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ABSTRACT

We continue to study structure and function in photosynthetic membranes and their components, principally with Rhodopseudomonas sphaeroides. We have discovered how to remove bacteriochlorophyll (bchl) from a specific binding site in the antenna pigment-protein complex B850, and to replace the bchl without denaturing the complex. This has never before been achieved for any photosynthetic chlorophyll-protein complex. It opens a substantial vista of experiments designed to elucidate the nature of the binding site. Our analyses of the B850 and B875 complexes cast doubt on the accepted ratios of bchl components, carotenoids and protein and hence on models based on these ratios. We have shown that Rp. sphaeroides contains a specific second quinone (Q_b) in contact with the primary photochemical electron acceptor (Q_a) and distinct from the molecules of a larger pool of quinone. We have resolved conflicting reports about the polypeptide composition of reaction centers from Rhodopseudomonas viridis. We have new data on the orientation, relative to the photosynthetic membrane, of cytochrome 552 in Rp. viridis and of the "shifting" component of carotenoid in Rp. sphaeroides. We have constructed and are using a new circular dichroism spectrometer.

INVESTIGATIONS AND RESULTS

We have continued our analyses of bchl and carotenoid content in the antenna complexes B850 and B875 of Rp. sphaeroides. B850 in vivo, and as isolated by the original method of Clayton and Clayton, shows absorption bands at 800 and 850 nm due to two distinct components of bchl bound to a set of two polypeptides. This is the form that has received the most study, especially by us and by K. Sauer and collaborators. These studies have indicated that the set of polypeptides carries two molecules of bchl in close dimeric interaction responsible for the absorption band at 850 nm and one molecule giving the band at 800 nm, plus one molecule of carotenoid. B850 isolated with dodecyl sulfate - polyacrylamide gel electrophoresis shows little or none of the 800 nm absorption band. When such B850 is exposed to a nonionic detergent such as lauryl dimethyl amine oxide (LDAO), it slowly regains the 800 nm band, apparently at the expense of a long-wave component of the 850 nm band Meanwhile the intensity of the dimer-like circular dichroism apectrum around 850 nm increases slightly. Free monomeric bchl, dislodged from the 850 nm component and kept soluble by the detergent, is bound at the "800 nm" site. This is shown by the fact that if purified bacteriochlorophyll is added along with LDAO the 800 nm band is regained much more rapidly than if LDAO alone is added to the "800-depleted" B850. We conclude that there is more bchl in the component giving 850 nm absorption than simply one type of dimer, and that the bchl giving the 800 nm band can be removed and reattached in simple ways without denaturing the antenna pigment-protein complex. Dodecyl sulfate removes this bchl , and LDAO allows its reattachment. Even in living cells a small proportion of the 800 nm sites are vacant and can be filled by adding LDAO if free bchl is available. We thus have an assay for the 800 nm binding site: the

capacity for regenerating the 800 nm band when bchl is added. This opens a vista of new experiments to probe the nature of this site, as by changing its ionic environment, attempting to attach molecules similar to bchl, etc This is the first time that any chlorophyll-protein complex (native) has been successfully treated so as to remove and add back a chlorophyll component without denaturing the system.

The analyses attending this work have shown that the "accepted" ratios, bchl(800):bchl(850):carotenoid:protein in B850, and bchl(875):carotenoid:protein in B875, are less secure than had been appreciated. Further and more careful analysis under well understood and controlled conditions are needed in order to give this aspect of our understanding a secure foundation.

Earlier we had shown, in reaction centers and membranes of Rp. sphaeroides, the following pattern of electron transfer from the quinone Q_a that acts as primary photochemical electron acceptor, on to a second quinone Q_b, and thence to a pool of quinone:

1st flash of light $\quad Q_a \xrightarrow{\text{photochem.}} Q_a^-$

$$Q_a^- + Q_b \longrightarrow Q_a + Q_b^-$$

2d flash $\quad Q_a + Q_b^- \xrightarrow{\text{photochem.}} Q_a^- + Q_b^-$

$\longrightarrow Q_a + Q_b^=$, followed by $Q_b^= \longrightarrow Q_b$ with transfer of two electrons (and $2H^+$) to quinone in the pool

3d flash $\quad$ Same as 1st flash

etc.

Thus the second quinone, Q_b, undergoes a binary oscillation in its state of reduction on consecutive flashes. Through experiments on the mode of action of orthophenanthroline as an inhibitor of this electron transport (reported earlier) we have drawn two significant conclusions. First, that the native photosynthetic membrane contains a specific "Q_b" distinct from the larger quinone pool. Second, that earlier reports of failure to see binary oscillations under physiological conditions (at moderately low redox potentials) can be attributed to insufficient dark-adaptation of the material between series of test flashes.

In contrast to the well defined reaction centers of Rp. sphaeroides, there have been conflicting reports on the protein composition of reaction centers isolated from Rp. viridis. By subjecting these reaction centers to polyacrylamide gel electrophoresis under a variety of carefully controlled conditions (temperature, detergent type and concentration, etc.) we have reconciled these discrepant reports, showing how they have arisen. The protein component of reaction centers from Rp viridis is similar to those of Rp. sphaeroides and other species of photosynthetic bacteria, showing three polypeptides in the range 20-35 kilodalton, plus one that retains heme and is either cytochrome 552 or a combination of cytochromes 552 and 558. After some technical difficulties we are obtaining data on the orientation, relative to the membrane, of the low potential cytochrome 552 to supplement earlier data on the orientation of the high potential cytochrome 558. This adds to our knowledge of the molecular architecture of the photosynthetic membrane in Rp. viridis.

We have continued study of the light-induced shift of absorption bands of carotenoid pigments in Rp. sphaeroides. The major carotenoid components associated with B875 and with B850 have their long axes at

about 50° from the plane of the photosynthetic membrane, as shown by measurements of linear dichroism of oriented preparations. In air-dried films of membrane fragments the shifting component is oriented more nearly perpendicular to the membrane. This finding is compatible with the idea that the shift is induced by an electrochemical gradient giving an electric field normal to the membrane.

We have completed construction of our circular dichroism spectrometer and it is performing very well. This capability frees us of the necessity of sending materials to other laboratories that have this capability.

We have prepared subcellular fractions from thermophilic cyanobacteria ("blue-green algae"), with chlorophyll bound to a particle consisting of about five distinct polypeptides, that retain their capacity for photosynthetic oxygen evolution. Studies with this kind of material will hopefully improve our knowledge of green plant Photosystem 2 and the chemistry of photosynthetic oxygen evolution. A detailed understanding of the mechanism of oxygen evolution could have a bearing on the problem of solar energy conversion through the photolysis of water.

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