A Reaction Center Mutant of
Rhodospirillum rubrum*

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Photosynthetic bacteria are especially suitable for a mutational analysis of the fundamental mechanisms of photosynthesis. Genetic techniques have, however, only recently been systematically introduced into the field of bacterial photosynthesis.\(^1\) Mutants of the photosynthetic bacterium *Rhodospirillum rubrum*, unable to grow photoheterotrophically, were isolated. This was accomplished by a method, whereby spontaneous mutants are enriched in the presence of 8.8 \(\mu\)g tetracyklin/ml under photoheterotrophic conditions (method suggested by B. Marrs, personal communication). One of the mutants obtained, B4, was grown semiaerobically to allow pigment synthesis, by gassing the medium continuously with 2.5% \(O_2\) and 5% \(CO_2\) in \(N_2\). Chromatophore fragments were obtained from the mutant by procedures used for the wild type (strain S1).\(^2\) Absorption spectra of these chromatophore fragments showed the apparent absence of a peak at about 800 nm, which is present in wild type membranes (Fig. 1) and is due to a component in the reaction center called P800. Spontaneous revertants, which have regained the capacity to grow photosynthetically, can be isolated from B4 with a frequency indicating that the phenotype is caused by a single mutation. All the revertants isolated also regained P800. Among the revertants are, however, some with a significantly diminished content of P800.

Although the mutant chromatophores did not catalyze photophosphorylation and light-induced reduction of cytochrome \(b\), energization of the membrane, measured as reduction of cytochrome \(b\), was still possible in the dark with inorganic pyrophosphate. Light-induced energization returned in revertant chromatophores.

Analysis of the membrane fragments from B4 with SDS-polyacrylamide gel electrophoresis showed that a protein band corresponding to a molecular weight of about 18 000 dalton was lacking. This band reappeared in revertants with fully regained P800. In order to certify which one of the three different low molecular weight polypeptides in the reaction center that might be missing, it was necessary to isolate and analyse the reaction center complex from

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Fig. 1. Near-infrared absorption spectra of chromatophores from strain S1 (solid line) and mutant B4 (broken line), recorded in a Cary 17 spectrophotometer.

the membrane fragments. This complex can easily be solubilized from wild type membranes by treatment with the detergent LDAO (dodecyl dimethylamine oxide). Treatment of chromatophores from the mutant with LDAO, however, did not result in any solubilization of the reaction center. A possible explanation could be the complete absence of the reaction center complex in the mutant membranes. This appeared not to be the case since the reaction center bacteriochlorophyll (P870) was present, as indicated by the ferricyanide minus ascorbate difference spectrum. Apparently, the absence of P800 has induced changes in the membrane, which makes the reaction center protected from the action of LDAO.

A closer study of the membrane, including the light-induced EPR signal due to excited P870, from the mutant and some different revertants is planned and might result in a considerable increase in our hitherto rather limited understanding of the role of the reaction center components in the primary light reaction.

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