STRUCTURAL CHANGES IN THE RIBOSOMES AND RIBOSOMAL PROTEINS OF RHODOPSEUDOMONAS PALUSTRIS

J.D. Mansour and C.S. Stachow
Department of Biology, Boston College, Chestnut Hill, Massachusetts

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Following a shift from anaerobic to aerobic growth conditions, R. palustris enters a transition period during which the ribosomes and ribosomal subunits assembled during anaerobic growth are degraded, and following adaption to the aerobic environment, ribosomes are reassembled. Characterization of the aerobic ribosomal subunits resulted in structural ribosomal differences in comparison to the anaerobic ribosomal proteins. Our results indicate that R. palustris utilizes two distinct populations of ribosomes, the type synthesized being dependent upon the existing growth conditions.

The facultative photoheterotroph, R. palustris, can be cultured photosynthetically under anaerobic conditions or aerobically in darkness. The organism can be shifted from one growth condition to the other, although a transition period of 100 hours is required for adaptation to the shifted growth condition. There is abundant evidence to suggest that the metabolism and structure of cells during aerobic growth differs significantly from that of photosynthetic growth (1, 2). Such changes in cellular metabolism and composition might also be reflected in the structure of the ribosomes, particularly in the ribosomal protein (3, 4). Changes in ribosome structure are supported by the investigations on E. coli, where concentration differences are observed in the r-proteins when the organism is cultured in different growth medias.

Bhatnagar and Stachow (5) have studied the ribosomal structure of R. palustris cultured anaerobically and have shown that the 29S and 46S ribosomal subunits are comprised of 23 and 28 different r-proteins, respectively. Accordingly, we have initiated studies to determine the r-protein composition of ribosomes assembled during aerobic growth of this organism, and have followed the fate of anaerobically synthesized ribosomes during the transition period.

MATERIALS AND METHODS

R. palustris was grown both anaerobically and aerobically in the minimal media described by Cohen-Bazire et al (6). The ribosomes and ribosomal subunits were isolated and purified according to that previously

reported (5). Ribosomal proteins were isolated by the method of Spitnik-Elson (7) and analyzed by urea-polyacrylamide gel electrophoresis as described by Reisfield et al (8). Gels were scanned in a Gilford 2400 spectrophotometer equipped with a linear transport (Gilford Instrument Laboratories Inc., Oberlin, Ohio).

For transition studies, R. palustris was grown anaerobically to an optical density of 400 (Klett-Summerson Colorimeter, 660 filter) and then shifted to aerobic conditions by placing in a New Brunswick Environmental Shaker set at 32°C at 200 rpm kept in total darkness. The transition period began immediately following the aerobic shift and continued for a 100 hour period. Cellular division was completely inhibited 24 hours following the shift and resumed at the end of the 100 hour period. Cells harvested following the shift to aerobic growth conditions but prior to the resumption of cellular division (100 hours) were designated as transition period cells, while those harvested after 100 hours were designated aerobic cells.

Crude extracts of R. palustris were used to study anaerobic, aerobic and transition period ribosomal profiles. The extracts were prepared by suspending harvested cells in low salt buffer (at a ratio of 1 gm wet weight per 10 volumes of buffer), disruption in a French pressure cell at 10,000 psi, followed by centrifugation for 30 minutes at 39,100 xg. A .1-.2 ml aliquot of this supernate was layered on 5 ml linear 15-30% sucrose gradients and centrifuged at 247,195 xg for 90 minutes. The gradients were continuously monitored through a Gilford 2400 recording spectrophotometer at 260 nm with the aid of an ISCO-density gradient fractionator (Instrumentation Specialty Co., Lincoln, Nebraska).

RESULTS

Investigation of the transition period of R. palustris indicate that the ribosomes and ribosomal subunits assembled during the anaerobic growth period are disassembled following a shift to aerobic growth conditions. The degradation of these ribosomes and ribosomal subunits, although not evident at 19 hours, is apparent 48 hours into the transition period (Figure 1). The ribosomal profile obtained from this time was found to differ greatly from the anaerobic control. Degradation of both the ribosomal subunits and the intact ribosome is evident by the decreased sedimentation values (12S, 37S, and 58S) obtained for these particles. The degradation process is further

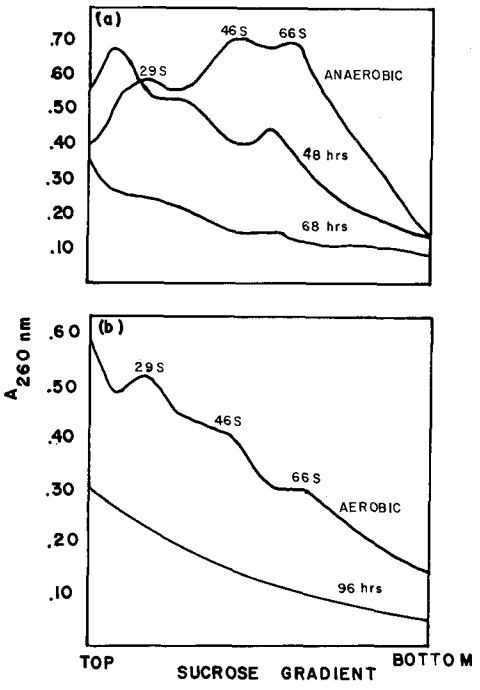

Figure 1. Sedimentation profile of R. palustris ribosomes and ribosomal subunits in sucrose gradients. Crude extracts were prepared from cells (a) anaerobically grown, 48 hours and 68 hours into the transition period; (b) 96 hours into the transition period and aerobically grown. The sedimentation values of the labeled subunits and ribosomes were previously determined using E. coli ribosomal subunits and ribosomes as markers on sucrose gradients.

illustrated at 68 and 96 hours where the ribosomes and ribosomal subunits are further degraded until no detectable ribosomes are observed. Following the adaption to aerobic growth conditions, ribosomes and ribosomal subunits having sedimentation values identical to ribosomes and ribosomal subunits synthesized during anaerobic conditions are assembled for active aerobic metabolism. These particles are assembled at the end of the transition period and are characteristically different from the anaerobic ribosomal profile in that the concentrations obtained for both the ribosomes and ribosomal subunits is always less than found during anaerobic growth conditions. This fact plus the data made available from the transition experiments, suggested that structural differences might exist between the ribosomes synthesized under anaerobic and aerobic conditions.

Polyacryamide gel electrophoresis studies indicate that the aerobic 29S

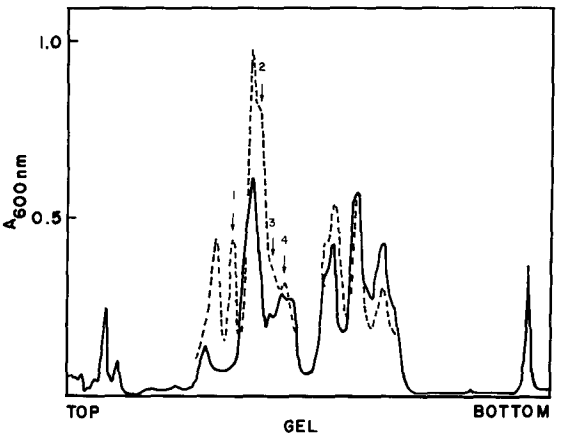

Figure 2. Urea-polyacrylamide (7.5%) gel electrophoresis profile of 29S ribosomal proteins extracted from anaerobically (—) and aerobically (---) grown R. palustris. 80 µg/ml of the total 29S ribosomal proteins from each growth condition was subjected to column electrophoresis and scanned at 600 nm in a Gilford 2400 recording spectrophotometer equipped with linear transport. Differences in the aerobic r-protein profile are designated by the numerals 1, 2, 3 and 4.

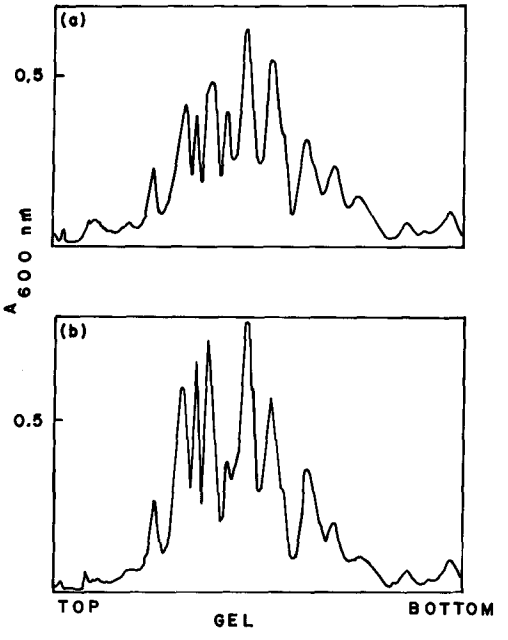

Figure 3. Urea-polyacrylamide (7.5%) gel electrophoresis profile of 46S ribosomal proteins extracted from (a) anaerobically and (b) aerobically grown R. palustris. The profiles were analyzed as described in figure 2.

ribosomal proteins differ from the anaerobic 29S ribosomal proteins in four areas of the r-protein profile (Figure 2). These differences included one additional r-protein not found in the anaerobic subunit composition, and three other r-proteins having different electrophoretic mobility. These latter r-proteins migrated at Rf values of .389, .414, and .439 in contrast to .417, .439, and .457 in the anaerobic polyacrylamide gel.

Examination and comparison of the 46S ribosomal proteins from both growth conditions resulted in no differences in the number of r-proteins nor in the electrophoretic mobility of the comparable proteins (Figure 3). However, as noted for the 29S subunit, concentration differences do exist between some of the anaerobic and aerobic 46S r-proteins.

DISCUSSION

The data presented in this report indicates that R. palustris utilizes two distinct populations of ribosomes during anaerobic and aerobic growth. Ribosomes synthesized during anaerobic growth conditions are degraded following a shift (transition period) to aerobic conditions. Prior to the onset of aerobic growth, ribosomes appear which are composed of altered 29S subunits.

The occurence of different r-proteins in the aerobic ribosomes raises questions as to their synthesis. It seems unlikely that the four aerobic r-proteins are the result of de novo synthesis during the transition period due to the extensive ribosome degradation and the absence of any detectable ribosomes. Because of this limitation, we feel that the r-proteins unique to ribosomes of aerobic cells are synthesized in either of the following manners.

The four different r-proteins found in the aerobic ribosome are present in the soluble pool during anaerobic growth. During adaptation to aerobic growth conditions, these proteins are incorporated into the aerobic ribosomes during the ribosome assembly process.

A second possibility is that following the breakdown of anaerobic ribosome subunits during the transition period, the r-proteins are modified by such processes as proteolysis or phosphorylation (9). When ribosomal subunits are re-assembled for aerobic metabolism, the modified anaerobic r-proteins are utilized.

The data made available from this study is not sufficient to assign a function to the new aerobic r-proteins. However, the fact that the different

r-proteins of the aerobic ribosome are associated with the 29S ribosomal subunit leads to speculation that these r-proteins are involved in some form of translational control to insure the synthesis of necessary aerobic proteins.

Currently, investigations are underway to determine the function and synthesis of the r-proteins specific to the aerobic ribosome.

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