Lateral Organization of Proteins in Bacterial Photosynthetic Membranes

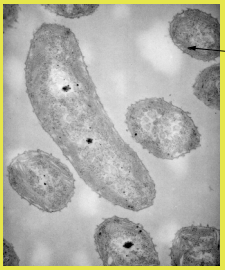
C. Mascle-Allemand[1], V. Prima[1], Rui Pedro Gonçalves[2], S. Scheuring[2] and James N. Sturgis[1]
[1]LISM UPR 9027, Institut de Biologie Structurale et Microbiologie, CNRS Marseilles FRANCE
[2]UMR 168 LRC CEA 34V Instiut Curie, Paris FRANCE

Introduction

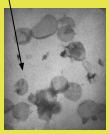
We have examined the photosynthetic membranes of the photosynthetic bacterium *Phaeospirillum molischianum* in an attempt to understand the organization of this apparatus. In particular we wish to understand the relationship between the light harvesting apparatus and reaction centers on the one hand and the cytochrome bc_1 and ATP synthase on the other hand. This is necessary as in several species extensive arrays of LH2 and core complexes have been observed while the other components appear to be absent.

Isolation of Membranes

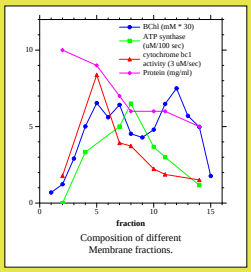
Photosynthetic membranes were isolated by centrifugation after a single passage of cells through a French press resemble membrane invaginations visible in cells.



Intra-cytoplasmic membranes
- In cells...
- Isolated



Membrane composition



Composition of different Membrane fractions.

Separation of membranes on sucrose density gradients.
In common with many other photosynthetic bacteria different membrane bands can be separated on sucrose density gradients.

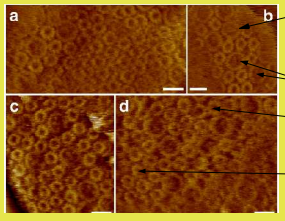
- The bacteriochlorophyll is associated with 2 distinct bands centered on fraction 5 and 12. The majority of the pigment is associated with the second band.
- Myxothiazol sensitive quinol-cytochrome c oxydo-reductase activity is associated with the band centered at fraction 5.
- Oligomycin sensitive ATP'ase activity is associated with a distinct membrane band centered at fraction 8.

There is therefore differential localization of the major components of the photosynthetic machinery.

Structural analysis

In common with other native photosynthetic membranes that have been examined (*Rsp. photometricum, Rb blasticus, Rb sphaeroides, Blc. viridis*)

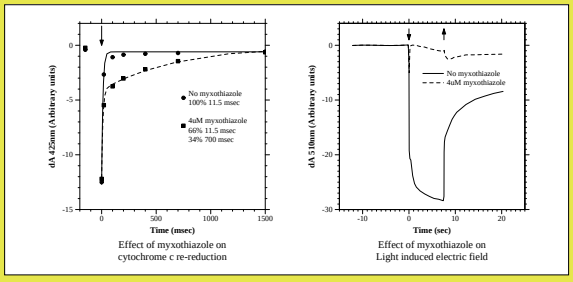
- Closely packed LH2 and core complexes are observed.
- No cytochrome bc_1 or ATP synthase was observed even over large areas.



- Reaction center – LH1 core complexes form closed ovals 9.5 nm x 8.5 nm.
- Core complexes are often grouped
- LH2 complexes form 8 fold symmetric rings of 4.8 nm diameter
- LH2 often form hexagonally packed arrays (a = 7.4 nm).

Functional analysis of cells

In whole cells cytochrome *c* re-reduction is rapid (τ=11,5 msec) and depends on the cytochrome bc_1 complex (after inhibition by myxothiazole τ= 700msec)



Effect of myxothiazole on cytochrome c re-reduction

Effect of myxothiazole on Light induced electric field

Membranes are hyper-polarized in light, indicative of good coupling, and part of this collapses very rapidly after illumination, indicating an active ATP synthase, This is inhibited by myxothiazole.

Conclusions

- Functionally linked proteins appear to be localized in structurally distinct membrane areas.
- Long distance proton and quinone movements seem necessary to explain our observations.
- This seems paradoxical in view of the high protein density around reaction centers.