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Modification of membrane composition in growing photosynthetic bacteria

Synthesis of energy-converting membranes in purple bacteria growing photosynthetically appears to be regulated in some way by the average energy flux[1,2]. Growth rate is, of course, also related to energy flux and, consequently, membrane synthesis and growth rate are ordinarily interdependent. Thus, in typical purple bacteria there is an inverse relation between growth rate (divisions/h) and quantity of membrane per unit of dry weight[2,3]. It has been suggested[2] that this results from operation of a "compensatory" control system, which, in effect, aids the photosynthetic cell in maintaining its chemical energy flux when the light intensity changes. Perhaps the most common situation encountered in this respect is the decrease in light intensity that inevitably occurs as the density of cells increases in a growing batch culture (usually referred to as "self-shading"). When the continuous light intensity diminishes beyond a certain point, the growth rate decreases and, per unit of dry mass, the quantities of bacteriochlorophyll and membrane increase[4]. This also occurs when cultures are exposed to saturating, but intermittent, light[1]. These two situations are probably closely related in that momentary self-shading of motile cells is, in effect, intermittent illumination. The physiological effects ensuing from exposure of cells to bright intermittent or dim continuous light have been interpreted[1] as regulatory responses initiated by conditions of energy stress.

The production of "additional" membrane as a response to energy stress might be viewed as a kind of "differentiation" of the cytoplasmic membrane. Experimental attempts with various photosynthetic bacteria to detect differences in chemical composition between the cytoplasmic and "additional" membrane, however, have given contradictory data[3]. In this communication, we present evidence for a chemical differentiation, in respect to phospholipid composition, of "additional" membrane in the bacterium *Rhodopseudomonas capsulata*.

R. capsulata (strain "St. Louis", American Type Culture Collection No. 23782) was grown in a synthetic medium (initial pH 6.8) containing 0.4% DL-malic acid,

0.1 % $(NH_4)_2SO_4$, 0.0001 % thiamine hydrochloride, and additional salts as specified by ORMEROD *et al.*[5]. For photosynthetic growth, the cultures were anaerobic (completely filled 170-ml flat-sided bottles) and were illuminated at 34° with Lumiline lamps to give continuous saturating illumination (>550 ft candles), continuous dim (40 ft candles), or intermittent saturating illumination (light source alternately turned on and off at 30-sec intervals by a recycling cam timer). For aerobic dark growth, cultures in erlenmeyer flasks (200 ml of medium per 1-l flask) were incubated at 30° on a rotary shaker (approx. 100 rev./min). Cells were harvested during the logarithmic growth phase, after at least four mass doublings under each experimental condition.

Protein content of cells was determined by the method of LOWRY *et al.*[6] on samples solubilized by treatment with 1 M NaOH at 100° for 15 min (crystalline bovine serum albumin used for standards). Bacteriochlorophyll was estimated from absorbance measurement at 775 nm of an acetone–methanol extract[7] of cells, assuming an extinction coefficient of 75 $mM^{-1} \cdot cm^{-1}$ (see ref. 8). Phosphorus in lipid fractions (see below) was determined by BARTLETT's[9] procedure, adapted for the Technicon Auto Analyzer.

Lipids were extracted from cells with methanol and chloroform as described by BLIGH AND DYER[10], evaporated to dryness under N_2 and dissolved in methanol–toluene (1:1, v/v) prior to analysis. Deacylation of the lipids was accomplished as follows: 1 ml of 0.2 M KOH (in methanol) was added to 1 ml of methanol–toluene solution of lipid (containing up to 4 μmoles of phosphorus) and the sample incubated for 2 h at 0°. 0.2 ml of 1 M acetic acid and 2 ml each of chloroform and water were then added, the mixture shaken thoroughly and then briefly centrifuged. The upper phase, containing the deacylated lipids, was removed and concentrated to about one third its original volume in a stream of N_2. The deacylated lipids were chromatographed using an anion-exchange analytical system with AG 1-X2 Bio-Rad Dowex resin in the formate form and an ammonium formate *plus* sodium borate elution gradient[11].

Fig. 1 shows the relation observed between total phospholipid and bacteriochlorophyll contents of *R. capsulata* cells grown so as to contain different quantities of energy-converting membranes. It is evident that a doubling in bacteriochlorophyll content is accompanied by an increase of only about 20 % in total phospholipid. Generally similar results have been described for cells of different bacteriochlorophyll content obtained by growth of cultures in darkness with different O_2 tensions[12]. Assuming that lipid phosphorus is a measure of membrane quantity and that the value at very low bacteriochlorophyll content is an approximate index of cytoplasmic membrane (or invariant integument structures), these data suggest that the bacteriochlorophyll to total membrane ratio changes as the amount of "additional" membrane changes. It would seem, however, that the bacteriochlorophyll content per unit of lipid phosphorus in "additional" (total lipid-P *minus* lipid-P at "zero" bacteriochlorophyll) membrane remains constant.

Anion-exchange chromatography analysis (see above) indicated that the deacylated phospholipids of *R. capsulata* had peak elution volumes which corresponded to those of the authentic deacylated phospholipids: glycerophosphorylglycerol, glycerophosphorylethanolamine and glycerophosphorylcholine. These three phospholipids accounted for essentially all of the lipid phosphorus, thus confirming previously reported analyses of *R. capsulata*[13]. Analyses of the (deacylated) phospholipids from

the experiments of Fig. 1 revealed a significant change in relative composition as a function of bacteriochlorophyll content of the cells. As shown in Fig. 2, the quantities of both glycerophosphorylethanolamine and glycerophosphorylglycerol appreciably increased with increase in bacteriochlorophyll content, while glycerophosphorylcholine remained quite constant. From these data, we conclude that "additional" membrane differs from "original" cytoplasmic membrane in phospholipid composition. It appears that membrane synthesized, in *R. capsulata* cells growing photosynthetically, in response to restriction of the energy flux is enriched in phosphatidylglycerol and phosphatidylethanolamine. In this connection, it should be noted that the possible existence of a continuous but chemically differentiated membrane in photosynthetic bacteria is indicated by the presence of two physically separable components in comminuted membrane preparations[14,15] (see also ref. 3).

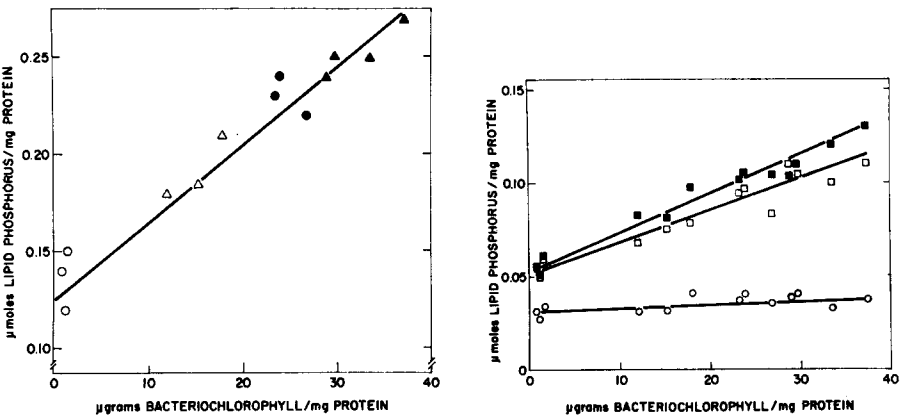

Fig. 1. Relation between lipid phosphorus and bacteriochlorophyll contents of *R. capsulata* cultures grown under the following conditions: ○, aerobically in darkness; △, 550 ft candles continuous light; ●, 550 ft candles intermittent light (see text); ▲, 40 ft candles continuous light.

Fig. 2. Relative quantities of the major (deacylated) phospholipids in *R. capsulata* cells as a function of bacteriochlorophyll content. The amount of each phospholipid was calculated by multiplying the percentage composition (obtained by chromatographic analysis) by the total lipid phosphorus value. ○, glycerophosphorylcholine; □, glycerophosphorylglycerol; ■, glycerophosphorylethanolamine.

Our conclusion in respect to bacteriochlorophyll content of "additional" membrane in *R. capsulata* is in harmony with certain earlier observations on *Rhodopseudomonas spheroides*[16] and *Rhodospirillum rubrum*[17] cells grown at light intensities below 2400 ft candles. It seems likely that the changes seen in phospholipid composition of *R. capsulata* are related to the operation of regulatory systems designed for efficient use of available biosynthetic resources[2]. The reasons for seemingly contradictory results and interpretations reported in the literature (*e.g.* see refs. 3 and 16–18) on membrane composition of photosynthetic bacteria are still not clear, and these may be due, in part, to basic differences among different genera and species. It is perhaps more likely that similar biosynthetic and regulatory mechanisms will be found in the various photosynthetic bacteria and that the apparent discrepancies derive mainly from lack of standardization of growth conditions, experimental procedures and bases of comparison.

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