Lipopolysaccharide of *Rhodocyclus purpureus*: Taxonomic Implication

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The lipopolysaccharide (O-antigen) of the phototrophic bacterium *Rhodocyclus purpureus* Ames 6770^T was isolated and chemically analyzed. Like the lipid A moiety of *Rhodospirillum tenue* lipopolysaccharide, the lipid A moiety of *Rhodocyclus purpureus* lipopolysaccharide contains arabinose, 4-amino-arabinose, glucosamine, glucosamine phosphate, and fatty acids. In addition, the fatty acid spectrum, which is comprised of β -C₁₀OH, C₁₄, and C₁₆, is identical to the fatty acid spectrum of *Rhodospirillum tenue* lipid A. The polysaccharide moiety (degraded polysaccharide) of *Rhodocyclus purpureus* lipopolysaccharide contains rhamnose, mannose, glucose, galactosamine, and D-glycero-D-mannoheptose in addition to L-glycero-D-mannoheptose and 2-keto-3-deoxyoctonate. The data on lipopolysaccharide composition indicate that the taxonomic relationship between *Rhodocyclus purpureus* and *Rhodospirillum tenue* is closer than the relationship between *Rhodocyclus purpureus* and *Rhodopseudomonas gelatinosa*.

Rhodocyclus purpureus Ames 6770 is the type strain and presently the only available strain of this recently described species of the *Rhodospirillaceae* (7, 8). *Rhodocyclus purpureus* is unique among the species of this family because of its characteristic half-ring-shaped cells, its immobility, and its strong requirement for vitamin B₁₂. As in *Rhodospirillum tenue*, the number of carbon sources utilized by this species is rather limited, although the utilization of cyclohexane carboxylate and benzoate is reminiscent of a similar capability of *Rhodopseudomonas palustris* (8). A close relationship of *Rhodocyclus purpureus* to *Rhodospirillum tenue* and *Rhodopseudomonas gelatinosa* is also indicated by the fine structure of the photo-synthetic membrane system, which consists of only the cytoplasmic membrane and a few invaginations. In addition, the carotenoids of *Rhodocyclus purpureus* belong to the rhodopinal series and are almost identical to those of purple and violet *Rhodospirillum tenue* strains (11).

Pfennig expressed (8) the hope that methods of molecular taxonomy would clarify the relationship of *Rhodocyclus purpureus* to the species of the *Rhodospirillaceae* mentioned above. Preliminary data on oligonucleotide cataloging of 16S ribosomal ribonucleic acid have confirmed a close relationship between *Rhodocyclus purpureus* and the *Rhodospirillum tenue*-*Rhodopseudomonas gelatinosa* group (4). A short cytochrome type similar to the cytochrome type of the *Rhodospirillum tenue*-*Rhodopseudomonas gelatinosa* group has been pre-

dicted for *Rhodocyclus purpureus* (2). In this paper we report the chemical composition of the lipopolysaccharide (O-antigen) of *Rhodocyclus purpureus*. Lipopolysaccharides, and especially the structure of lipid A as the most conserved region of the heteropolymer, have taxonomic value within the *Rhodospirillaceae* (15).

MATERIALS AND METHODS

Bacteria and isolation of the lipopolysaccharide. *Rhodocyclus purpureus* Ames 6770^T was obtained from the German Collection of Microorganisms, Göttingen, Federal Republic of Germany, as strain DSM 168. Cells were grown at 30°C in medium containing (per liter of distilled water) 0.5 g of KH₂PO₄, 0.8 g of NH₄Cl, 0.4 g of MgSO₄ · 7H₂O, 0.05 g of CaCl₂ · 2H₂O, 3 g of sodium pyruvate, 1 g of yeast extract, 1 ml of trace element solution SL8 (1), and 1 ml of a vitamin B₁₂ solution (2 mg/100 ml).

This medium was adjusted to pH 6.8 and was autoclaved without pyruvate, which was added later from a 10% filter-sterilized solution together with vitamin B₁₂. For mass cultures, 10-liter carboys were used; these cultures were illuminated by two 100-W spotlights. Cells were collected at the early stationary growth phase, washed once with water, and lyophilized before storage at -80°C. Lipopolysaccharide was isolated by the phenol-chloroform-petroleum ether (PCP) method (3), followed by extraction of the remaining cells with hot phenol-water (16).

Analytical methods. The methods used for liberation of neutral sugars from lipopolysaccharide and their analysis by thin-layer chromatography and as alditol acetate derivatives by gas-liquid chromatography have been described elsewhere (14). 2-Keto-3-deoxyoctonate was liberated by hydrolysis in 1% acetic acid at 100°C for 3 h, separated by high-voltage paper electro-

TABLE 1. Chemical compositions of the lipopolysaccharide, degraded polysaccharide, and lipid A fractions of *Rhodocyclus purpureus* Ames 6770^T

Constituent	% Of dry wt		
	Lipopolysaccharide fraction	Degraded polysaccharide fraction	Lipid A fraction
Neutral sugars			
Rhamnose	9.8	13.9	2.4
Mannose	8.6	9.1	0.5
Glucose	5.2	6.2	1.6
L-Glycero-D-mannoheptose	6.4	6.9	0
D-Glycero-D-mannoheptose	8.0	12.1	0
Arabinose	2.1	Tr	3.2
2-Keto-3-deoxyoctonate	+ ^a	+	Tr
Amino sugars			
Glucosamine	1.8	0	13.0
Glucosamine-phosphate	0.4	0	2.7
Galactosamine	1.6	2.7	Tr
4-Amino-L-arabinose	+	0	+
Fatty acids			
β-C ₁₀ OH	6.0	0	4.1
C ₁₄	1.7	0	3.4
C ₁₆	2.6	0	5.4
C ₁₂	0.5	0	0.8
Unknown	0.5	0	0.8
Total phosphorus	1.0	0.5	1.4

^a +, Present but not quantified.

phoresis in buffer containing pyridine, acetic acid, and water (10:4:86, vol/vol; pH 5.3), and detected by periodate-thiobarbituric acid staining (13). Uronic acids, which were liberated by treatment with 1N H₂SO₄ at 100°C for 4 h, were stained by alkaline silver nitrate after separation by high-voltage paper electrophoresis, as described above. Amino sugars, which were liberated by treatment with 4 N HCl at 110°C for 16 h, were identified and determined with a Durrum model D-500 automatic amino acid analyzer. 4-Amino-arabinose was liberated by treatment with 10 N HCl at 100°C for 15 s (10) and was separated by high-voltage paper electrophoresis in buffer containing pyridine, formic acid, acetic acid, and water (1:1.5:10:90 vol/vol; pH 2.8). This amino sugar was visualized by heating the electropherogram to 100°C for 10 min or by staining with ninhydrin. In both cases the amino sugar stained orangish red. The fatty acids of the lipopolysaccharide were determined as their methyl esters by gas-liquid chromatography, using two different columns (2.5% Castorwax on Chromosorb G [80 to 100 mesh] and 15% EGSS-X on Gaschrom P [100 to 120 mesh]). Phosphorus was determined by the method of Lowry et al. (6).

Degradation of lipopolysaccharide. Lipopolysaccharide was degraded by treatment with 1% acetic acid at 100°C for 3 h; this treatment yielded the lipid A fraction and the so-called degraded polysaccharide fraction (5, 14). The degraded polysaccharide fraction was further purified by ultracentrifugation at 105,000 × *g* for 4h.

RESULTS

Isolation of lipopolysaccharide. The lipopolysaccharide of *Rhodocyclus purpureus* Ames

6770^T could be extracted by the PCP method from whole cells with a yield of about 1% of cell dry weight. Additional lipopolysaccharide was obtained by extracting the PCP-extracted cells with hot phenol-water. This lipopolysaccharide fraction, which was obtained in the water phase with a yield of 5% (based on cell mass dry weight), was contaminated with glucan (up to 50% of the dry weight).

Chemical analysis. The results of a chemical analysis of the lipopolysaccharide isolated by the PCP method are summarized in Table 1. The neutral sugars found were rhamnose, mannose, glucose, arabinose, and two heptoses (L-glycero-D-mannoheptose and D-glycero-D-mannoheptose), as revealed by thin-layer chromatography and gas-liquid chromatography. 2-Keto-3-deoxyoctonate in the *Rhodocyclus purpureus* lipopolysaccharide was tentatively identified by the detection of a periodate-thiobarbituric acid-positive compound that migrated identically to authentic 2-keto-3-deoxyoctonate during high-voltage paper electrophoresis. There was no evidence for the presence of significant amounts of uronic acids. An analysis with the amino acid analyzer revealed the presence of large amounts of glucosamine, glucosamine phosphate, and galactosamine. An additional amino sugar migrated identically to authentic 4-amino-L-arabinose during high-voltage paper electrophoresis (*M*_{GlcN} = 1.14). Like the standard, this sugar stained characteristically orangish red after ei-

FIG. 1. The three regions and their characteristic constituents of the lipopolysaccharides from *Rhodocyclus purpureus*, *Rhodospirillum tenue* and *Rhodopseudomonas gelatinosa*. A. distinction between the O-chains and the core (core substituents) is hypothetical for *Rhodocyclus purpureus*. Rha, Rhamnose; Man, mannose; Glc, glucose; D,D-Hep, D-glycero-D-mannoheptose; L,D-Hep, L-glycero-D-mannoheptose; KDO, 2-keto-3-deoxyoctonate; AraN, 4-amino-arabinose; Ara, arabinose; P, phosphate; GlcN, glucosamine. The parentheses indicate that the presence of a sugar is strain specific. In *Rhodospirillum tenue* lipid A an additional, unsubstituted glucosamine is bound to C₃ of the glucosamine backbone.

ther heating (100°C, 10 min) or ninhydrin spraying.

A gas-liquid chromatographic analysis of the fatty acids (as methyl ester derivatives) on two different columns showed that β -C₁₀OH was the main fatty acid and that C₁₄ and C₁₆ were also present. Other fatty acids were found in only small amounts. The lipopolysaccharide contained phosphorus (Table 1).

Lipopolysaccharide obtained by the phenol-water procedure had a composition similar to that of lipopolysaccharide isolated by the PCP method (data not shown). The contaminating glucan could be destroyed by α -amylase treatment of the fraction.

Degradation of lipopolysaccharide. By using mild acid hydrolysis (1% acetic acid, 100°C, 3 h), the lipopolysaccharide was cleaved into the so-called degraded polysaccharide and lipid A moieties. The yields were about 50 and 5% of the lipopolysaccharide dry weight, respectively (the low yield of lipid A might be explained by a loss of material in the washing steps during isolation of lipid A).

The degraded polysaccharide fraction contained the bulk of the neutral sugars of the lipopolysaccharide except arabinose. The latter sugar was found in this fraction only in trace amounts. The degraded polysaccharide also contained most of the galactosamine of the lipopolysaccharide but no glucosamine and no fatty acids. On the other hand, the lipid A fraction contained all of the fatty acids, glucosamine, glucosamine phosphate, and 4-amino-arabinose of the lipopolysaccharide. Arabinose and phos-

phorus were highly enriched in the lipid A fraction compared with the degraded polysaccharide fraction. The lipid A fraction was not completely free of lipopolysaccharide or degraded polysaccharide, as indicated by the presence of residual rhamnose or mannose in this fraction.

DISCUSSION

The chemical composition of the lipopolysaccharide of *Rhodocyclus purpureus* Ames 6770^T is similar in a number of ways to the chemical compositions of the lipopolysaccharides of *Rhodospirillum tenue* strains. This is most obvious from the presence of a neutral sugar, arabinose, in the lipid A fractions of both *Rhodocyclus purpureus* and *Rhodospirillum tenue* (14). In *Rhodospirillum tenue* lipid A, D-arabinose was identified as a substituent at the phosphate group of the reducing end of the glucosamine backbone structure (12). In addition, 4-amino-arabinose, which substitutes the phosphate of the nonreducing glucosamine residue of *Rhodospirillum tenue* lipid A (11), is also present in *Rhodocyclus purpureus* lipopolysaccharide. This amino sugar has not been found in the lipopolysaccharides of other *Rhodospirillaceae* species studied (15). The similarity between the lipid A of *Rhodocyclus purpureus* and the lipid A of *Rhodospirillum tenue* even extends to the fatty acid spectrum. Both species uniformly contain β -C₁₀OH, C₁₄, and C₁₆ as the main fatty acids (14). We conclude that *Rhodocyclus purpureus* and *Rhodospirillum tenue* have the same

characteristic type of lipid A with a specific fatty acid spectrum. Recently, D-arabinose was detected also in lipid A from *Yersinia pestis* (S. Minka, Ph.D. thesis, Université de Lyon, Lyon, France, 1980).

A comparison of the polysaccharide moieties (degraded polysaccharides) of the *Rhodocyclus purpureus* and *Rhodospirillum tenue* lipopolysaccharides reveals further similarities. Like *Rhodocyclus purpureus*, some, but not all, *Rhodospirillum tenue* strains have L-glycero-D-mannoheptose and D-glycero-D-mannoheptose as main constituents in their O-antigens (14). A part of the L-glycero-D-mannoheptose belongs to a common R-core region in the *Rhodospirillum tenue* O-antigens (9), whereas additional residues of this sugar and all of the D-glycero-D-mannoheptose residues are constituents of strain-specific O-chains of the respective lipopolysaccharides (9). However, there is no correlation between the occurrence of the two epimers of glycero-D-mannoheptose in the lipopolysaccharides described above and the carotenoid compositions of the respective strains. Despite the above described similarities in the compositions of the degraded polysaccharides, there is at least one main difference between *Rhodocyclus purpureus* and *Rhodospirillum tenue*: the relatively large amounts of rhamnose, mannose, and galactosamine in the *Rhodocyclus purpureus* O-antigen indicate the presence of repeating units in the O-chains of this lipopolysaccharide, whereas the short O-chains of *Rhodospirillum tenue* lipopolysaccharides probably do not consist of repeating units (9).

Thus, the data on lipopolysaccharide composition confirm the close relationship between *Rhodocyclus purpureus* and *Rhodospirillum tenue* (8). The relationship between *Rhodocyclus purpureus* and *Rhodopseudomonas gelatinosa* appears to be much less pronounced (Fig. 1). The lipopolysaccharides of *Rhodopseudomonas gelatinosa* are different in the following respects (15): they have no arabinose and no 4-amino-arabinose in their lipid A, the fatty acid spectrum is different, they have D-glycero-D-mannoheptose but no L-glycero-D-mannoheptose in their polysaccharide moiety, and they lack O-specific chains.

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