THE LOCATION OF THE CAROTENOID IN THE B800–850 LIGHT-HARVESTING PIGMENT–PROTEIN COMPLEX FROM *RHODOPSEUDOMONAS CAPSULATA*

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1. Introduction

The light-harvesting apparatus of the purple non-sulphur photosynthetic bacteria *Rhodopseudomonas capsulata* consists of 2 types of pigment–protein complexes (B870 and B800–850) [1,2]. The B800–850 complex is more stable than the B870 complex, easier to purify and is consequently better characterised [3–8].

In vivo the B800–850 complex probably exists as aggregates of a basic minimal unit. This minimal unit consists of 3 molecules of bacteriochlorophyll, 1 molecule of carotenoid and 1 each of 3 polypeptides with mol. wt 14 000, 10 000 and 8000 [3,6].

Two of the bacteriochlorophyll molecules are associated with the 850 nm absorption while the third is responsible for the 800 nm absorption [6,7]. The pigments are only bound to the 2 low molecular weight polypeptides [3].

In [9] mild trypsin digestion of chromatophores, from a photosynthetically incompetent mutant of *Rps. capsulata*, strain Y5, (this strain has only the B800–850 and lacks the B870 and reaction centre complexes), initially selectively degraded the 800 nm absorption leaving the 850 nm absorption relatively intact.

Here we have been able to use mild pronase digestion of *Rps. capsulata* chromatophores to show that in the B800–850 complex the carotenoid is associated with the 8000 mol. wt polypeptide.

2. Materials and methods

Cells of *Rps. capsulata*, strain Z1, were grown photosynthetically with succinate as the sole carbon source. The cells were harvested and then disrupted by passage through a French pressure cell. Chromatophores were isolated from the broken cells by differential centrifugation [10] and resuspended in 20 mM Tris–HCl (pH 8.0).

The B800–850 light-harvesting complex was solubilised and purified as in [3] except that the sucrose density gradient centrifugation step was omitted.

For the proteolysis the chromatophores were diluted with 20 mM Tris–HCl (pH 8.0) to give 2.5 mg protein/ml. The digestion was carried out at 37°C in the dark and was initiated by the addition of pronase (Sigma type V1, 20 μg/mg membrane protein). The protein concentration was determined by the tannin assay [11].

At various times during the pronase digestion pairs of samples were removed. One aliquot was immediately diluted 20-fold with Tris buffer and its absorption spectrum was recorded on Pye-Unicam SP8000 and SP500 spectrophotometers. The other aliquot was immediately denatured by boiling for 1 min following the addition of an equal volume of denaturing solution (50 mM Tris–HCl (pH 8.0), 2% sodium dodecyl sulphate (SDS), 2% β-mercaptoethanol and 0.1% bromophenol blue).

The denatured aliquots were analysed by SDS–polyacrylamide slab gel electrophoresis as in [12], using a linear concentration gradient of acrylamide (10–16.5%). The gels were stained with Kenacid blue R and the individual tracks were scanned at 550 nm using a Gilford 240 spectrophotometer equipped with a linear transport device. The molecular weights of the polypeptides were estimated by calibration of the gels with the following protein standards: bovine serum albumin (68 000), alcohol dehydrogenase (41 000), myoglobin (17 200) and equine cytochrome *c* (11 700).

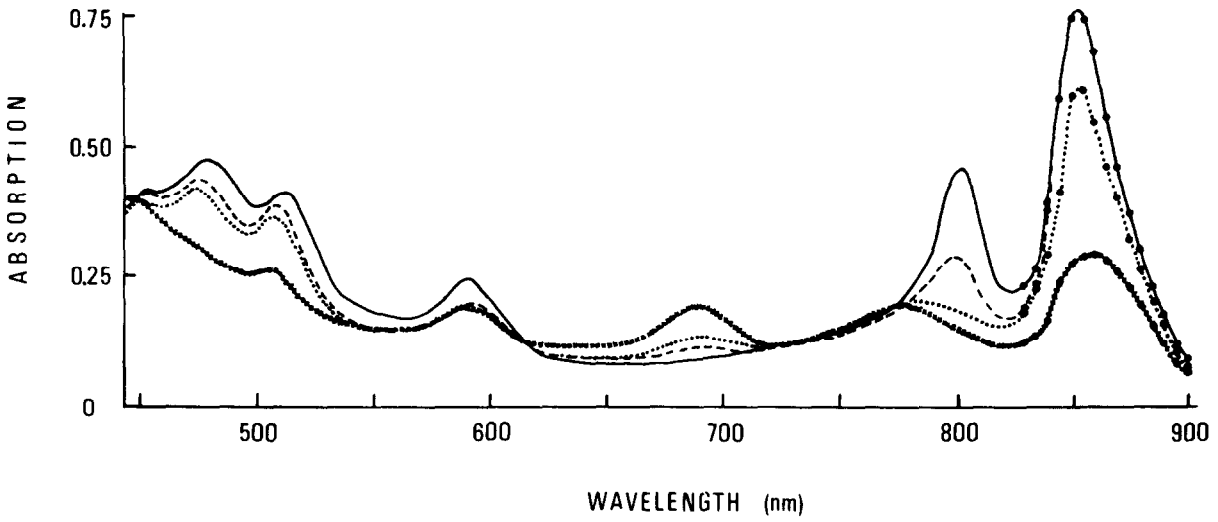

Fig.1. The absorption spectra of *Rps. capsulata* chromatophores at various times during the incubation with pronase. (——) 0 min; (– – –) 45 min; (...) 150 min; (xxx) 270 min after the addition of pronase. The absorption spectra of the untreated and 45 min treated samples were coincident from 840–900 nm. Spectra from 430–830 nm were recorded on the SP8000 and from 830–900 nm at 5 nm intervals on the SP500 (absorbance values measured on the SP500 are shown by the solid circles). Incubation conditions are given in section 2.

3. Results and discussion

Fig.1 shows the changes in the absorption spectrum of chromatophores, from 430–900 nm, at various times after the addition of pronase. As described [9] mild proteolysis of chromatophores results in a sequential degradation of the two bacteriochlorophyll absorption peaks associated with the B800–850 light-harvesting complex. Initially only the 800 nm spectral form is degraded. Then after prolonged pronase digestion the 850 nm absorption is removed.

In addition to these effects in the infrared, absorption changes induced by pronase treatment are seen in the region where the carotenoids absorb. The effect of pronase digestion upon absorption spectrum of the carotenoids is best visualised in a difference spectrum (fig.2). As the 800 nm bacteriochlorophyll absorption band disappears a proportion of the absorption due to the carotenoids undergoes a blue shift.

In fig.3 the time course of the loss of absorption at 800 nm and 850 nm are compared with the blue shift in the carotenoids. The blue shift of the carotenoid absorption bands and the degradation of the 800 nm bacteriochlorophyll band show similar kinetics and precede the decrease in absorption at 850 nm. The blue shift of the carotenoid absorption bands

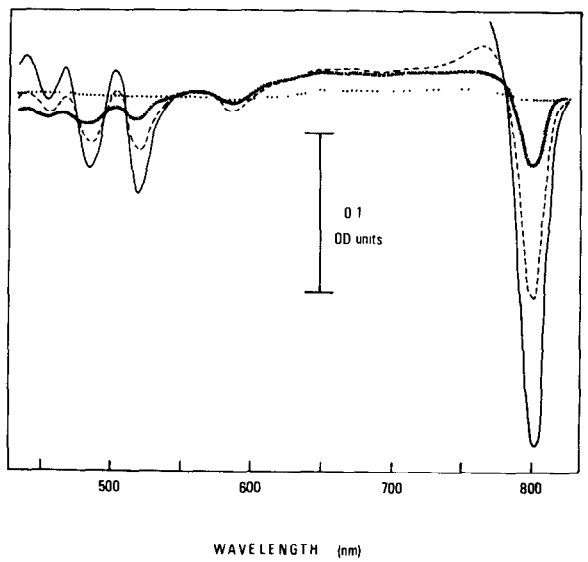

Fig.2. Difference spectra of pronase treated minus untreated *Rps. capsulata* chromatophores. (●●●) 0 min; (xxx) 8 min; (– – –) 24 min; (——) 65 min after the addition of pronase. Details of the incubation conditions are given in section 2.

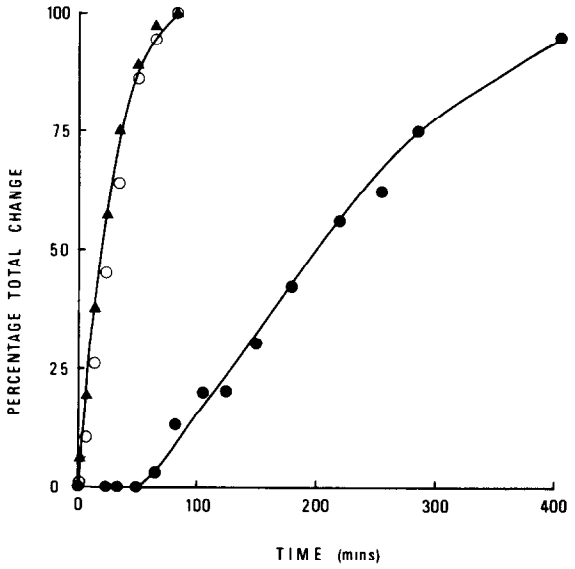

Fig.3. Time course of pronase-induced degradation of the B800–850 bacteriochlorophyll absorption bands and the blue shift of the carotenoid spectrum in *Rps. capsulata* chromatophores. The total absorption change was taken as the maximum change observed during a 12 h incubation with pronase. Further addition of pronase at this stage had no effect on the chromatophore absorption spectrum. The disappearance of the 800 nm band was followed by the absorption difference 830–800 nm (▲——▲). Degradation of the 850 nm bacteriochlorophyll was followed by direct measurement at this wavelength (●——●). The blue shift of the carotenoid bands was measured by the peak to trough absorption difference at 505–522 nm (○——○). Conditions of the incubation with pronase are given in section 2.

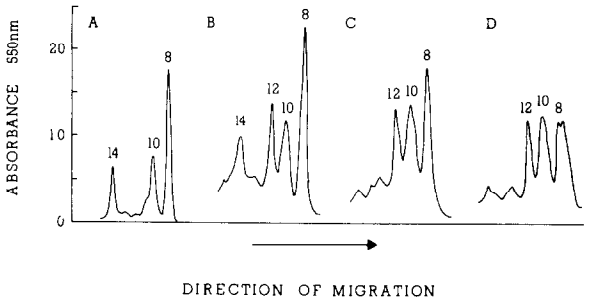

Fig.4. Polypeptide pattern in SDS–polyacrylamide gel electrophoresis of the light-harvesting region of pronase treated *Rps. capsulata* chromatophores. (A) Untreated, purified B800–850 complex; (B) chromatophores incubated with pronase for 0 min; (C) 45 min; (D) 150 min. Gel electrophoresis was carried out as in section 2 on samples taken from the same experiment as depicted in fig.1.

reflects the removal of the carotenoid from its binding site on the protein. Similar blue shifts are obtained, for example, if a sample of the isolated B800–850 light-harvesting pigment–protein complex is denatured.

The effect of the pronase digestion upon the polypeptides of the light-harvesting proteins was examined by polyacrylamide gel electrophoresis. Fig.4 shows representative gel scans of the light-harvesting polypeptide region of membrane samples taken periodically during the same pronase digestion as depicted in fig.1. Four polypeptides are clearly seen and as described [3] the 12 000 mol. wt polypeptide represents B870 and the 14 000, 10 000 and 8000 mol. wt polypeptides represent B800–850. The 14 000 mol. wt polypeptide is very rapidly degraded and its loss is complete before any large absorption changes in the near infrared are seen. The 8000 mol. wt polypeptide is degraded before the 10 000 mol. wt polypeptide while the 12 000 mol. wt B870 polypeptide is largely unaffected.

These results support the conclusions drawn in [9] that the 800 nm bacteriochlorophyll is bound to the 8000 mol. wt polypeptide while the 850 nm bacteriochlorophylls are bound to the 10 000 mol. wt polypeptide. The similar kinetics of loss of the 800 nm absorption and the blue shift of the carotenoid would put the carotenoid present in the B800–850 complex also on the 8000 mol. wt polypeptide. However, it is clear from comparing the time course of the loss of the 8000 mol. wt polypeptide with the degradation of the 800 nm absorption band that the 800 nm band is lost before the complete removal of the 8000 mol. wt polypeptide. This probably means that only limited proteolysis is required for the pigments to be dislodged from this polypeptide.

It is difficult to directly rule out the possibility that the carotenoid which is undergoing the blue shift is bound to either the reaction centre or B870. However, several lines of evidence make this suggestion untenable. The B870 polypeptide is unaffected by the pronase digestion during the time course of either the blue shift of the carotenoid or the loss of the 800 nm absorption. Also there is no loss of B870 absorption during this period of the digestion. Over the period of the digestion when the 800 nm absorption is being removed reaction centre photochemistry and the reaction centre carotenoid band shift are

completely unaffected (G. D. W., R. J. C., G. J. L., submitted).

We conclude that the carotenoid associated with the B800–850 complex from *Rps. capsulata* is located, together with the 800 nm bacteriochlorophyll on the 8000 mol. wt polypeptide.

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