COMPARATIVE STUDIES ON TWO REACTION CENTER PREPARATIONS FROM *RHODOPSEUDOMONAS SPEHEROIDES* Y

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1. Introduction

In our previous studies [1] we have isolated from a wild type *Rps. spheroides* strain a purified reaction center, i.e., a solubilized complex devoid of light harvesting BChl and containing the photooxidizable BChl, P 865, in a functional state. A cationic detergent, CTAB, was used for extracting the reaction center particles from the bacterial membranes, and a non ionic one (Triton X-100 or Brij 35) for maintaining solubility. CTAB was observed to slowly inactivate the preparation. Hence, we tried recently to prepare reaction centers using instead a non-ionic detergent, LDAO. This detergent has been used extensively to isolate reaction centers from a carotenoidless mutant, *Rps spheroides* R-26 [2, 3] but was apparently unsuitable for carotenoid-containing strains, in which separation of reaction centers from light-harvesting BChl could only be achieved by immunological techniques [4]. In this paper we describe the preparation of LDAO-reaction center, and compare some of its properties to those of CTAB-reaction center.

2. Materials and methods

Bacteria were grown, as described previously, in the synthetic 'L, 17 μM iron' medium [1]. 48 hr-grown cells were disrupted and purified chromatophores (i.e., free of ribosomes) prepared, vide [1].

Abbreviations: Rps spheroides: *Rhodopseudomonas spheroides*; CTAB: cetyltrimethylammonium bromide; LDAO: lauryldimethylamine oxide; BChl: bacteriochlorophyll; Bpheo: bacteriopheophytin; SLS: sodium lauryl sulfate.

For preparing CTAB-reaction centers, the published method [1] was modified as follows: chromatophores were incubated with CTAB at 26°C for 30′ in 0.1 M sodium phosphate buffer, pH 7.5 (final concn: CTAB, 0.5%; A 850 nm, 65; protein, 4.5 mg/ml). The mixture was centrifuged 10′ at 16 000 g, and the pellet discarded. The supernatant (called 'crude reaction center' in table 1) was treated at 5°C with ammonium sulfate (47 g/100 ml); after a 10′ centrifugation at 27 000 g the precipitated reaction center fraction was recovered, dissolved and dialyzed against 0.1 M sodium phosphate buffer, 0.2% Brij 35, pH 7.5. Any insoluble material was then eliminated by a 10′ centrifugation at 27 000 g. The final purification was achieved following either A) or B): A) successive ammonium sulfate precipitations at steadily decreasing salt concentration, as used by Feher [2], from 30

Table 1
Specific activity and yield* of CTAB and LDAO reaction centers

	CTAB-reaction center	LDAO-reaction center
Specific activity		
Purification method: A	11.8	14.0
B	9.2; 8.5 (1 exp)	14.3
Yield:		
Crude reaction center	28%	25%
Purified reaction center	6%	6%

* The specific activity is expressed in moles P 865 per g protein. These were determined as described in [1]. The yield is expressed in % protein recovered in reaction center as compared to chromatophore protein used as starting material. Purification methods A) and B) are described in text. All values are mean values determined from at least 3 experiments except as noted.

g/100 ml to 20 g/100 ml, redissolving at each step the precipitate in the minimal amount of 0.1 M sodium phosphate buffer, pH 7.5 and finally dialysis of the product against the same buffer containing 0.2% Brij 35, B) centrifugation for 24 hr at 314 000 *g*, solution of the pellet in 0.1 M sodium phosphate buffer, 0.2% Brij 35, pH 7.5, and chromatography on a Sepharose 6 B column in the same buffer.

For preparing LDAO-reaction centers, chromatophores were incubated with the detergent at 26°C for 10′ in 0.1 M sodium phosphate buffer, pH 7.5 (final concn: LDAO, 0.25%; *A* 850 nm, 50; protein, 3.3 mg/ml); the mixture was centrifuged at 5°C, 10′ at 16 000 *g*, and the supernatant further centrifuged 2 hr at 150 000 *g*. The two pellets were discarded. The final supernatant ('crude reaction center' in table 1) was treated at 5°C with ammonium sulfate (22 g/ml). The reaction center was recovered after a 10′ centrifugation at 27 000 *g*, redissolved and dialyzed in 0.1 M sodium phosphate buffer, 0.3% LDAO, pH 7.5. Insoluble material if present was eliminated by a 10′ centrifugation at 27 000 *g*. The final purification was as described above, except that 0.3% LDAO was used instead of 0.2% Brij 35, using either A) successive ammonium sulfate precipitations (from 22 g/100 ml to 16 g/100 ml) or B) Agarose 6B chromatography (without prior centrifugation).

The total lipid was extracted with chloroform–methanol 2:1, v/v, by the Bligh and Dyer modified method [5], dried in vacuum and weighed. Total phosphorus was determined in the lipid extract by the method of Bartlett [6], and phospholipid content was derived from the phosphorus analysis, by multiplying by a factor of 25. Lipid components were examined by thin layer chromatography (silica gel G plates (Merck); solvent system: chloroform-acetone-methanol-actic acid-water 50:70:10:10:5, v/v, or acetone–acetic acid–water 100:2:1, v/v). The dried plates were first stained with iodine vapors or Rhodamine 6 G for total lipid detection, then sprayed with ninhydrin to reveal aminolipids, and with phosphomolybdate for phospholipid staining [7]. Lipid standards, test for choline phosphorus and R_f values from the literature [8] were used for identification of the lipids.

Ubiquinone and haem were determined as described in [1]. For SDS-gel electrophoresis, the reaction centers were first freed from lipids, then redissolved in 0.1 M sodium phosphate buffer (or 0.1 M Tris buffer), 2% SDS, 1% β-mercaptoethanol, pH 7.5, and heated for 1′ at 100°C. Thet were then dialyzed overnight at 25°C against 0.01 M sodium phosphate buffer (or 0.01 M Tris buffer), 0.1% mercaptoethanol, pH 7.5. Polyacrylamide gel electrophoresis was then performed as described elsewhere [9].

3. Results and discussion

The preparation procedures have been tested in several experiments (⩾ 5 in each case) and found to be reliable. We should emphasize that a most crucial point is the choice of the incubation conditions: temperature, and relative concentration of detergent relative to that of BChl (or protein) were noted as crucial parameters. Thus, attempts to isolate reaction centers with LDAO at 5°C, even after prolonged incubations at various concentrations, were negative. At 26°C, the concentration ratio, LDAO/BChl (or protein), had to be kept in a narrow range for obtaining a reaction center without severe contamination by other BChl complexes. This was also true for CTAB: at 26°C incubation used was found to give better results than the initial procedure at 5°C [1], as regarded reproducibility and degree of purity.

In both cases, the purification eliminated pigmented contaminants, viz: oxidized BChl (absorbing at 680 nm), a 410 nm absorbing compound (see [1]), and a unbleaching BChl absorbing at 800 and 845 nm. Table 1 gives some characteristics of the preparations. The specific activity was highest with LDAO, and independent in that case of the purification method used. The specific activity of the CTAB reaction center, higher than previously obtained [1] was better with ammonium sulfate purification than with Agarose chromatography. We should note that the LDAO reaction center migrated as a single symmetrical band on Agarose, whereas the CTAB reaction center chromatographed as two fractions of similar specific activities (see table 1) but of unequal magnitude, the major component being the slowest. In this latter case, analyses were done on the major fraction only.

The absorption spectra of the purified reaction centers are given in fig. 1. In both, the bleaching of the 865 nm band by light was total; recovery of ab-

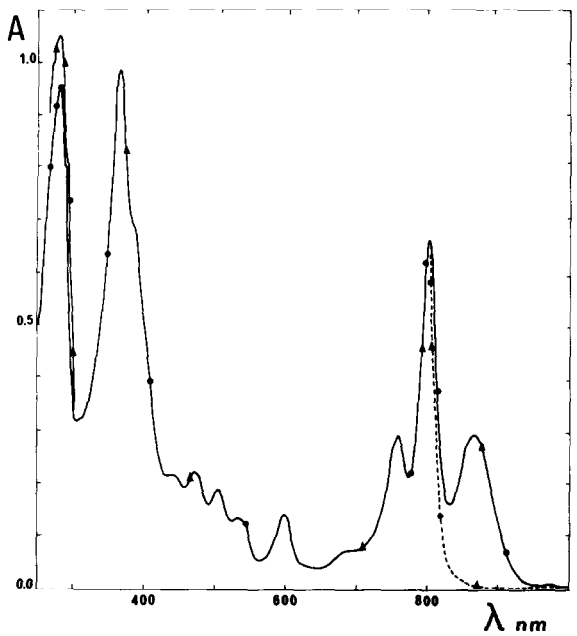

Fig. 1. Absorption spectrum of the purified reaction centers prepared with CTAB (▲) and LDAO (●), in the reduced state. Dashed line: bleaching of the 865 nm band during actinic illumination.

sorbance in the dark was extremely slow (at the difference from crude preparation), so purified reaction centers were routinely reduced by dithionite in order to determine P 865 content. Irrespective of the detergent used, the spectra were identical, both in position and in relative intensity of the absorption bands (except for the aromatic 285 nm band whose intensity varied with specific activity). This indicated a similar pigment composition. In both cases, thin layer chromatography showed the presence of Bpheo, spheroidence, and spheroidenone (in trace amounts) besides BChl; the relative contents of these pigments relative to that of BChl did not depend on the nature of the detergent (unpublished experiments).

The particle weight of the LDAO reaction center (determined by gel filtration as described elsewhere [1]) was 170 000, a value quite similar to that (150 000) found previously for the CTAB reaction center [1]. As already noted [1] this rather high value (as compared to the protein molecular weight) could be due in part to the presence of bound detergent and (or) lipid. In fact reaction centers were found to contain lipids, in similar amounts, rather

Table 2
Total lipid and phosholipid contents of reaction centers and chromatophores (mean values over 4 experiments)

	Reaction center		Chromatophores
	CTAB	LDAO	
Total lipid (mg/g dry weight)	477	613	525
Phospholipid content (mg/g dry weight)	133	133	258
(mg/g protein)	285	365	655

than chromatophores (table 2). However the proportion of phospholipids was decreased by a factor of 2 in reaction centers, which indicated that roughly half of the phospholipids were replaced by CTAB or LDAO. No qualitative difference in lipid composition was found between the two reaction centers. The bound detergents were easily detected in both cases, as they migrated in the solvent system used. The major lipids were identified as phosphatidylethanolamine, phosphatidylglycerol, phosphatidylcholine, and a ninhydrin-positive component, which was probably the ornithine–lipid noted by others [10]. A phospholipid similar in R_f value to diphosphatidylglycerol was present in small amounts.

Both reaction centers contained ubiquinone and very low amounts of -c type haem, but no -b type haem. The molar ratios per P 865 were respectively: 1.7 and 2.5 UQ, and 0.12 and 0.09 -c type haem for LDAO and CTAB reaction centers. These values were much lower than those found earlier for CTAB reaction center preparations of lower specific activity [1].

From the particle weight (see above) and the ratio of lipid to dry weight (table 2), one may estimate the maximum weight of protein per particle: values of 66 000 and 78 000 daltons were obtained respectively for LDAO and CTAB reaction centers. Comparative experiments on the protein components were done by SDS-gel electrophoresis. Again no qualitative difference between the two reaction centers was found. When the electrophoresis was performed in phosphate buffer, results identical to those already published [9] were obtained: that is, a major protein species (M_w = 30 000) was observed. When Tris buffer was

used instead of phosphate, this band was split into three components (M_w = 22 000, 24 000 and 27 000), in agreement with the observations of other authors [2, 3].

These results clearly show that the compositions of the reaction center particles extracted with either CTAB or LDAO are essentially the same. Thus the two detergents seem to attack the chromatophore membrane in a identical fashion. The LDAO procedure has distinct advantages over that using CTAB in that it permits easier and better purification. A comparison between the reaction centers from the carotenoid-less mutant R-26, extensively studied (see [11] for a review) and from the wild type Y strain, is now possible and important to accomplish. For the present the major difference between them is the presence in the wild type strain-reaction center only of phospholipids and carotenoids. Further studies on the pigment and the protein components of the wild type reaction center, now in prospect, should show if this difference is unique.

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