PHOTOCHEMICAL REACTION CENTRES FROM *RHODOPSEUDOMONAS CAPSULATA ALA PHO*$^+$

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1. Introduction

During the past few years, much attention has been paid to the preparation, by use of surface active agents, of reaction centre particles from chromatophores of a number of species of photosynthetic bacteria. Thus preparations have been made from *Rhodopseudomonas spheroides* [1–3] *Rps. viridis* [2,4] and *Rhodospirillum rubrum* [5] using Triton X-100 [1,5], sodium dodecyl sulphate [2,4] and cetyl trimethyl ammonium bromide [3].

More recently, reaction centre preparations have been obtained which are substantially smaller than those mentioned above, and contain fewer components in association with the reaction centre itself. This has been achieved [6–8] using the zwitterionic detergent LDAO (lauryl dimethyl amine oxide) on blue green (carotenoid less) mutants of *Rps. spheroides* [6,7] and *R. rubrum* [8]. However, to date, no reaction centre preparations have been prepared from *Rps. capsulata.* The recent isolation of a blue green mutant of this species, termed *Ala pho*$^+$, by Drews et al. [9] has enabled us to prepare reaction centres from this species essentially by the method of Clayton and Wang [6].

2. Materials

The original culture of *Rps. capsulata Ala pho*$^+$ was a gift from Professor G. Drews. The cells were grown in batch culture, harvested in late log phase, and stored at −20°C, essentially as previously described [10].

Ammonyx LO (lauryl dimethyl amine oxide) was a generous gift from A.B.M. Industrial Products Ltd., Woodley, Stockport, Cheshire.

Aluminium oxide (activity grade I, anionotropic) was purchased from M. Woelm, Eschwege, Germany. All other chemicals used were analar grade or of the purest grade available commercially.

3. Methods and results

Reaction centres were prepared by the method of Clayton and Wang, as outlined in table 1. To prepare chromatophores the cells were broken in the French press at a pressure of 16 tons/inch^2. The more usual breaking pressure of 10 tons/inch^2 used to break wild type strains of *Rps. capsulata* did not break a significant number of cells, thus necessitating the use of this higher pressure.

The purified reaction centres were resuspended in 10 mM Tris–HCl, pH 7.5 with 2% LDAO, and stored at −20°C.

Spectra were recorded on a Cary 14 spectrophotometer. As shown in fig. 1, the measuring beam of this instrument was sufficiently intense to cause a significant photobleaching of the P870 peak, and other associated spectral changes. This could be overcome by the addition of *N*-methylphenazonium methosulphate (PMS) (40 μM) and sodium ascorbate (100 μM) which kept the reaction centres fully reduced. Spectra of fully oxidised preparations were obtained by the addition of potassium ferricyanide (100 μM).

In the region 400 nm–700 nm the light oxidised minus dark reduced spectra of the reaction centres is

Table 1
Scheme for the preparation of reaction centres from *Rps. capsulata Ala pho*$^+$.

CELL SUSPENSION

French pressure cell at 16 tons/inch^2,
centrifuge 20 000 *g* for 20 min, discard pellet,
centrifuge supernatant 140 000 *g* for 90 min.

CHROMATOPHORE SUSPENSION

Adjust *A* at 870 nm to 50,
add 1% LDAO, layer onto 0.25 M and 0.5 M sucrose,
centrifuge 200 000 *g* for 90 min

PELLET OF EXTRACTED MEMBRANES

BULK BACTERIOCHLOROPHYLL –PROTEIN COMPLEX AT 0.25 M/0.5 M SUCROSE INTERFACE

SUPERNATANT REACTION CENTRES

50% $(NH_4)_2SO_4$ 7000 *g* for 20 min.

Discard supernatant,
redissolve levitate in 2% LDAO,
10 mM Tris–HCl pH 7.5.

50% $(NH_4)_2SO_4$ 7000 *g* for 20 min.

Discard supernatant,
redissolve levitate,

35% $(NH_4)_2SO_4$ 7000 *g* for 20 min.

Discard levitate

Bring supernatant to 60% $(NH_4)_2SO_4$ saturation
7000 *g* for 20 min.

LEVITATE OF PURIFIED REACTION CENTRES

Redissolve in 2% LDAO, 10 mM Tris–HCl pH 7.5

All operations were carried out in dim light at room temperature.

identical with the ferricyanide oxidised minus PMS ascorbate reduced reaction centre spectrum. This is also true for reaction centres prepared from *Rps. spheroides* R_{26}. (D. Crowther and R. Prince, unpublished observation). We have therefore used ferricyanide and PMS ascorbate to mimic light minus dark spectra over the range 900 nm–500 nm in the Cary 14 as shown in fig. 2, since for technical reasons we are unable to measure the light induced change above 700 nm. The cytochrome content of the reaction centres was estimated on the basis of reduced minus oxidised spectra in the region of 555–540 nm, using dithionite and ferricyanide added to the reaction centres. No cytochromes could be detected in the

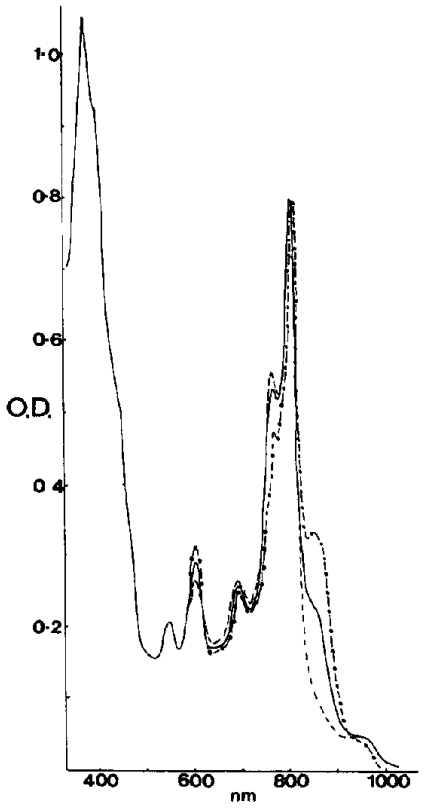

Fig. 1. Cary 14 spectrum of reaction centres from *Rps. capsulata Ala pho*$^+$. Reaction centres (2.9 μM calculated using A_{mm} at 870 nM = 113 [6]) suspended in 10 mm Tris–HCl, pH 7.5, 2% LDAO, supplemented as indicated by PMS (40 μM) and sodium ascorbate (100 μM) or potassium ferricyanide (100 μM): (——) no additions; (·—·—·) plus PMS ascorbate; (-----) plus ferricyanide.

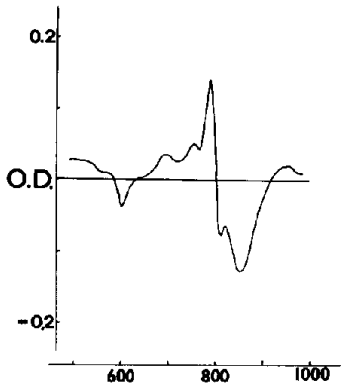

Fig. 2. Oxidised minus reduced Cary 14 spectrum of reaction centres from *Rps. capsulata Ala pho*$^+$. Reaction centres (2.5 μM) reduced with PMS-(40 μM) and sodium ascorbate (100 μM) or oxidised with potassium ferricyanide (100 μM).

reaction centres (<1 cyt/20 BChl) or the bulk bacteriochlorophyll–protein complex fraction, but cytochromes were present in the pellet of the 200 000 *g* spin. These results, and identical ones obtained by us for *Rps. spheroides* R_{26} reaction centres are in agreement with the data of Clayton and Yau [11].

Quinones were estimated by the method of Griffiths et al. Essentially this involved a total lipid extraction into acetone and methanol followed by extraction into petrol ether, and subsequent column chromatography on Alumina to separate ubiquinones from other lipids. Ubiquinone was estimated by the difference spectra ± sodium borohydride at 275 nm [11]. This method estimated ~ 1.5 ubiquinones per reaction centre. A similar extraction procedure but using *Rps. spheroides* R_{26} reaction centres also indicated ~ 1.5 ubiquinones per reaction centre, and this is in contrast to Clayton and Yau [11]. However, we have used a total lipid extraction with methanol and acetone while Clayton and Yau extracted freeze-dried preparations with iso octane. We have used iso octane and have been unable to extract all the quinones present in the reaction centre preparation, although this solvent does remove 10–15% of the quinones present which is in agreement with Clayton and Yau [11].

4. Discussion

The reaction centre preparations described here are essentially similar to those prepared with LDAO from *Rps. spheroides* [6, 7] and *R. rubrum* [8] in that the reaction centre complex is substantially free of secondary electron carriers other than quinones. There are, however, several changes in the positions of the spectral peaks, making the reaction centres described here green compared with the 'ice-blue' of those from *Rps. spheroides.*

On excitation by flash or continuous light, these reaction centres were able to photooxidise added reduced mammalian cytochrome *c* (c.f. [13]) and also added *Rps. capsulata* cytochrome c_2 (R.C. Prince, R.J. Cogdell and A.R. Crofts in preparation) at rates

comparable to those observed *in vivo*. *Ortho*phenanthroline (4 mM) inhibited cytochrome oxidation in continuous light presumably by inhibiting electron flow between primary and secondary electron acceptors (D. Crowther and R.C. Prince unpublished observation). Added 1,4-napthoquinone (100 μM) stimulated cytochrome *c* oxidation in continuous light.

Antiserum prepared against *Rps. spheroides* R_{26} reaction centres showed no cross reaction with those from prepared *Rps. capsulata*, *Ala pho*$^+$ (R.J. Berzborn, G. Hauska and R.C. Prince, unpublished observation). This result is similar to that of Wang and Clayton [8] who found no cross-reaction of this serum with reaction centres from *R. rubrum*. Thus although the three reaction centre preparations are very similar, they do not necessarily share antigenic determinants.

Acknowledgements

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