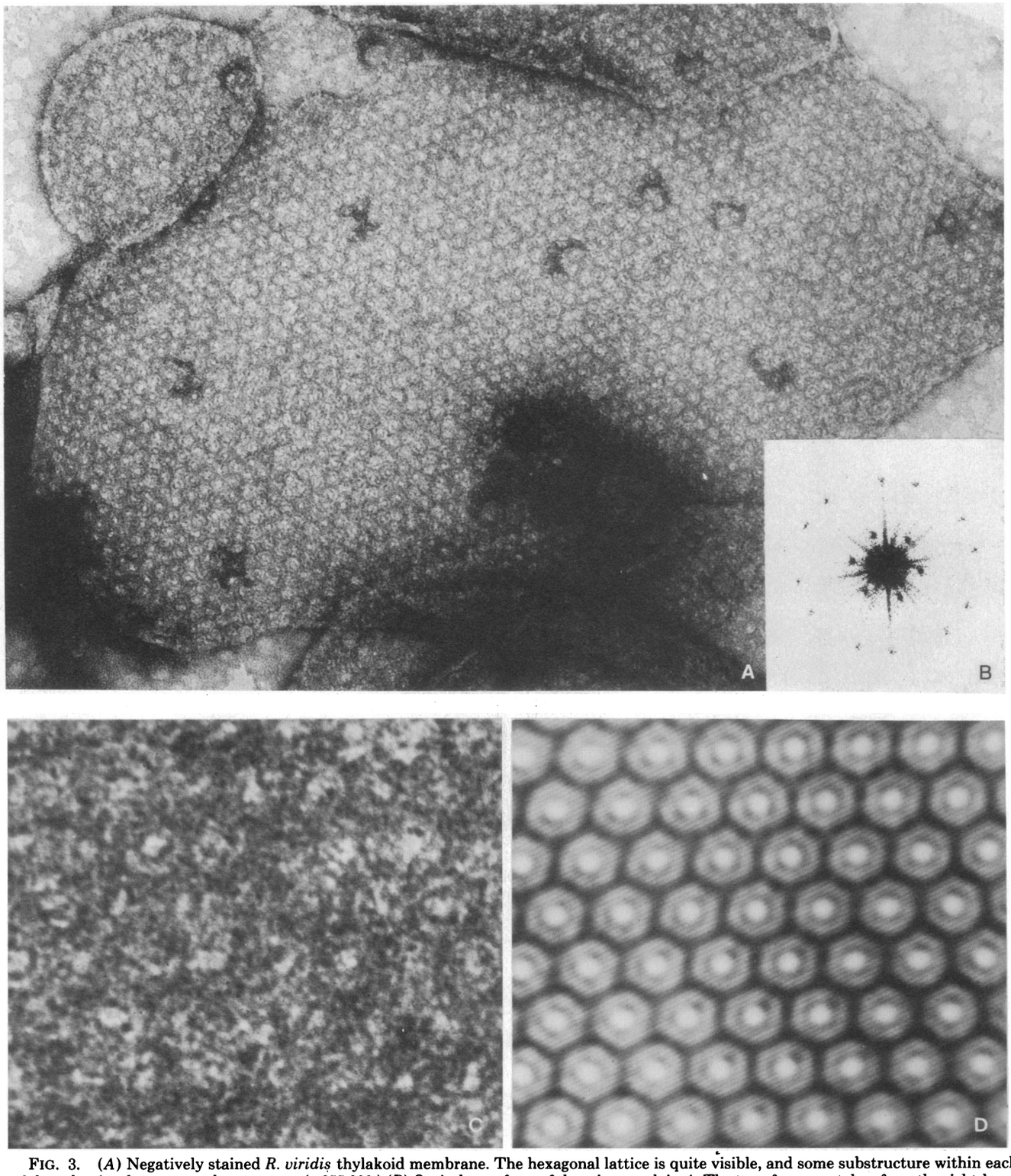

FIG. 3. (*A*) Negatively stained *R. viridis* thylakoid membrane. The hexagonal lattice is quite visible, and some substructure within each of the subunits also seems to be apparent. (×255,000.) (*B*) Optical transform of the micrograph in *A*. The transform was taken from the right-hand side of the micrograph, from an area approximately 15 units in diameter delineated by a circular mask (15 units = 15 × 110 Å repeat distance). Third-order diffusion maxima indicating a resolution of 32 Å are observable. (*C*) Higher magnification view of the negatively stained membrane shown in *A*. (×1,000,000.) (*D*) Digitally filtered image obtained from *C* with the use of the transform (*B*). Central regions of low electron density are surrounded by rings (also of low density) that display hexagonal substructure. The center-to-center distance is 110 Å.

final image, this image reflects a composite of both membrane surfaces. In Fig. 4 I have illustrated how this might in fact take place, namely, how the membrane images obtained from negative staining might be reconciled with the surface views obtained from shadowing.

Freeze-fracturing presents an initial problem, owing to the fact that the lattice structure is barely visible in freeze-fractured membrane preparations (Fig. 2*A*). However, the regular lattice of the membrane may provide an answer to how such images are obtained. The passage of a fracture plane through the

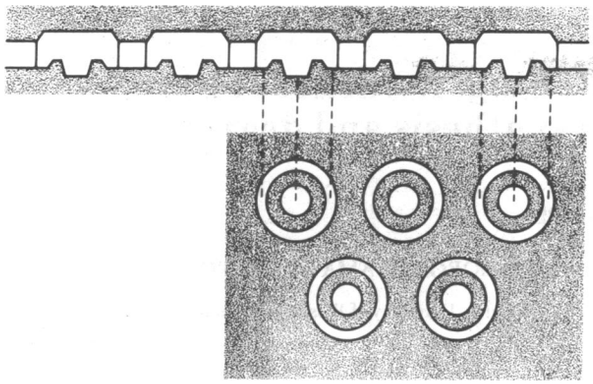

FIG. 4. All images of the membrane, thin sections, freeze-fracture and deep-etch replicas, and negative staining, seem to be consistent. This diagram illustrates how differences in structure at both surfaces of the membrane (which are indicated by the results of deep-etching) account for the unusual image of the *R. viridis* thylakoid seen in negative staining.

central regions of a biological membrane occurs primarily because this region is held in place under normal circumstances by hydrophobic interactions, which are weakened at low temperatures (8, 16). One might therefore expect particles to be observed on membrane fracture faces in regions where transmembrane structures are held together by other than hydrophobic forces (such as electrostatic, ionic, and covalent bonds). The organization of regular subunits within the *R. viridis* thylakoid membrane may be such that strong covalent or electrostatic forces hold together all of the macromolecules making up a single structural unit in the hexagonal lattice, and hydrophobic interactions are important only in maintaining the attachment between neighboring structural units. The fracturing process would then be expected to separate adjacent subunits in a more or less random fashion, leaving a fracture face in which particles were randomly distributed on the hexagonal surface of an underlying membrane.

From each of these approaches a single view of the *R. viridis* thylakoid membrane is obtained, and by pooling the observations a three-dimensional view of a single membrane subunit can be suggested (Fig. 5). Using 50 Å for the thickness for the membrane and 110 Å as the diameter of the hexagonal unit, a total volume for such a subunit of approximately 390,000 $Å^3$ can be calculated. On the basis of an approximate density of 1.4 g/cm^3, a mass for the entire subunit of 350,000 daltons is estimated. The polypeptide composition of this membrane is not known at this writing; however, if the *R. viridis* reaction center polypeptides and light-harvesting components prove to be similar to those of other photosynthetic bacteria, the entire

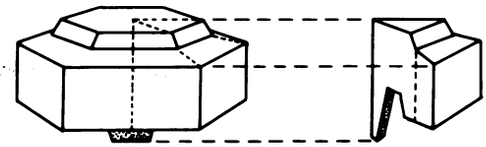

FIG. 5. Individual subunits within the membrane of this photosynthetic bacterium seem to have sixfold symmetry. An approximate volume for such a structure can be calculated, and a mass of 350,000 daltons can be estimated.

reaction complex of the membrane should be easily contained within each of the structural subunits of the *R. viridis* thylakoid membrane.

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