Regular paper

Studies on the light-harvesting complexes from the thermotolerant purple bacterium *Rhodopseudomonas cryptolactis*

Evelyn Halloren¹, Gerry McDermott¹, J. Gordon Lindsay¹, Clare Miller¹, Andrew A. Freer², Neil W. Isaacs² & Richard J. Cogdell¹

¹Division of Biochemistry and Molecular Biology, University of Glasgow, Glasgow G12 8QQ, UK; ²Department of Chemistry, University of Glasgow, Glasgow G12 8QQ, UK

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Abstract

The antenna complexes from *Rps. cryptolactis* have been isolated and purified. *Rps. cryptolactis* contains two types of variable antenna complex, B800-850 and B800-820 as well as the 'core' B875 antenna complex. The variable antenna complexes contain more than two types of antenna apoprotein, and have a Bchla:carotenoid ratio of ~2:1. They can both be crystallised, but the B800-820 complex is the easiest with which to get relatively large single 3-D crystals (up to 0.5 mm in each dimension).

Introduction

*Rhodopseudomonas cryptolactis* ATCC 49414 is a newly described thermotolerant purple bacterium with an optimum growth temperature of 40 °C (Stadtwald-Demchick et al. 1990). When this species is grown anaerobically in the light its photosynthetic apparatus is housed in and on intracytoplasmic lamellar membranes. This new species was drawn to our attention by Howard Gest, because it is another example of a species of purple bacteria, like *Rhodopseudomonas acidophila* (Cogdell et al. 1983) and *Rhodopseudomonas palustris* (Hayashi et al. 1982 and Evans et al. 1990), which produces two types of variable antenna complex (i.e. B800-850 and B800-820). Stadtwald-Demchick et al. (1990) described how when *Rps. cryptolactis* cells were grown at high-light the major antenna component was the B800-850 complex, while when the cells were grown at low-light this was replaced by the B800-820 complex.

We were interested to look at the antenna complexes for two reasons. Firstly, we have a long-standing interest in trying to understand the structural basis of the spectral variation between the different bacterial antenna complexes (Cogdell et al. 1983; Hayashi et al. 1982; Evans et al. 1990 Thornber 1970) and, secondly, we try to routinely screen all new bacterial antenna complexes for their suitability for crystallisation (Cogdell et al. 1991). We describe below our initial isolation and characterisation of the antenna complexes from *Rps. cryptolactis* and show that both the B800-820 and the B800-850 complexes can be crystallised.

Materials and methods

Cells of *Rps. cryptolactis* were grown anaerobically in the light at 38–40 °C in the medium described by Stadtwald-Demchick et al. (1990). The cells were either grown at 10 Wm⁻² (high-light) or at 0.2 Wm⁻² (low-light). Following growth, the cells were harvested by centrifugation and resuspended in 20 mM MES, 100 mM KCl pH 6.8, and either used immediately or stored at −20 °C until required. The cells grew rather slowly, especially at the low-light intensity, taking 1–2 weeks to achieve sufficient density for harvesting. This presented a major problem. Following advice from Howard Gest we have found that growing cells to early to log phase at high-light then down shifting them...
to low-light is a much more effective way of inducing the synthesis of the B800-820 complex for large-scale isolation and purification.

The photosynthetic membranes were isolated by breaking cells in a French pressure cell (at 154 MPa), in the presence of 1–2 mM MgCl₂ and a little DNase, and subsequent centrifugation. A low speed spin at 12,000 × g for 20 mins pelleted most of the membranes and the remainder was spun down by centrifugation at 150,000 × g for 1 h. Both pellets were combined and resuspended in 20 mM Tris HCl pH 8.0. Initially the antenna complexes were isolated and purified by a combination of sucrose density gradient centrifugation and ion exchange chromatography on DEAE cellulose (Whatman DE52). Typically the membranes were adjusted to an OD of 50 cm⁻¹ at the major NIR bacteriochlorophyll absorption band, and then solubilised by the addition of 1% v/v of LDAO (lauryldimethylamine-N-oxide). Any unsolubilised material was then removed by a low speed centrifugation at 12,000 × g for 10 mins, the supernatant was diluted ~3 × with 20 mM Tris HCl pH 8.0 and loaded onto a DE52 column (2.5 cm by 15 cm). The column was loaded to about one-third of its capacity and the antenna complexes were eluted with increasing concentrations of NaCl in 20 mM Tris HCl pH 8.0, 0.1% v/v LDAO. The first complex eluted is the RC-B875 ‘core’ complex, which is followed by the variable antenna complexes. Elution of the complexes begins at 100 mM NaCl and is complete at 250 mM NaCl. However the two fractions do not separate completely and mixed fractions, collected between the two main fractions were separated by sucrose gradient centrifugation. The solubilised complexes were layered onto a step-gradient containing 0.6, 0.4, 0.3 and 0.2 M sucrose made up in 20 mM Tris HCl pH 8.0, 0.1% v/v LDAO. The gradient was spun at 150,000 × g overnight at 8 °C. Two bands are obtained. The top band is the variable antenna complex and the bottom band is the ‘core’ RC-B875 conjugate. This method works well for membranes that only contain B800-850 or only B800-820 as the ‘variable’ antenna complexes. When the cells were grown initially at high-light then down-shifted to low-light, the membranes contained both complexes, even though B800-820 was the major component. In this case, the method described above failed to separate the B800-820 complexes from the B800-850 ones. It was found by trial and error that if the ion exchange step was carried out on DEAE-sephacel this separation could be achieved. When the complexes are chromatographed on DEAE-sephacel under identical conditions as described above for DE52 they elute in a different order (the reason for this is unclear). In this case, the main B800-820 fraction comes off first, followed by the RC-B875 ‘core’ complex and finally the B800-850 complex.

For the crystallisation studies the antenna complexes were further purified by a combination of passage through a 1 metre sephacryl-G200 molecular sieve column in 0.1% v/v LDAO, 20 mM Tris HCl pH 8.0 and a second DE52 column (conditions as described above). Also when required, the detergent was exchanged on a mini-DE52 column, usually poured in a Pasteur pipette. The complex for detergent exchange was loaded onto this column, washed for 5–6 column volumes with detergent-free 20 mM Tris HCl pH 8.0, and then eluted in 20 mM Tris HCl pH 8.0, 300 mM NaCl, 1%