Sites of phospholipid biosynthesis during induction of intracytoplasmic membrane formation in *Rhodopseudomonas sphaeroides*

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Abstract. A rapid, gratuitous and cell-division uncoupled induction of intracytoplasmic photosynthetic membrane formation was demonstrated in low-aeration suspensions of chemotrophically grown *Rhodopseudomonas sphaeroides*. Despite a nearly 2-fold increase in phospholipid levels, no significant increases were detected in the specific activities of CDP-1,2-diacyl-sn-glycerol:sn-glycerol-3-phosphate phosphatidylintransferase (phosphatidylglycerophosphate synthase, EC 2.7.8.5) and CDP-1,2-diacyl-sn-glycerol: L-serine O-phosphatidylintransferase (phosphatidylerserine synthase, EC 2.7.8.8), the first committed enzymes of anionic and zwitterionic phospholipid biosyntheses, respectively. The distribution of phosphatidylglycerophosphate and phosphatidylerserine synthase activities after rate-zone sedimentation of cell-free extracts indicated that intracytoplasmic membrane phospholipids were synthesized mainly within distinct domains of the conserved cytoplasmic membrane. Labeling studies with 32Pi and L-[3H]phenylalanine suggested that preexisting phospholipid was utilized initially as the matrix for insertion of intracytoplasmic membrane protein that was synthesized and assembled de novo during induction.

Key words: *Rhodopseudomonas sphaeroides* — Photosynthetic membrane assembly — Phosphatidylglycerophosphate synthase — Phosphatidylerserine synthase

When the facultative phototrophic bacterium, *Rhodopseudomonas sphaeroides* is grown under phototrophic conditions, a system of bacteriochlorophyll a (BChl)-containing intracytoplasmic membranes is formed which is continuous with an essentially unpigmented cytoplasmic membrane (Drews and Oelze 1981; Kaplan and Arntzen 1982; Niederman and Gibson 1978). In contrast, when grown chemoheterotrophically under high aeration, the synthesis of both BChl and the intracytoplasmic membrane is greatly repressed and essentially all of the cytoplasmic membrane is observed at the cell periphery in close apposition to the cell wall (Peters and Cellarius 1972; Brown et al. 1972). This response to changes in oxygen tension as well as the ease with which these membranes can be isolated (Niederman et al. 1981) has made *R. sphaeroides* and the other facultative phototrophs useful for studies of photosynthetic membrane induction.

In concentrated suspensions of chemoheterotrophically grown *R. sphaeroides*, the formation of the intracytoplasmic membrane can be induced without a significant lag at reduced oxygen tension in the dark (Takemoto and Lascelles 1973; Niederman et al. 1976). Under these circumstances, the intracytoplasmic membrane is assembled gratuitously and because very little cell division occurs, formation of the cell wall and peripheral cytoplasmic membrane is largely eliminated. In a previous study (Niederman et al. 1976), it was demonstrated in intracytoplasmic-membrane derived chromatophore preparations isolated at various stages of induction that BChl-protein complexes are assembled in a stepwise manner; accordingly, the cores of the photosynthetic units (B875 light-harvesting and reaction center complexes) were inserted first followed by the peripheral B800 — 850 light-harvesting complex.

In the present study, the biosynthesis of phospholipids has been examined during intracytoplasmic membrane induction in such cell suspensions. Studies in synchronous cells have suggested that phospholipids are not synthesized within the intracytoplasmic membrane (Cain et al. 1981) but, instead, are only incorporated during cell division (Lueking et al. 1978). In contrast, it is shown here that a continuous and cell-division uncoupled incorporation of phospholipids into developing intracytoplasmic membrane occurred in the induced cells. These studies, together with the intracellular localization of CDP-1,2-diacyl-sn-glycerol: sn-glycerol-3-phosphate phosphatidylintransferase (phosphatidylglycerophosphate synthase, EC 2.7.8.5) and CDP-1,2-diacyl-sn-glycerol: L-serine O-phosphatidylintransferase (phosphatidylerserine synthase, EC 2.7.8.8), the first committed enzymes of anionic and zwitterionic phospholipid biosyntheses, respectively, suggest that phospholipid components of the developing intracytoplasmic membrane are synthesized mainly within discrete domains of the conserved cytoplasmic membrane.

Materials and methods

Intracytoplasmic membrane induction and radioisotopic labeling procedures. *Rhodopseudomonas sphaeroides* NCIB 8253 was grown chemoheterotrophically on a gyrotory shaker and intracytoplasmic membrane formation was induced at low aeration in the dark as described by Niederman et al. (1976). Aliquots of the concentrated cell suspension were
removed for determination of dry weight, viable cell counts on agar plates, and cellular levels of BCHl (Clayton 1966) and phospholipid (Onishi and Niederman 1982), as well as for isolation of membranes in enzyme distribution and labeling studies (see below).

For continuous labeling studies $^{32}$P$_1$ (0.2 $\mu$Ci/ml) and L-$^{[3}$H$]$phenylalanine (0.1 $\mu$Ci and 42 $\mu$g/ml) were added at the start of induction to chemoheterotrophically grown cells concentrated to an O.D. at 680 nm of 2.0 (1-cm light path) in low-phosphate medium (Lascelles and Sivilagi 1963). Chromatophores were isolated by differential and rate-zone sedimentation (Niederman et al. 1976) and protein, total phosphorus and radioactivity were determined as in Brogle and Niederman (1979).

For pulse-chase studies, cells grown chemoheterotrophically in the presence of $^{32}$P$_1$ (0.3 $\mu$Ci/ml) to an O.D. at 680 nm of 1.0 were resuspended to an O.D. of 2.0 in fresh medium containing $^{32}$P$_1$ at the same specific radioactivity. The cells were incubated in the dark under low aeration as described above and after 10 h, they were pulsed with 2-$^{[3}$H$]$glycerol (2 $\mu$Ci/mmoll for 10 or 1 min and chased with a 10$^3$-fold excess of unlabeled glycerol for 180 and 40 min, respectively. In each experiment, aliquots of cells were removed at the end of the pulse and in the first experiment, at 30, 60 and 180 min thereafter, and at 10, 20 and 40 min in the second experiment. At 1 h prior to each pulse, the temperature of the culture was lowered from 30 to 15$^\circ$C to slow metabolic processes and phospholipid diffusion. Under these circumstances, BCHl synthesis continued at a reduced rate comparable to cultures incubated at 15$^\circ$C from the onset of induction; rates of BCHl synthesis were 0.50 $\mu$g/h·ml$^{-1}$ in cells maintained at 30$^\circ$C, 0.26 $\mu$g/h·ml$^{-1}$ at 15$^\circ$C and 0.14 $\mu$g/h·ml$^{-1}$ after the shift from 30 to 15$^\circ$C. The O.D. of the cultures at 680 nm indicated that little cell division occurred at either 15 or 30$^\circ$C.

For the isolation of membranes from the pulsed and chased cells, aliquots were poured over crushed ice in the presence of chloramphenicol (10−15 $\mu$g/ml culture) and a 10$^3$-fold excess of unlabeled glycerol and maintained in a salt-ice water bath until processed. Washed cells were disrupted in a French-pressure cell and subjected to rate-zone sedimentation on sucrose density gradients (Niederman et al. 1976) and protein, total phospholipid and radioactivity of chromatophore membranes were determined by turnover since labeling of both phospholipids (Onishi and Niederman 1982) and proteins (Niederman et al. 1979) for the isolation of chromatophores, a cell-wall enriched fraction, and an upper pigmented band which contained fragments of the cytoplasmic membrane as well as pigmented membrane invaginations. The isolated membrane fractions were precipitated with cold 5% trichloroacetic acid and lipids were extracted as in Onishi and Niederman (1982). Specific radioactivities were calculated directly from $^{3}$H/$^{32}$P ratios since $^{32}$P levels provided a measure of total phospholipid. For detection of pulse-chase relations, the specific radioactivities for individual membrane fractions were divided by those for the total lipids of the respective crude extracts as in Niederman et al. (1979). Pulse-labeling of cellular phospholipid with 2-$^{[3}$H$]$glycerol was shown to be terminated by the chase with excess unlabeled glycerol since no further incorporation of radioactivity occurred during a 180-min chase following a 10-min pulse; in contrast, in an unchased culture, incorporation continued linearly for 40 min and increased about 4-fold by 180 min. The kinetics of pulse-chase labeling of phosphatidylglycerol, phosphatidylethanolamine and phosphatidylycholine, isolated as in Onishi and Niederman (1982), suggested that in low-aeration cells, these phosphoglycerides were also synthesized from CDP-diacylglycerol (Cain et al. 1983). During the chase, the respective $^{32}$P-phospholipid distributions in the upper pigmented, chromatophore and cell-wall enriched bands remained at 40.6±3.2, 32.8±4.4 and 26.6±2.7% of the total radioactivity in these fractions.

Enzyme assays. Phosphatidylglycerophosphate synthase activity was determined essentially as described by Hayashi et al. (1976). The assay mixture contained 0.1 M Tris-HCl buffer, pH 8.4, 0.1 M MgCl$_2$, 0.1 M glycerol, 0.2 mM CDP-diacylglycerol (kindly supplied by G. M. Carman) dispersed in Triton X-100 (final concentration, 5.0 mM), 0.5 mM sn-$^{[2}$H$]$glycerol-3-phosphate (5 to 10 Ci/mmol, diluted to 1,250 cpm/nmol) and membrane protein in a final volume of 0.1 ml. After a 20-min incubation at 30$^\circ$C, the reaction was terminated with 0.5 ml of acidified methanol and lipids were extracted in 1:1.5:3 (vol/vol) methanol:chloroform:1 M MgCl$_2$. Tubes were vortexed 1 min and centrifuged at 5,000 rpm for 10 min. The chloroform-insoluble phase was removed by aspiration, washed twice with 5 mM MgCl$_2$, evaporated to dryness at 45$^\circ$C and resuspended in Maxifluor scintillation fluid (J. T. Baker Chemical Co., Phillipsburg, NJ, USA); radioactivity was determined in a Beckman LS-3150T liquid scintillation counter.

Phosphatidylserine synthase activity was assayed essentially as described by Raetz and Kennedy (1972) in a mixture that contained 0.1 M Tris-HCl (pH 8.1), 0.1 M MgCl$_2$, 0.1 M glycerol, 0.2 mM CDP-diacylglycerol dispersed in Triton X-100 (final concentration 1.25 mM), 0.5 mM L-$^{[3}$H$]$serine (5−25 Ci/mmol, diluted to 1,350 cpm/nmol) and membrane protein in the above final volume. After a 30-min incubation at 30$^\circ$C, product extraction and determination of radioactivity were performed essentially as described above.

One unit of enzyme activity was defined as the amount of enzyme converting 1 nmol of sn-glycerol-3-phosphate or L-serine to chloroform soluble product in 1 min at 30$^\circ$C. Product identification, further assay details and characterization of synthase activities will be described elsewhere (Radcliffe et al., in preparation).

Results

To assess the extent to which phospholipids and proteins of the developing intracytoplasmic membrane arise from preexisting cytoplasmic membrane, chemoheterotrophically grown R. sphaeroides was resuspended in fresh medium containing $^{32}$P and L-$^{[3}$H$]$phenylalanine and intracytoplasmic membrane formation was induced under low aeration. These experiments were not complicated significantly by turnover since labeling of both phospholipids (Onishi and Niederman 1982) and proteins (Niederman et al. 1979) is essentially stable. A 3 h intervals between 7 and 16 h, aliquots of the cell suspensions were harvested and chromatophores were purified by differential and rate-zone sedimentation. It had been shown by Niederman et al. (1976) that during this time period, the amount of intracytoplasmic membrane increases by ~5-fold. Figure 1 shows that over this same interval, in which the specific BCHl content of chromatophores increased nearly 1.4-fold, the specific radioactivity of chromatophore phospholipid doubled, whereas that of chromatophore protein remained essentially unchanged.