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Permalink
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Publication Date
1977-09-01

UC-63

LBL-6758
Preprint   c.1

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September 1977

LBL-6758
c.1

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PHOTOSYNTHETIC MEMBRANES

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The challenge of collecting light from the sky efficiently and economically is becoming increasingly urgent for our energy-consuming society. The phenomena of green plant photosynthesis are frequently cited as the response to this challenge developed via biological evolution. The performance of photosynthetic organisms in gathering sunlight and converting it into chemical free energy is enormously impressive when compared with the best efforts of technology to date. What are the lessons that we can learn from careful examination of natural photosynthesis? Can these lessons be applied in designing and constructing solar energy devices to provide future generations with convenient, portable and economic forms of power? Can we accomplish these objectives without prohibitive costs in terms of initial investment, consequences to ecology and the environment, or major disruptions of the organization of society and the distribution of populations? These are some of the questions that are being addressed by the planners of new programs to meet the energy needs of the future.[1]

Before describing how photosynthetic organisms accomplish the task of converting photons of visible light into energy-rich chemical products (wood, foodstuffs and eventually coal and petroleum), it is meaningful to explore a familiar analogy. Persons acquainted with astronomy are well aware that, while a pinhole camera can be used to make an acceptable picture of the heavens, large diameter telescopes that increase enormously the capture cross-section for incoming light are required to enable very weak or very distant stellar objects to be recorded. Telescopes the size of the largest reflectors in California (5.1 meter diameter) or in the USSR (6.0 meters) have reached nearly the physical limit of such devices, however. Astronomers are proposing a new generation of more powerful optical telescopes

constructed as arrays of smaller collectors that send their signals in
a coherent fashion to a computer for processing and integration. It is
probably no accident that the absorption, collection and utilization of
light by plants and photosynthetic bacteria has evolved into a system that
bears a remarkable similarity to the astronomers' scheme.[2]

Photosynthetic Energy Conversion

The light reactions that characterize photosynthesis are photoredox
reactions that convert the electromagnetic energy of light into chemical
free energy, ultimately in the form of biochemical substances--carbohydrates,
fats, proteins, nucleic acids, etc.--that make up the plant itself. The
rate of this photochemistry increases with increasing light intensity,
but as for enzymatic reactions there is a saturation level that is reached
when the influx of photons exceeds the turnover rate of the slower bio-
chemical steps in the complex electron transport chain. The photochemical
events involve the transfer of electrons one at a time from chlorophyll
(Chl) molecules to electron acceptors. If each Chl present in the chloro-
plast had this photochemical role to play, then the situation would be
analogous to an astronomer using a pinhole camera. In fact, plants and
photosynthetic bacteria are much more frugal with their complex and expensive
electron transport machinery, because only 1% or less of the Chl is typically
committed to the photo "reaction centers". The remainder serves as a chemically
passive antenna that collects additional light by absorbing photons and
transferring the resulting electronic excitation to the reaction centers.
This clearly permits photosyntheis to occur efficiently at much lower light
intensities than would be true if each reaction center had only its intrinsic

Chl to gather light. The analogy with the telescope lens or reflector is straightforward.

Beyond this simple analogy, however, there is increasing evidence that arrays of photosynthetic units in intracellular membranes act cooperatively to generate transmembrane electric fields and to pump ions from one side to the other.[3] The fields and ion gradients provide an important part of the chemical potential necessary to drive the endergonic biosynthetic reactions. The detailed ordering of these membrane arrays and the nature and interactions of the subunits are the subject of intensive investigation.[4]

Membrane Organization and Function

It is of major importance that in all known photosynthetic organisms the light reactions occur within membranes inside the cells. The membranes are complex biochemically, for they contain the chlorophyll pigments, the reaction center components and most of the molecules of the electron transport chain.[5] In addition, there are accessory pigments like carotenoids and phycobilins, a variety of colorless lipids, quinones that participate in electron transport, and an array of proteins that incorporate an assortment of several dozen distinct polypeptide chains. Furthermore, these proteins include functional prosthetic groups such as the hemes of cytochromes, iron-sulfur centers in ferredoxins and copper in plastocyanin. Manganese atoms, bound in an unknown way into the membranes, are essential to the mechanism of molecular oxygen evolution in higher plants.

The "minimum molecular weight" of a photosynthetic unit of photosynthetic bacteria is about 0.7×10^6 daltons; in higher plants it is nearly 2×10^6 daltons. The concentration of chlorophyll pigments, which constitute over 5% by weight of the membranes, approaches 0.1 molar. The absorption

of light by these membranes is sufficiently great that stacks of 20 or

so thylakoids (enclosed internal membranes of chloroplasts) that occur

in the grana of chloroplasts (see Fig. 1) appear to be black in the light

microscope. The low level of light that penetrates the leaf canopy of

a mature forest is a measure of the thoroughness with which photons are

captured.

Electron microscopy and a variety of biochemical studies demonstrate

that the internal membranes seen in Fig. 1 are closed surfaces like flattened

balloons, and they serve to separate inside and outside spaces. (The terms

"inside" and "outside" are used here in a relative sense; both spaces are

apparently completely internal to the chloroplast or to the bacterial cell.)

Electron microscopy of the surfaces of the membranes (Fig. 2) shows distinctions

in surface structure that characterize the different faces.[7] These differences

are supported by studies of the localization of biochemical reactivity

or of accessibility to labelling reagents.[8,9] One consequence of the inside-

outside polarity is the ability of photosynthetic membranes to generate

trans-membrane electric fields and to pump ions upon illumination.[10,11]

Because of the transport of H^+ from the outside to the inside of the thylakoids,

differences of more than 2.5 pH units can be built up across the membranes.

The electric potentials generated[12] appear to reach magnitudes of 100 milli-

volts, and together with the ion gradients they constitute an important

form of stored chemical potential. The fields and ion gradients are thought

to be the driving force for the phosphorylation of ADP to ATP, which is

one of the major energy-storing accomplishments of the photosynthetic light

reactions.

Several of the thylakoid surfaces visualized in Fig. 2 show evidence
of containing particles, some of them in regular two-dimensional arrays.
It is tempting to relate these to the components of the light reactions,
especially because they appear to be about the right size (18.5 x 15.5
x 10 nm in chloroplast membranes) to correspond to the photosynthetic units
as defined in kinetic experiments. Their detailed structure lies beyond
the limits of resolution of the electron microscope, however, and at present
it is necessary to use more indirect physical and optical spectroscopic
methods to probe the molecular arrangement within the photosynthetic membranes.

Absorption Spectra

The chlorophyll, carotenoid and other pigment molecules present in
photosynthetic membranes tend to dominate studies using optical absorption,
fluorescence and circular dichroism spectroscopy. Reactive molecules such
as cytochromes or quinones exhibit absorption that is usually several orders
of magnitude weaker in intensity than that of the chlorophylls. As a conse-
quence, in the important studies designed to determine their role in the
photoreactions, the "active" species are usually observed by sensitive
difference spectroscopy (light minus dark, oxidized minus reduced, etc.).

The chief photosynthetic pigments--chlorophylls, carotenes and xantho-
phylls--occur in the membranes as distinct molecules, not covalently linked
to other membrane components. This is demonstrated by the ease with which
the pigments can be extracted intact by organic solvents or certain detergents.
At the same time the molecules are not distributed uniformly through the
membrane as in a simple solution. They occur in well-defined environments
such that, using more gentle methods, they can be dissected from the membrane

with their surroundings essentially intact.[4] It is from studies of such
membrane subunits that we are beginning to learn the nature of the chief
building blocks.

When chlorophyll is extracted from photosynthetic membranes into
an organic solvent, there are distinctive changes in the absorption and
fluorescence spectra, a several-fold increase in fluorescence yield and
a dramatic alteration in the circular dichroism.[2] The most extensive and
interpretable changes occur between 650 and 750 nm for the chlorophylls
(Chl a and Chl b) of higher plants and between 750 and 1050 nm in the near
infrared for the bacteriochlorophylls (BChl a and BChl b) of the purple
photosynthetic bacteria. The molecular structures of the two most important
pigments are shown in Fig. 3. In each case the absorption bands in the
long wavelength region, designated Q_y bands by spectroscopists, occur at
longer wavelengths than for the same molecules in solution. The magnitude
of the shifts is best appreciated on an energy scale. For Chl a in a solvent
such as diethyl ether the Q_y band occurs at 15,100 cm^{-1}, whereas in chloro-
plast membranes it is decreased in energy by 300 to 800 cm^{-1}. (In crystalline
Chl a the decrease is as large as 1600 cm^{-1}, but there is no evidence for
the presence of crystalline chlorophylls in vivo.) For BChl, where the
Q_y band occurs at 13,000 cm^{-1} in ether solution, the absorption changes
are larger and more dramatic. Not only are large shifts observed, by as
much as 1600 cm^{-1} and 3200 cm^{-1} in bacteria containing BChl a and BChl
b, respectively; but the Q_y transition exhibits multiple components in
the absorption spectra of the bacterial membranes (Fig. 4A). Similar absorp-
tion band splittings are seen for chloroplast membranes, but the components
overlap strongly and are more difficult to resolve. It has been proposed
that these multiple bands simply reflect Chl or BChl molecules in separate

and different environments in the membranes.[13,14] However, there is a more intriguing likelihood that they arise from interactions of an excitonic nature between neighboring chlorophyll molecules and, hence, reflect the energy and geometry of the interacting pigment molecules in a much more specific way.[15]

Circular Dichroism

To resolve this question we have applied circular dichroism (CD) spectroscopy, which indicates the molecular interactions more directly. The differential absorption of left- and right-circularly polarized light $(\Delta A = A_L - A_R)$ occurs only when there is chirality (asymmetry) in the system. The (bacterio)chlorophyll molecules all possess intrinsic chirality owing to the presence of asymmetrically substituted carbon atoms, as shown in Fig. 3. As a consequence, solutions of the chlorophylls all exhibit circular dichroism. The CD spectra of chlorophylls in polar organic solvents exhibit two characteristic features: (1) the intensity of the circular dichroism, ΔA, is small relative to the absorbance, A, at any wavelength, with $\Delta A/A$ characteristically in the range $(0.6 - 2.6) \times 10^{-4}$; and 2) the shape of each CD spectral band resembles that of the corresponding absorption band, although the CD may be either positive $(A_L > A_R)$ or negative $(A_L < A_R)$ for each band.[16]

CD spectra can be measured also for suspensions of membranes derived from chloroplasts or photosynthetic bacteria. (There are problems in looking at whole leaves or intact chloroplasts because of distortions owing to the strong light-scattering by such materials.[17]) For the same overall concentration of the chlorophylls, the CD signals of the membranes are typically larger, $\Delta A/A = (5 - 20) \times 10^{-4}$, and have more complex band shapes

than those observed for simple solutions of the same pigments. As seen

in Fig. 4B, there are characteristically one or more zero-crossings (where

$\Delta A = 0$) within the band envelope, such that the CD changes sign from one

portion to the next. Such features are known to be a consequence of dipole-

dipole coupling between similar or identical chromophores that are asym-

metrically arranged near one another.[13] In other words, it is evidence

for aggregation of chlorophylls within the membranes. The same qualitative

features (increased CD amplitudes and zero-crossings) are observed for

Chl or BChl dimers in solution,[15,16] for example, although analysis of

the spectra indicates that such solution dimers do not occur in vivo.

More generally, the number of zero-crossings within the band envelope and

the relative amplitudes of the different components are direct and sensi-

tive indicators of the number of molecules interacting and, more partic-

ularly, of their relative orientation and arrangement within the aggregate.

A Bacteriochlorophyll Protein Complex of Known Structure

Recent studies provide strong evidence that most of the chlorophyll

in vivo occurs in an aggregated form that is closely associated with and

probably surrounded by protein.[4,19] The clearest example of this is a water-

soluble BChl-protein from green photosynthetic bacteria that was isolated

and crystallized by Olson and coworkers.[20] This is an antenna component

that is unusual in that it is not membrane bound. Detailed analysis of

the X-ray diffraction[21] of the crystals shows that the molecule consists

of 3 identical subunits, each containing 7 BChl molecules, for a total

of 21 BChl in a molecular weight of 145 kd. The BChl molecules are collected

together inside a thin coat of protein, mostly single-layered in an anti-

parallel β sheet structure--much like marbles in a sack. The center-to-

center distance between nearest neighbor BChl molecules within one sub-
unit is 1.2 - 1.4 nm, which is rather large compared with that in chloro-
phyll crystals. It appears that the central Mg atoms are coordinated by
protein side chain groups or water, rather than by other BChl molecules
in the complex.

The absorption and CD spectra, particularly at low temperature, for
this BChl-protein[22] show multiple components and zero-crossings, respec-
tively, within the Q_y band envelope. (Fig. 5) Now that the detailed arrange-
ment of the pigment molecules is known, it is possible to calculate the
spectroscopic properties to calibrate the quantum mechanical models of
the excitonic interactions. Preliminary calculations show that the observed
band splittings are reasonable for exciton coupling at the known distances
of separation in this BChl-protein.[22]

Photosynthetic Membrane Components

During the past decade the dissection of photosynthetic membranes
into their components has revealed a heirarchy of structure. Certain proteins
that are loosely attached can be largely removed by washing with aqueous
buffers. For chloroplast membranes these lightly bound species include
some of the carbon-fixing enzymes, a portion of the copper-protein plasto-
cyanin and the "soluble" plant ferredoxin. Addition of a chelating agent
like EDTA serves to detach the phosphorylation coupling factor[23,24] a
large (325 kd) complex of 8 or 9 peptides that appears to be located on
the "outer" surface of the membranes and linked by a bridge that requires
the presence of divalent cations. Selective use of organic solvents, depend-
ing on their polarity, serves to induce differential extraction of the
carotenes, quinones, xanthophylls, chlorophylls, and the colorless lipids.

In some of these cases the limited extent of membrane disruption can be demonstrated through the reconstitution of "activity" by adding back the missing component.[25]

The most widely used approach of membrane dissection involves the use of detergents or other amphiphilic compounds. These agents presumably substitute for the normal lipid-protein interactions that hold the membrane together. By using Triton X-100 or lauryl dimethylamine oxide (LDAO) to treat the intracytoplasmic membranes of photosynthetic bacteria, for example, Clayton and coworkers[26,27] were able to obtain preparations of reaction centers as isolated protein complexes free from the antenna pigments. These reaction center complexes retain the ability to carry out the primary photochemistry. Further studies showed that they contain 4 BChl and 2 BPheo per center (BPheo, bacteriopheophytin, is identical to BChl, but with the central Mg atom replaced by two hydrogens), three peptides with molecular weights of 21, 23, and 28 kd, ubiquinone molecules and an iron atom,[28] as illustrated in Fig. 6. Reaction centers can be obtained with or without attached cytochromes; either way they are able to carry out the light reactions with high efficiency.

The majority of the BChl, which constitutes the antenna array of the membranes, appears in a separate BChl-protein complex that has no photo-chemical activity.[19] In most cases involving detergent treatment a portion of the pigment (up to 1/3) is unavoidably solubilized free of protein. In at least one case this appears to derive from an unstable complex that dissociates slowly during the separation procedure,[4] but there remains the possibility that some of the pigment pool occurs in the membrane not in direct association with protein.

In the case of chloroplast membranes a clean separation of the antenna pigment from the reaction centers has not yet been accomplished. Nevertheless, Chl-proteins with well-defined stoichiometries have been obtained especially by Vernon,[29] Boardman,[30] Thornber[4] and coworkers. Some of these contain reaction centers, and others appear to be portions of the antenna without photochemical activity. There is increasing evidence that these Chl-proteins exist as complexes in the native membranes and are not simply artefacts resulting from rearrangements during the detergent treatment. This is easiest to demonstrate for the particles that contain active reaction centers, but Lynn Austin in my laboratory at Berkeley has used CD spectroscopy to demonstrate that it is probably true for antenna BChl-proteins as well.[31]

Reaction Centers and Antenna Bacteriochlorophyll-Proteins

Our studies involved two variants of the photosynthetic bacterium Rhodopseudomonas spheroides. We used the wild-type organism and a mutant species (designated R-26) that is missing the carotenoids spheroidene and spheroidenone, which occur in addition to BChl in the antenna complement of the wild-type organism. Because these carotenoids absorb light in the visible region between 400 and 520 nm (Fig 4A), the two organisms have a markedly different appearance in culture flasks--the wild-type is deep purplish-red and the mutant is blue-green in color. The resulting differences in absorption spectra are not restricted to the carotenoid regions, however, for there are significant alterations in the Q_y transition region of BChl absorption. While the main absorption occurs between 850 and 860 nm in both cases, the wild-type organism has an additional peak at 800

and a shoulder at 870 nm (Fig. 4A) that are missing in the mutant. Nevertheless, the CD spectra are quite similar in this region. In each case there is a large double CD that is negative at long wavelengths, has a zero crossing near the absorption maximum at 850 nm and becomes positive on the short wavelength side. (See Fig. 4B for the wild-type CD spectrum.) The magnitude of $\Delta A/A$ for these components is about 8×10^{-4}, which is about 10 times that for BChl in organic solvents.

Austin used a detergent treatment somewhat modified from that of Clayton and Wang[27] to prepare fractions containing purified reaction centers or antenna BChl-proteins. The absorption and CD spectra of the reaction center complexes have been studied in detail,[32-34] and they provide evidence of extensive excitonic interactions among the associated BChl and BPheo molecules. (Fig. 7). Not only are there resolved absorption bands at 860, 800, and 760 nm, but the 800 nm band is complex, with a shoulder seen at 810 nm at low temperature,[35] and with zero-crossings in the CD spectrum. Furthermore, these features change when the reaction centers are photoactivated to initiate electron transfer.[32] In the intact chromatophores or bacteria the spectral contributions of the reaction centers are not apparent because they contribute only a few percent to the absorption and the CD.

The antenna BChl-protein fraction exhibits distinctive absorption and CD spectra (Fig. 8) that are quite different from those of the reaction center fraction but resemble the correspinding spectra of the chromatophore fraction. This is true for both the mutant and the wild-type organism. Each antenna BChl-protein has a strong absorption maximum near 855 nm

associated with a double CD feature, negative to long wavelength. The wild-type antenna BChl-protein has the absorption band at 800 nm that is missing in the mutant; the 800 nm band is associated with a weak negative single CD.[31] Because of the close correspondence of the spectral features we feel that the arrangement of the BChl molecules in these isolated antenna complexes is essentially unchanged from the in vivo configuration.

The antenna BChl-proteins appear to be quite simple in structure. Austin found that each contains two copies apparently of a single polypeptide of about 8.5 kd. The molecular weight of the polypeptide was confirmed by polyacrylamide gel electrophoresis, amino acid analysis, sedimentation velocity and gel filtration. (Tonn, et al. recently reported two different peptides associated with the antenna from the wild-type of Rps. spheroides.[35] We do find that the intact BChl-protein contains two polypeptide chains per complex, but we have been unable to resolve molecular weight differences between them, either for the wild-type or the mutant organism.[31]) Associated with the two peptide chains there are two BChl molecules in the R-26 mutant and three BChl molecules in the wild-type antenna BChl-protein. Although they are not covalently attached, the BChl molecules do appear to be inside the protein and not readily accessible by solvent.

The differences between the absorption and the similarities between the CD spectra of the two complexes can be related nicely to the stoichiometry differences by assuming that both the mutant and the wild-type complexes contain a pair of closely-coupled BChl molecules responsible for the 855 nm absorption band and the associated double CD, and that the additional BChl molecule in the wild-type complex is responsible for the 800 nm absorption band seen in its spectrum. The evidence suggests that the latter molecule

does not interact strongly with the other two, either because it is peripheral or because its orientation is unfavorable. In fact, it seems remarkable that such a small complex (20 kd) can so readily delete one of the 3 BChl molecules in the wild-type antenna protein without causing profound structural changes and altering the relation of the other two BChl to one another. Another puzzle is the nature of the genetic mutation that puts a block in the terminal stages of carotenoid synthesis and, at the same time, deletes one of three BChl molecules from each antenna complex.

A Pebble-Mosaic Membrane Model

An important guide to current thinking about membrane structure is the fluid-mosaic model formulated by Singer and Nicolson[37] in 1972. In this view active biological membranes consist of a basic lipid bilayer in which the membrane proteins are partially or wholly immersed, depending on the extent and distribution of the hydrophobic amino acid side chains. Some membrane proteins appear to protrude through both surfaces of the lipid matrix, some through only one, and others have no demonstrable accessibility from the aqueous phase. The entire arrangement is "fluid" in the sense that individual proteins are relatively free to move about in two dimensions in the lipid bilayer matrix.

The photosynthetic membranes are unusual in having a relatively low content of lipids (27% in the case of chromatophores from Rps. spheroides) involved in the bilayer structure.[38] Furthermore, they often show extensive arrays of ordered particles (Fig. 2) that appear to be protein complexes. To pictorialize these properties of chloroplast membranes I formulated the Pebble-Mosaic Model,[2] which is a variation of the Singer-Nicolson model. It is relevant to chloroplast thylakoids, to the intracytoplasmic membrane of photosynthetic bacteria, to the bacteriorhodopsin-containing purple

membrane of <u>Halobacterium</u> <u>halobium</u>, to rod-outer segments of visual cells, to mitochondrial intracytoplasmic membranes--in fact, to any membranes where interprotein interactions are dominant.

In the case of <u>Rps.</u> <u>spheroides</u> we consider the intracytoplasmic membrane to consist of a two-dimensional array or mosaic of individual photosynthetic unit complexes. An illustration of this model is shown in Fig. 9. Each of these complexes consists of a defined set of components (antenna BChl-proteins, cytochromes, quinones, etc.) associated with a reaction center and joined together by the lipid matrix. The fact that the ordered arrays of particles occur irregularly may mean that the photosynthetic membranes retain a certain aspect of fluidity as the arrays break up and reform.[39]

A listing of the major known membrane-bound components for <u>Rps.</u> <u>spheroides</u> is given in Table I, with approximate stoichiometries and molecular weights for each. Put together, these make a complex of about 750 kd, which corresponds rather closely to the estimated weight (650-800 kd) of sub-chromatophore particles that can be visualized in the electron microscope.[40]

The arrangement of the components is critical for efficient photosynthetic energy conversion. The BChl of the antenna must be able to transfer energy efficiently to the reaction centers. One direct indicator of this transfer is the fact that BChl fluorescence is much more depolarized coming from chromatophores than from the isolated BChl-proteins.[31] Furthermore, a number of experiments suggest that there may be excitation transfer not only within, but also between adjacent photosynthetic unit complexes.[41]

Orientation of Reaction Centers in the Membranes

An important aspect of membrane structure is the orientation of the
components and their disposition with respect to the inside and outside
surfaces. Studies using polarized light show that, not only are the BChl
antenna pigments oriented with respect to the membrane plane,[42] but the
reaction center pigments are as well.[43] In the case of chloroplast membranes,
Anne McGuire, Charles Dismukes, and Robert Blankenship in my laboratory
at Berkeley have recently carried out electron paramagnetic resonance
studies, including spin polarization or CIDEP studies, on oriented mem-
branes.[44] These show that certain of the paramagnetic species (probably
quinone derivatives in one case and iron-sulfur proteins in another) that
serve as electron acceptors have fixed orientations, both with respect
to the membrane plane and with respect to counter radicals (Chl$^+$) produced
in the primary photochemical charge separation. Undoubtedly these fixed
orientations and arrangements are essential to producing the electric fields
across the membranes that lead to vectorial pumping of ions and eventually
to photophosphorylation.[45]

Confirmation of these distinctions between inside and outside surfaces
comes from studies using chemical labelling reagents or antibodies to partic-
ular membrane proteins. Furthermore, accessibility by substrate molecules
that are either natural (ADP is phosphorylated on the "outside" surface)
or artificial (electron donors and acceptors) gives additional information
concerning the location of membrane components. To cite one example, anti-
bodies to polypeptides of the bacterial reaction center complex have been
prepared and attached to the dense iron-core protein, ferritin, which can
be seen in electron microscope pictures.[46,47] These ferritin-labelled
antibodies interact with accessible sites on the surface of chromatophore

fragments or intracytoplasmic membranes of bacterial cells with ruptured cell walls or perforated membranes. The studies are enriched by the fact that the membrane surface that faces the periplasmic space between the membrane and the limiting bacterial cell wall (i.e., the surface facing outwards in the cell) is inverted when chromatophores are prepared and ends up on the inside of these small, closed membrane vesicles.[48] From such studies it appears that (1) antibodies to reaction center proteins react with the outer chromatophore surface, but only after the ATPase phosphorylation complex has been removed from the outer surface using EDTA,[46] (2) antibodies to a mixture of the two smaller reaction center peptides (21 and 23 Kd) react at both membrane surfaces, but antibodies to the largest (28 Kd) peptide react only at the surface that ends up on the outside of the chromatophores,[47] and (3) antibodies to cytochrome c_2, a membrane-bound electron transport component, appear to be located on the opposite surface, inside the chromatophores.[49] From studies such as these we can begin to fill in some of the details in membrane models, such as the one shown in Fig. 9.

Solar Energy Conversion

Analysis of the photosynthetic light reactions in terms of physical chemistry suggests a set of design criteria that would be desirable for solar energy converters for commercial power production. These include (1) spectral absorption of nearly all wavelengths of photochemically active light incident at the surface of the earth; (2) rapid conversion of electronic excitation into separation of electrical charge; (3) stabilization against wasteful charge recombination, ultimately by separating chemical species across an impermeable membrane with a minimum expenditure of the "stored"

energy and entropy; and (4) subsequent conversion of a high fraction of
electrical and ion gradient forms of chemical potential into stable "chemical"
products obtained from readily available reactants (e.g., H_2O and CO_2).
This is a big order, and it is true that commercial solar energy units
do not need to incorporate all of these features. The solar cells used
on space probes and satellites convert solar energy directly into electrical
energy with efficiencies as high as 15%. Nevertheless, biological photo-
synthetic organisms do provide targets of efficiency, economy and scale
arising from ingenious and intricate characteristics that we may well use
to our profit.

Aknowledgement

Table I. Principal Components of the Subunit of Membranes from the
Photosynthetic Unit of <u>Rhodopseudomonas</u> <u>spheroides</u>, wild type.

		Molecular Weight (Kd)
Reaction center proteins		$\begin{cases} 28 \\ 23 \\ 21 \end{cases}$
Cytochrome c_{551}		44
Cytochrome b_{558}		60
Cytochrome b_{560}		60
Antenna proteins	(25-30) x 8.5	250
BChl + BPheo	(60-80)	70
Carotenoids		28
Ubiquinone	(8)	4
Phospholipids	(20%)	150
Carbohydrate		30
	Total	770

Figure 1. Electron micrograph of a thin section through a leaf of spinach.
The cell walls separating two adjacent cells run across above the
center of the picture. The chloroplast shown in the lower portion
contains internal membranes (dark staining) where the chlorophyll
and the photochemical apparatus are located. (Figure is from an
article by R. B. Park.[6])

Figure 2. Freeze-fracture electron micrograph showing fracture faces asso-
ciated with the photosynthetic membranes (thylakoids) of a chloro-
plast. The large particles, which appear in organized arrays in
some regions of the sample, are spaced at intervals of about 17
nm. (Figure was kindly provided by Prof. R. B. Park, University
of California, Berkeley)

Figure 3. Chlorophyll a and bacteriochlorophyll a molecular structures. These
magnesium porphyrins contain extensive regions of π-electron density
(stippling) that are the origins of the visible absorption transi-
tions. Centers of asymmetric substitution adjacent to the porphyrin
system give rise to intrinsic circular dichroism.

Figure 4. Spectra of BChl a in diethyl ether solution (dashed curves) and
of chromatophores of the photosynthetic bacterium Rhodopseudomonas
spheroides, wild-type (solid curves). A) Absorption spectra.
B) Circular dichroism. Path lengths, 1 cm.

Figure 5. Absorption and CD spectra of a bacteriochlorophyll-protein complex
from green bacteria at room temperature (dashed) and at 80 K (solid
curves). Improved resolution at the lower temperature reveals
multiple CD components. (Figure is from Philipson and Sauer.[22])
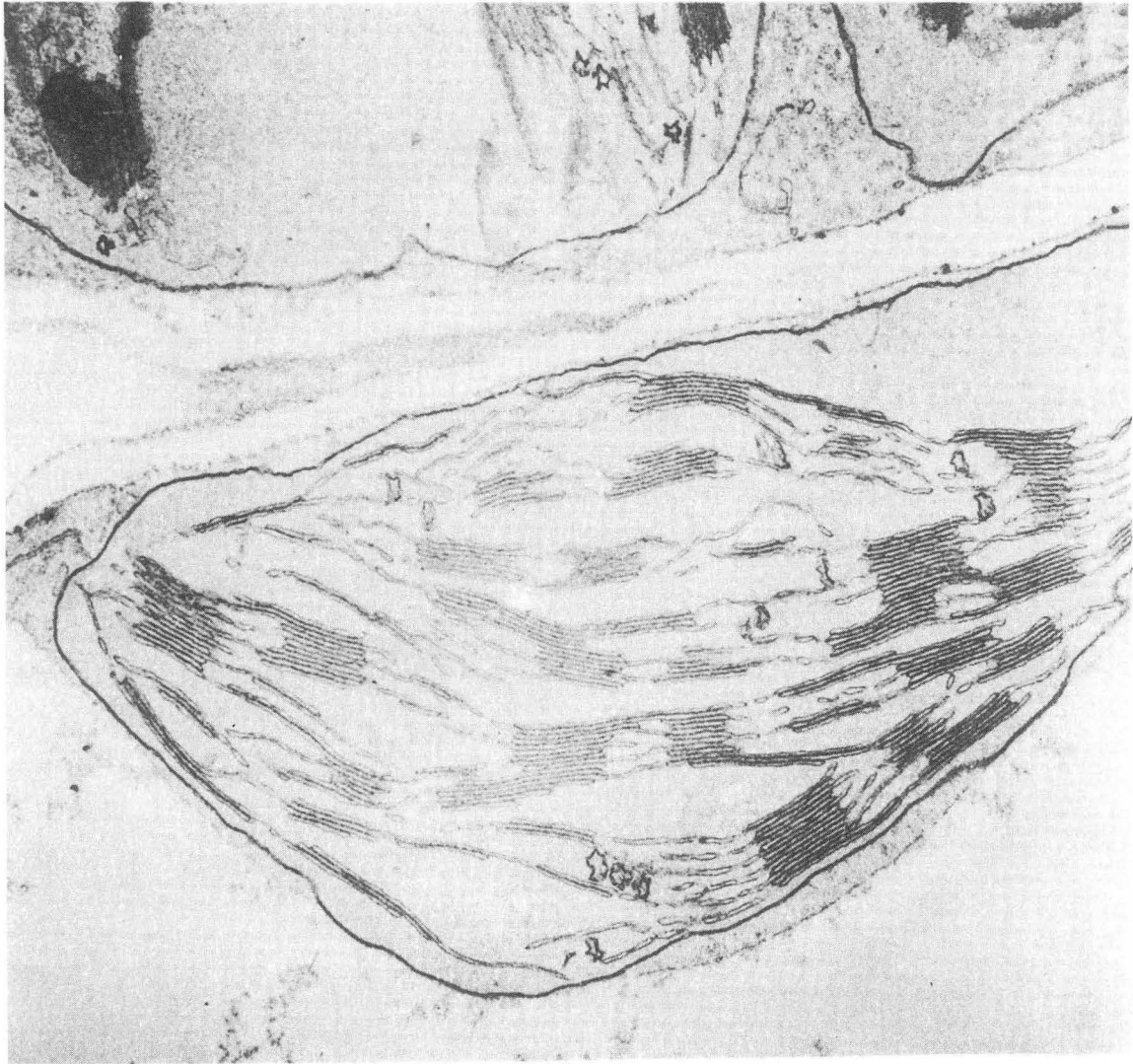
Figure 6.   Hypothetical model of the reaction centers of photosynthetic
             bacteria, showing the association of 4 BChl and 2 BPheo with 3
             peptides and an iron-ubiquinone complex.

Figure 7.   Absorption and CD spectra of reaction centers isolated from Rps.
             spheroides (R-26 mutant). Reaction center activity is demonstrated
             by the differences between spectra measured while the sample is
             under actinic illumination (dashed) and an unilluminated sample
             (solid curves).

Figure 8.   Absorption and CD spectra of an antenna BChl-protein (solid) iso-
             lated from the wild-type of Rps. spheroides, compared with the
             spectra of intact chromatophores (dashed curves) from the same
             organism.

Figure 9.   Model of the assembly of intracytoplasmic membranes of photosyn-
             thetic bacteria. Reaction centers (left) form complexes with cyto-
             chromes and, with 12-15 antenna BChl-proteins attached, make up
             a basic photosynthetic unit of the active membranes. The units
             are assembled with lipids in a two-dimensional sheet that possesses
             the asymmetry required for ion pumping and electric-field formation.

References

1.   R.K. Clayton, Brookhaven Symposia in Biology 28, 1-13 (1977).

2.   K. Sauer, in Bioenergetics of Photosynthesis, Govindjee, Ed., Academic Press, N.Y., 1975. pp 115-181.

3.   A. T. Jagendorf, in Bioenergetics of Photosynthesis, Govindjee, Ed., Academic Press, N.Y. 1975. pp 413-492.

4.   J.P. Thornber, R.S. Alberte, F.A. Hunter, J.A. Shiozawa and K.-S. Kan, Brookhaven Symposia in Biology 28, 132-148 (1977).

5.   A. Trebst, Annu. Rev. Plant Physiol. 25, 423-458 (1974).

6.   R.B. Park, in Plant Biochemistry, J. Bonner and J.E. Varner, Eds., Academic Press, N.Y., 1965, pp 124-150.

7.   D. Branton and R.B. Park, J. Ultrastructure Res. 19, 283-303 (1967).

8.   E.G. Uribe, Biochemistry 11, 4228-4235 (1972).

9.   R.T. Giaquinta, R.A. Dilley, B.R. Selman and B.J. Anderson, Arch. Biochem. Biophys. 162, 200 (1974).

10.   W. Junge and H.T. Witt, Z. Naturforsch. 23b, 244-254 (1968).

11.   J.B. Jackson and A.R. Crofts, FEBS Lett 4, 185-189 (1969).

12.   P. Graber and H.T. Witt, Biochim. Biophys. Acta 423, 141-163 (1976).

13.   C.S. French, J.S. Brown and M.C. Lawrence, Plant Physiol. 49, 421-429 (1972).

14.   J. J. Katz, J.R. Norris and L.L. Shipman, Brookhaven Symposia in Biology 28, 16-54 (1977).

15.   E.A. Dratz, A.J. Schultz and K. Sauer, Brookhaven Symposia in Biology 19, 303-318 (1967).

16.   C. Houssier and K. Sauer, J. Am. Chem. Soc. 94, 779-791 (1970).

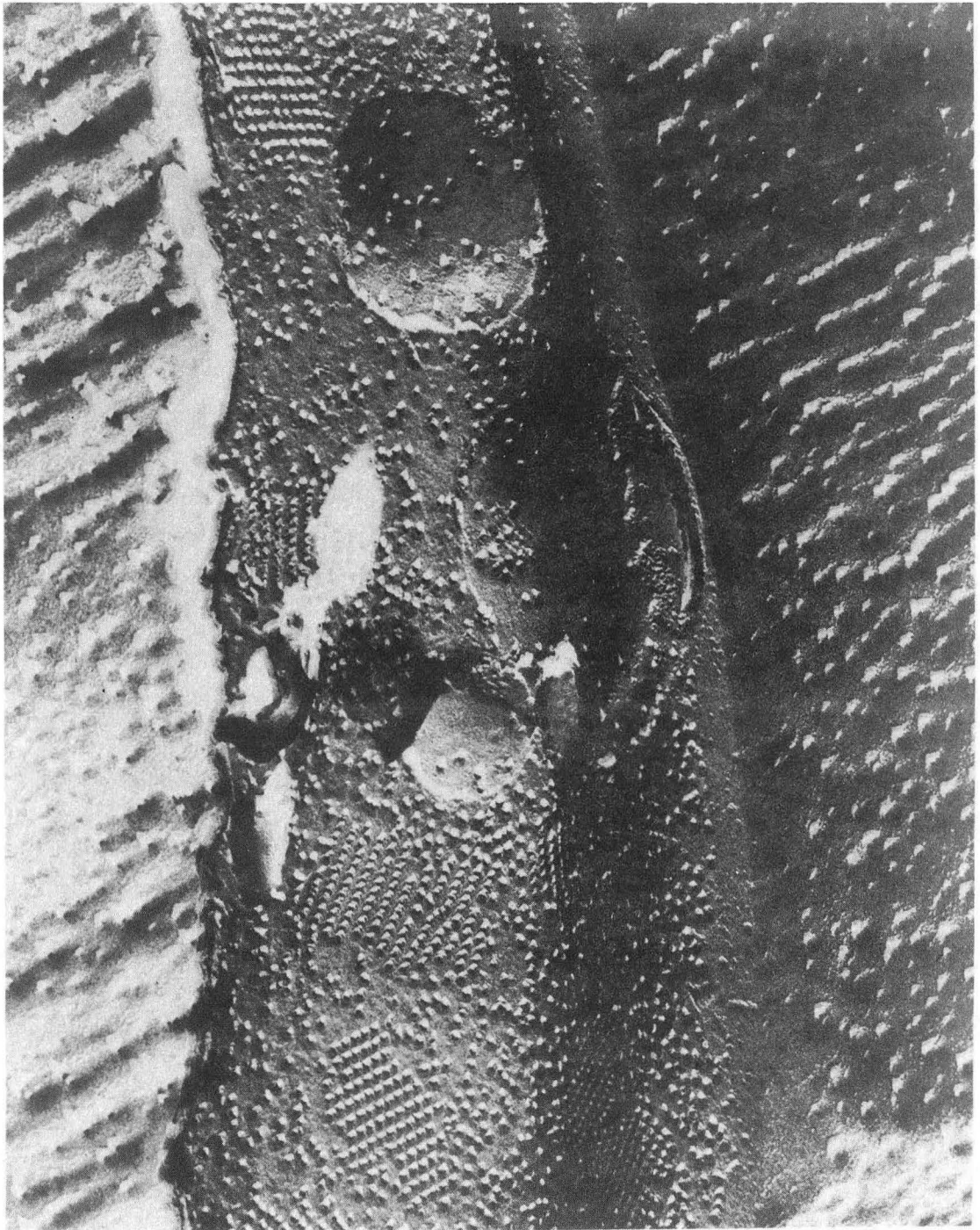17.   K.D. Philipson and K. Sauer, Biochemistry 12, 3454-3458 (1973).

18. I. Tinoco, Jr., Radiation Research 20, 133-139 (1963).

19. R.K. Clayton and B.J. Clayton, Biochim. Biophys. Acta 283, 492-504 (1972).

20. J.M. Olson, Methods in Enzymology 23, 636-644 (1971).

21. R.E. Fenna and B.W. Matthews, Brookhaven Symposia in Biology 28, 170-182 (1977); Nature 258, 573-577 (1975).

22. K.D. Philipson and K. Sauer, Biochemistry 11, 1880-1885 (1972).

23. F. Farron, Biochemistry 9, 3823-3828 (1970).

24. N.Nelson, Biochim. Biophys. Acta 456, 314-338 (1976).

25. P.M. Wood, H.N. Bhagavan and F.L. Crane, Plant Physiol. 41, 633-640 (1966).

26. D.W. Reed and R.K. Clayton, Biochem. Biophys. Res. Commun. 30, 471-475 (1968).

27. R.K. Clayton and R.T. Wang, Methods in Enzymology 23, 696-704 (1971).

28. M.Y. Okamura, L.A. Steiner and F. Feher, Biochemistry 13, 1394-1403 (1974).

29. L.P. Vernon and E.R. Shaw, Methods in Enzymology 23, 277-289 (1971).

30. J.M. Anderson and N.K. Boardman, Biochim. Biophys. Acta 112, 403-421 (1966).

31. L.A. Austin, Ph.D. Thesis, University of California, Berkeley, Lawrence Berkeley Laboratory Report LBL-5512 (1976).

32. K. Sauer, E.A. Dratz and L. Coyne, Proc. Natl. Acad. Sci. US 61, 17-24 (1968).

33. K.D. Philipson and K. Sauer, Biochemistry 12, 535-539 (1973).

34. D.W. Reed and B. Ke, J. Biol. Chem. 248, 3041-3045 (1973).

35. G. Feher, Photochem. Photobiol. 14, 373-387 (1971).

36. S.J. Tonn, G.E. Gogel and P.A. Loach, Biochemistry 16, 877-885 (1977).

37. S.J. Singer and G. L. Nicolson, Science 175, 720-731 (1972).

38. A. Gorchein, A. Neuberger and G. H. Tait, Proc. Roy. Soc. (London) B170, 229-246 (1968).

39. L.A. Staehelin, J. Cell Biol. 71, 136-158 (1976).

40. D.W. Reed and D. Raveed, Biochim. Biophys. Acta $\underline{283}$, 79-91 (1972).

41. R.K. Clayton, J. Theoret. Biol. $\underline{14}$, 173-186 (1967).

42. J. Breton, Biochem. Biophys. Res. Commun. $\underline{59}$, 1011-1017 (1974).

43. A. Vermeglio and R.K. Clayton, Biochim. Biophys.Acta $\underline{449}$, 500-515 (1976).

44. G. C. Dismukes, A. McGuire, R.E. Blankenship and K. Sauer, Biophys. J., submitted.

45. A.R. Crofts, C.A. Wraight and D.E. Fleischmann, FEBS Letters $\underline{15}$, 89-100 (1971).

46. D.W. Reed, D. Raveed and M. Reporter, Biochim. Biophys. Acta $\underline{387}$, 368-378 (1975).

47. G. Feher and M.Y. Okamura, Brookhaven Symposia in Biology $\underline{28}$, 183-194 1977).

48. K. Matsuura and M. Nishimura, Biochim. Biophys. Acta $\underline{459}$, 483-491 (1977).

49. R.C. Prince, A. Baccarini-Melandri, G.A. Hauska, B.A. Melandri and A. R. Crofts, Biochim. Biophys. Acta $\underline{387}$, 212-227 (1975).

XBB 7411-7521

Fig. 1

XBB 7411-7522

Fig. 2

Phytyl

CHLOROPHYLL A

Phytyl
or
Geranylgeranyl

BACTERIOCHLOROPHYLL A

Fig. 3
K. Sauer

ABSORPTION

CD
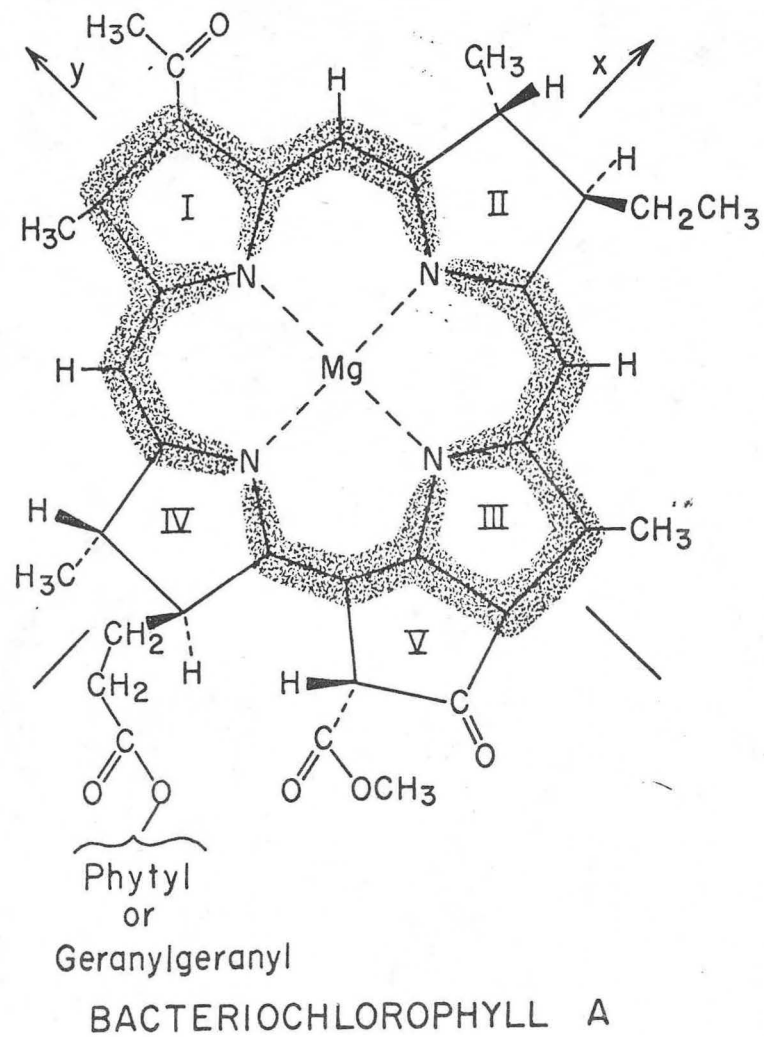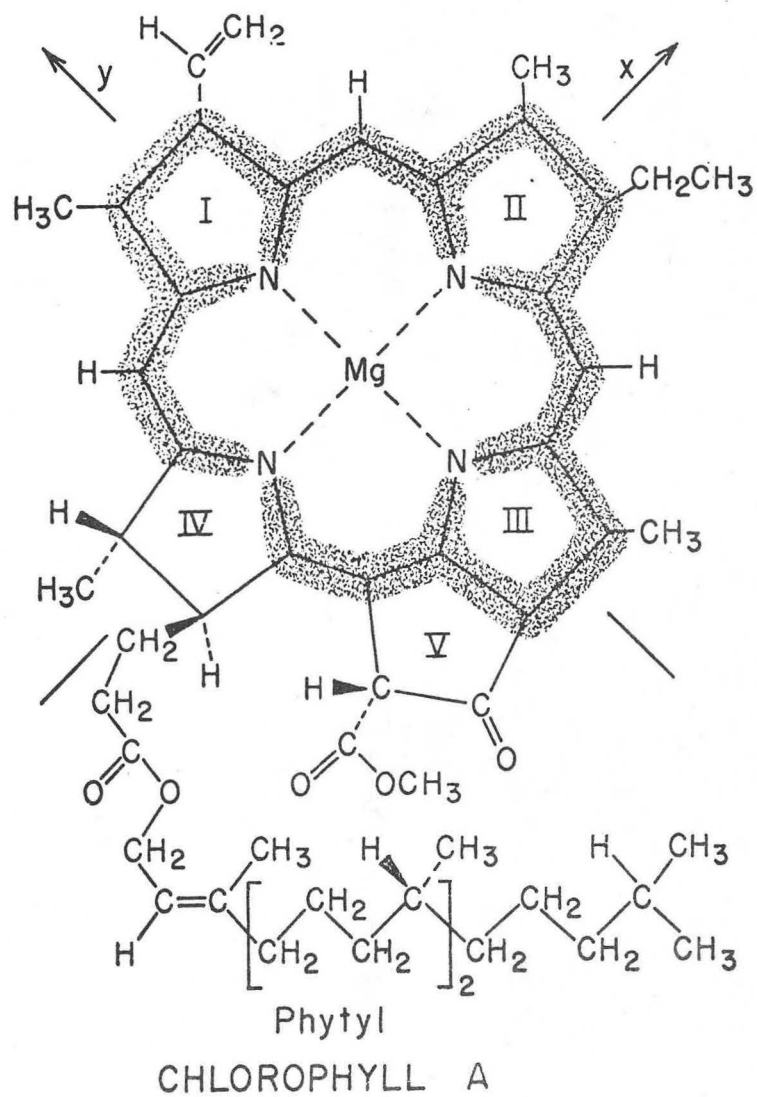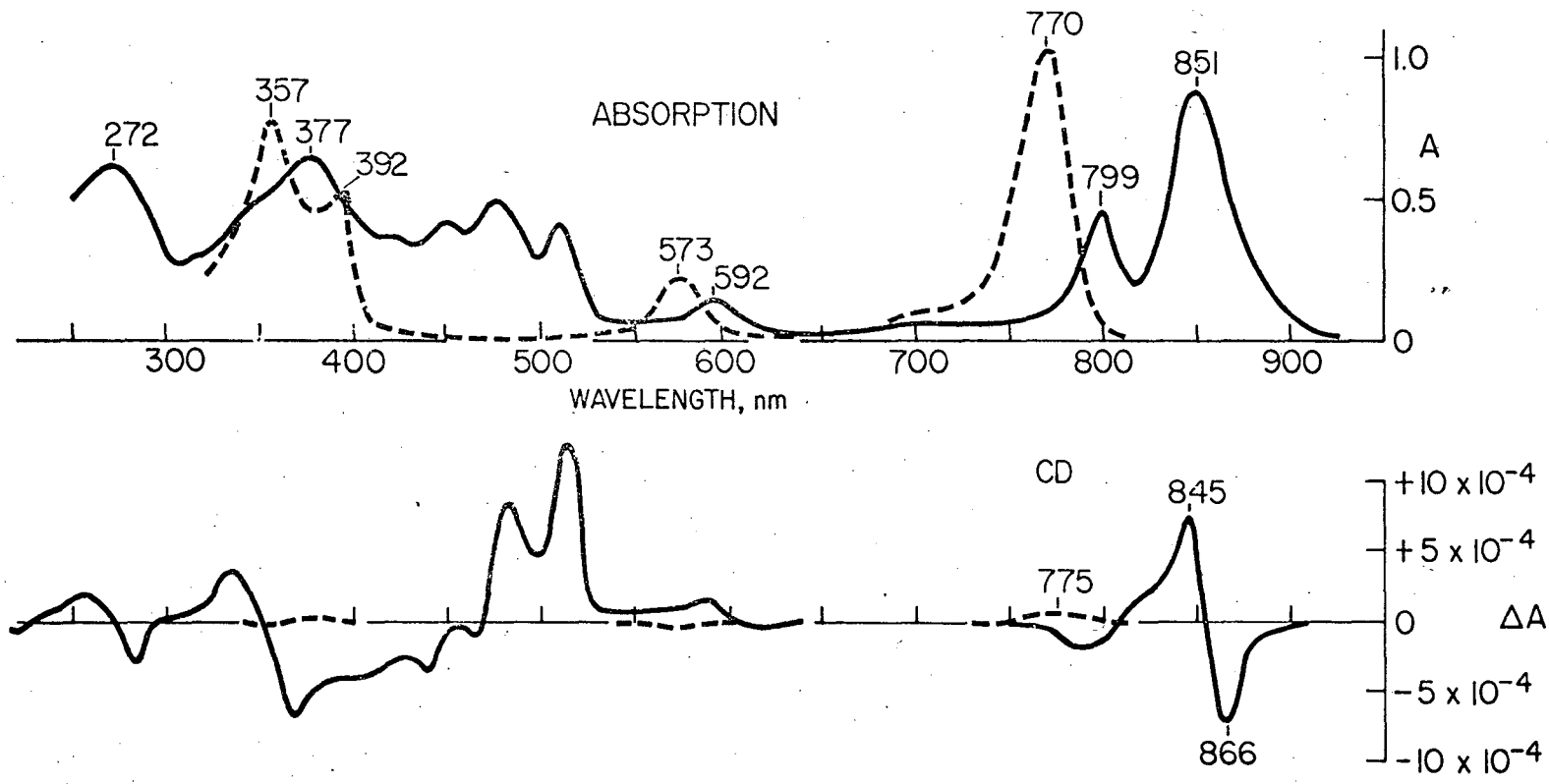
XBL 778-4596

Fig. 4
K. Sauer

ABSORPTION
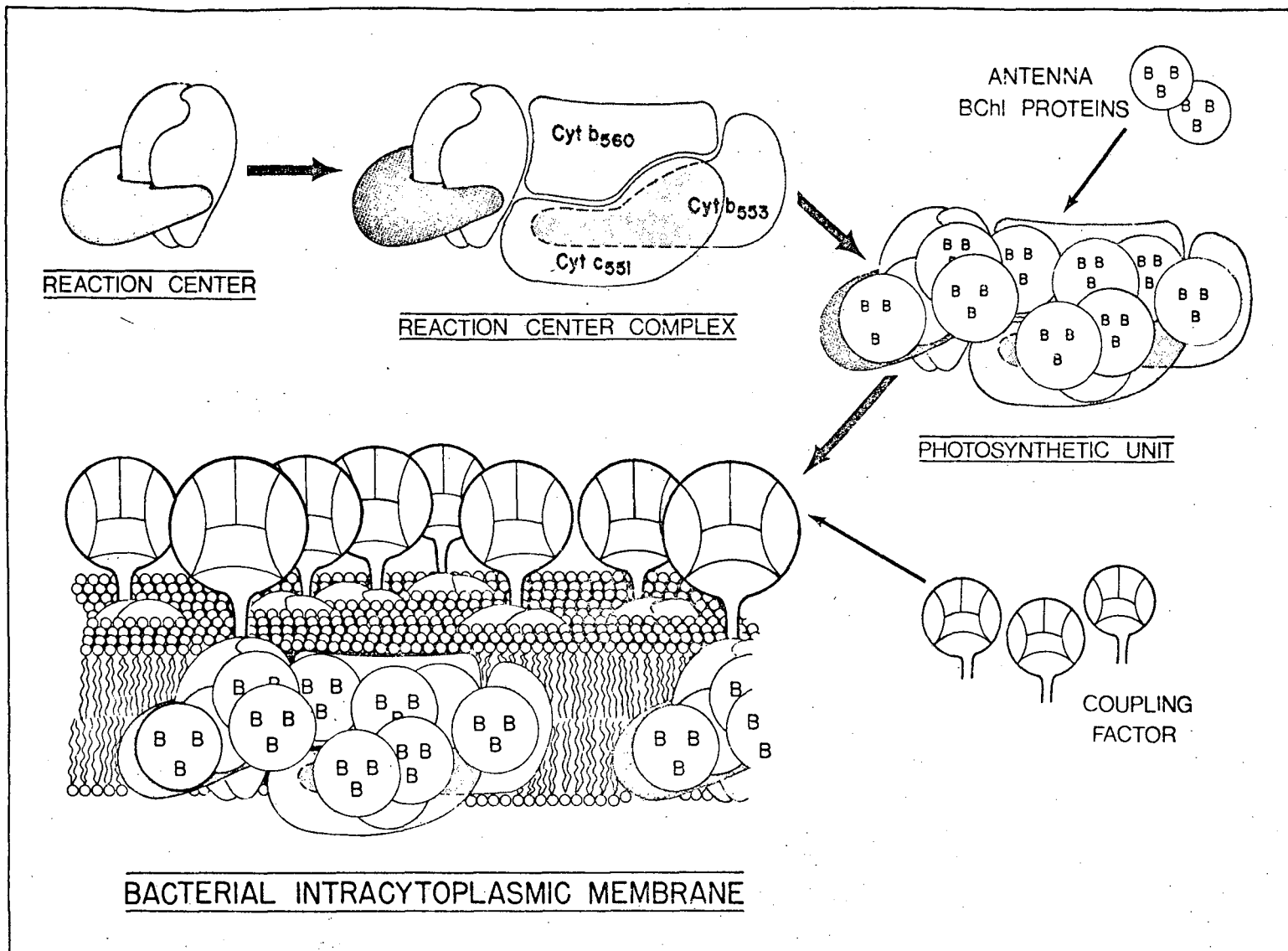
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XBL 711-5022 A

Fig. 5
K. Sauer

REACTION CENTER

XBL 776-4461

REACTION CENTERS

Rps. spheroides R-26

Fig. 7
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XBL 767-6068 A

R. spheroides wild type

Chromatophores — — — —
Antenna BChl protein —————

Fig. 8
K. Sauer

ANTENNA BChl PROTEINS

REACTION CENTER

Cyt b₅₆₀

Cyt b₅₅₃

Cyt c₅₅₁

REACTION CENTER COMPLEX

PHOTOSYNTHETIC UNIT

COUPLING FACTOR

BACTERIAL INTRACYTOPLASMIC MEMBRANE

XBL 779-4616

-34-

Fig. 9

K. Sauer