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# THE ISOLATION AND PARTIAL CHARACTERISATION OF THE LIGHT-HARVESTING PIGMENT-PROTEIN COMPLEMENT OF *RHODOPSEUDOMONAS ACIDOPHILA*

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**The photosynthetic membranes of two strains of *Rhodopseudomonas acidophila* (7750 and 7050) have been resolved into their constituent light-harvesting pigment-protein complexes. Four different types of antenna complexes (B880, B800-830 and two types of B800-850) have been isolated and partially purified. In each case the light-harvesting pigments (bacteriochlorophyll *a* and carotenoids) are bound to rather low molecular weight polypeptides (in the 5000–9000 region).**

## Introduction

In most species of photosynthetic bacteria the components required for the 'light reactions' are localised in and on the intracytoplasmic membranes. The pigment-protein complexes which make up the photosynthetic unit (the light-harvesting complexes and the photochemical reaction centres) constitute the majority of the proteins in these membranes. It is therefore important for a detailed understanding of both the structure and function of the photosynthetic membrane to characterise these pigment-protein complexes.

In the present study we described the isolation and partial characterisation of the antenna pigment-protein complexes from *Rhodopseudomonas acidophila. Rps Acidophila* is a purple non-sulphur photosynthetic bacterium and it takes its name from the fact that the optimal pH for its growth is pH 5.2 [1]. The major light-absorbing pigments in *Rps. acidophila* are bacteriochlorophyll *a* (BChl *a*) [1] and carotenoids of the 'normal spirolloxanthin series' [2].

The structure of the antenna complexes from purple photosynthetic bacteria has recently been reviewed [3]. A typical antenna complex consists of a rather small number of BChl *a* and carotenoid molecules non-covalently bound to low molecular weight, hydrophobic polypeptides. These complexes are generally identified by the positions of their absorption maxima in the near-infrared region of the spectrum due to BChl *a*. Based on this criterion there appear to be two main types of antenna complexes, those with a single strong absorption maxium in the near infrared (e.g., B890 from *Rhodospirillum rubrum* [4]) and those with two strong absorption maxima (e.g., B800-850 from *Rps. sphaeroides* [5,6]). The present investigation of the antenna complexes from *Rps. acidophila* is also part of our attempts to gain a detailed understanding of a wider range of light-harvesting pigment-protein complexes, to see whether this division of antenna complexes into two major groups is a useful generalisation. A preliminary report of some of this data been presented elsewhere [7].

Abbreviation: BChl, bacteriochlorophyll.

## Materials and Methods

*Growth of the cells*

Two wild-type strains of *Rps. acidophila* (7750 and 7050) were used in the present study. Both strains were kindly provided by Professor N. Pfennig, Institute of Microbiology, Göttingen. The cells were grown anaerobically in the light at 30°C in Pfennig's medium [1] with succinate as the sole carbon source. Usually cells were grown in flat-sided 250-$cm^3$ bottles and harvested in late log phase. The light intensity at which the cells were grown was varied between 100 and 2000 lx (measured with an United Detector Technology, Model 40X, Optometer (Santa Monica, CA) light meter, fitted with its photometric filter).

Cells were also grown aerobically in a rotary incubator. In this case 2.5 $cm^3$ of a photosynthetically grown culture were used to inoculate 500 $cm^3$ of medium in a 2 l conical flask. The incubator was set at 30°C and to shake at 150 cycles/min.

After growth the cells were harvested, washed and resuspended in 20 mM Tris-HCl, pH 8.0. The cells were then either used immediately or stored frozen at −20°C until they were required. Their absorption spectra were recorded on a Zeiss DMR 21 spectrophotometer. This spectrophotometer is ideal for use with whole cells, which are rather turbid, since the cuvette position is very close to the light detector. The concentration of protein was determined by the method of Lowry et al. [8] in Göttingen and by the Tanin method [9] in Glasgow. The concentration of BChl *a* was determined by extraction with 7 : 2 (v/v) acetone/methanol, using a millimolar extinction coefficient of 76 $cm^{-1}$ at 772 nm [10].

*Isolation and characterisation of the light-harvesting pigment-protein complexes*

The washed cells were broken by passage through a French pressure cell at 10 ton/$inch^2$ in the presence of a little DNAase. The disrupted membranes were then collected by centrifugation at 12000 × *g* for 20 min. Unlike *Rps. sphaeroides*, *Rps. acidophila* has lamellar, intracytoplasmic membranes (see Fig. 1). Cell disruption therefore yields mainly large membrane fragments rather than chromatophores.

The antenna complexes were isolated by solubilising the membranes with lauryldimethylamine *N*-oxide and subsequent fractionation by column chromatography on Whatman DE52 DEAE-cellulose. A considerable degree of variation was encountered in the chromatography from batch of cells. Therefore, the purification method described below is only an indication of the required procedure rather than a precise description. This problem has been discussed at length elsewhere [11].

The membranes were resuspended in 20 mM Tris-HCl, pH 8.0, to give an absorbance at 850 nm of 50 $cm^{-1}$. This solution was then made 1% (v/v) with lauryldimethylamine *N*-oxide, and incubated with stirring, at room temperature for 10 min. The unsolubilised material was then removed by a low-speed centrifugation at 12000 × *g* for 10 min. The solubilised fraction was collected, diluted five times with 20 mM Tris-HCl, pH 8.0, and loaded onto a column of DE52 cellulose, equilibrated with 20 mM Tris-HCl, pH 8.0. Best results are obtained with the column loaded to 1/3 of its capacity. The various complexes were then eluted by an NaCl gradient of 0–300 mM made up in 20 mM Tris-HCl, pH 8.0, 0.2% (v/v) lauryldimethylamine *N*-oxide. Typically a given complex was collected, diluted and further purified by passage over a second DE52 column. The different pigment-protein complexes have different carotenoid compositions. This is very convenient, since this gives them a different colour and they can be easily distinguished by eye, during the chromatography.

The carotenoid content of the isolated pigment-protein complexes was determined by standard procedures [2,12]. The absorption spectra of the complexes were recorded on an SP8000 recording spectrophotometer (wavelengths up to 850 nm) and on an SP500 spectrophotometer (wavelenghts above 850 nm). Their polypeptide composition was determined by electrophoresis on SDS gradient (11.5–16.5%) polyacrylamide gels using the method of Laemmli [13] as previously described [14].

## Results and Discussion

Two strains of *Rps. acidophila*, 7050 and 7750, were used in the present study. These two strains

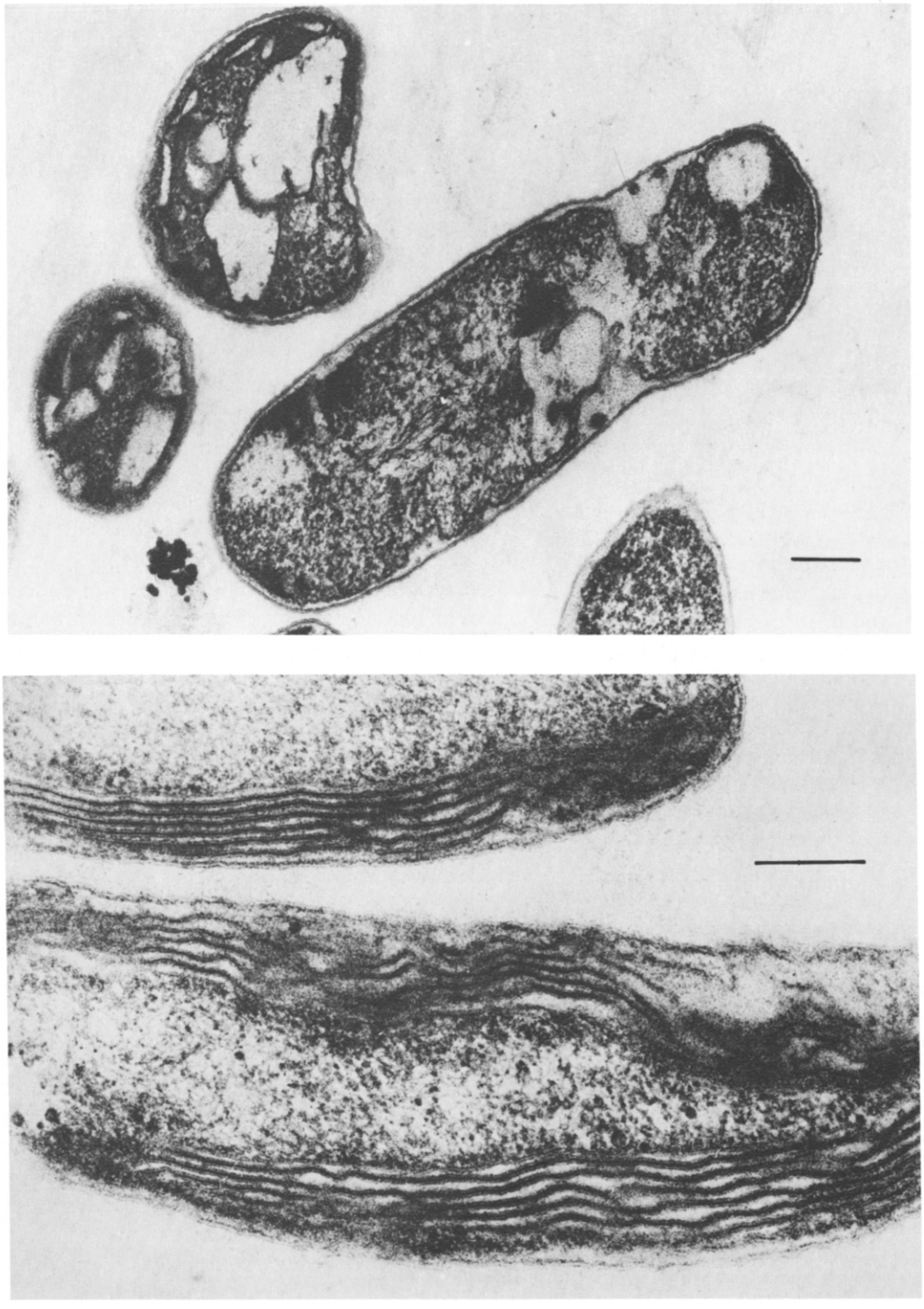

Fig. 1. Electron micrographs of cross-sections through whole cells of *Rps. acidophila* 7050. (A) Cells grown aerobically. The scale bar represents 200 nm. (B) Cells grown photosynthetically at 400 lx. The scale bar represents 200 nm. The cells were visualised by negative staining with 4% (w/v) uranyl acetate.

differ in their response to growth at different light intensities [15] and their content of pigment-protein complexes (see below). The use of these two strains therefore provides extra information upon the complement of the antenna complexes in *Rps. acidophila*.

When *Rps. acidophila* is grown aerobically the cells have very few intracytoplasmic membranes (Fig. 1A) and a low BChl *a*/protein ratio (Table I). The near-infrared absorption spectrum of aerobic cells (illustrated in Fig. 2 for strain 7050, but the same is true for strain 7750) shows only one major BChl *a* absorption band at approx. 885 nm. In contrast, photosynthetically grown cells have an extensive system of intracytoplasmic membranes (Fig. 1B) (lamellae in this case) and an increased BChl *a* content (Table I). The exact value of the BChl *a*/protein ratio depends upon the light intensity at which the cells are grown.

Associated with the increase in BChl *a* content in the photosynthetically grown cells is an increase in the complexity of the near-infrared absorption spectrum of the cells. This is illustrated in Fig. 2 for cells from strain 7050. Cells grown at 400 lx show additional maxima/shoulders in the near-infrared at approx. 800, 830 and 850 nm, while those grown at 2000 lx have two major absorption bands in the near-infrared with peaks at approx. 800 and 850 nm. We show below that this large increase in BChl *a* content is associated with the appearance of new types of antenna pigment-protein complexes.

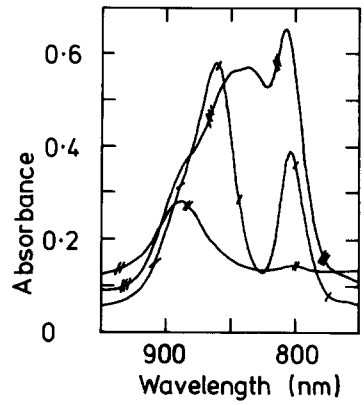


Fig. 2. The near-infrared absorption spectra of whole cells of *Rps. acidophila* strain 7050 grown under different conditions. (/) Cells grown photosynthetically at 2000 lx. (//) Cell grown aerobically. (///) Cells grown photosynthetically at 400 lx. Different amounts of cells were used in each case so that the spectra could be recorded on the same absorbance scale.

TABLE I

THE SPECIFIC BChl *a* CONTENT OF WHOLE CELLS OF *RPS. ACIDOPHILA* 7050 GROWN AEROBICALLY AND ANAEROBICALLY AT DIFFERENT LIGHT INTENSITIES

The results presented here are from a single experiment. The absolute values of the specific BChl *a* content vary from run to run but the trend depicted here is always the same. Values of [BChl *a*] were obtained using the method of Lowry et al. [7].

| Light intensity (lx) | [BChl *a*] (μg/mg protein) | Strain |
|---|---|---|
| Aerobic | 1.6 | 7050 |
| 100 | 68.5 | 7050 |
| 400 | 52.9 | 7050 |
| 1000 | 35.7 | 7050 |
| 2000 | 28.2 | 7050 |
| Aerobic | 1.6 | 7750 |
| 100 | 74.8 | 7750 |
| 400 | 54.5 | 7750 |
| 1000 | 36.9 | 7750 |
| 2000 | !8.3 | 7750 |

In parallel with these alterations in the BChl *a*/protein ratio in the cells, dependant upon the growth conditions, there are also dramatic changes in the carotenoid composition [15]. These changes are largest in strain 7050. In this strain the aerobic cells are orange-red, the cells grown at 400 lx are deep purple and the cells grown at 2000 lx are orange/brown.

The absorption spectrum of cells of strain 7750 (data not shown) is not so variable as that of strain 7050. At all the light intensities for growth that were used in this study the near-infrared absorption spectrum of photosynthetically grown cells for strain 7750 was very similar to that of the 2000 lx cells from strain 7050, and the 7750 cells were always orange/brown in colour.

The light-harvesting complexes from *Rps. acidophila* can be fully resolved following solubilisation of the photosynthetic membrane with the zwitterionic detergent lauryldimethylamine *N*-oxide, and the whole cell absorption spectrum may be completely understood in terms of the

presence of these antenna complexes (see below).

The absorption spectra of the isolated, purified antenna pigment-protein complexes are presented in Fig. 3. The first pigmented complex which is eluted from the DE52 column is the B880 complex which also appears to contain reaction centres (Fig. 3A). This complex has an absorption maximum in the near-infrared at approx. 885 nm and appears to correspond to the antenna complex which dominates the absorption spectrum of the weakly pigmented aerobic membranes (Fig. 2). This complex has a red/brown colour and is always eluted first from the DE52 column irrespective of which strain of *Rps. acidophila* is being fractionated.

When 2000 lx-grown cells of strain 7750 are used in this preparation procedure the next and final complex eluted from the column is the type I B800-850 antenna complex (fig. 2A). This complex has an orange/brown colour and is spectrally very similar to the B800-850 light-harvesting complexes which have been isolated from *Rps. sphaeroides* [5,6] and from *Rps. capsulata* [16]. It is characteristic of this type of B800-850 complex that the absorbance at approx. 850 nm is usually between 1.5- and 2-times larger than the absorbance at 800 nm. A similar type I B800-850 antenna complex can be obtained from 2000 lx cells of strain 7050. However, we have concentrated upon strain 7750 for the isolation of this complex, since with strain 7750 there is no possibility of contamination with the other antenna types which are also present in strain 7050.

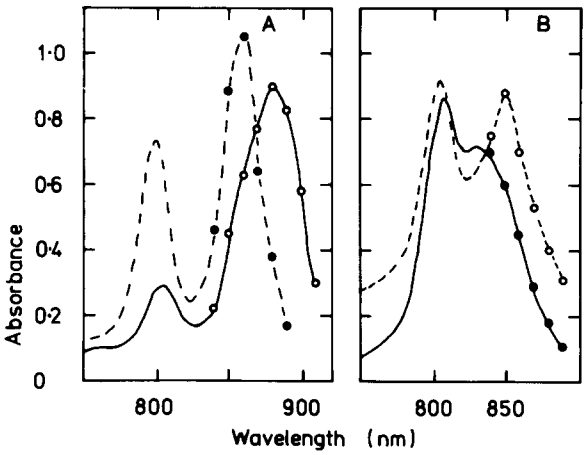


Fig. 3. The near-infrared absorption spectro of the isolated light-harvesting pigment-protein complexes from *Rps. acidophila*. In each case the complexes were suspended in 20 mM Tris-HCl at pH 8.0, 0.1% v/v lauryldimethylamine *N*-oxide. The circles show where the recording spectrophotometer stopped and the spectra were continued point by point in the SP500 spectrophotometer. (A) Complexes from strain 7750 grown photosynthetically at 2000 lx: (●— — —●) type I B800-850, (○ ——— ○) B880. (B) complexes from strain 7050 grown photosynthetically at 400 lx: (○- - - - -○) type II B800-850 this spectrum was raised to make it more clearly visible, (●———●) B800-830.

Two points can be emphasised from the fractionation of strain 7750 membranes. Firstly, the near-infrared absorption of the whole cells can be adequately accounted for in terms of the absorption spectra of the isolated complexes, and secondly the isolated antenna complexes retain their integrity during their isolation and purification. Monomeric BChl *a* in organic solvents absorbs maximally in the near-infrared at approx. 770 nm [10] but in the antenna complexes its near-infrared absorption bands are strongly red shifted. This red shift if fully reversed if the antenna complexes are denatured (data not shown). The position of the near-infrared absorption bands is therefore a good indication of the correct pigment-protein interaction within these complexes and because the near-infrared absorption maxima of the isolated antenna complexes are very nearly the same as those seen in the whole cells from which they were derived, it is clear that they have retained their native structure.

Fractionation of 400 lx-grown strain 7050 cells yields two further types of antenna pigment-protein complex, type II B800-850 and B800-830 (Fig. 3B). The type II B800-850 antenna complex is typified by the absorbance at 800 and 850 nm being very nearly equal, and is therefore very similar to the B800-850 complex from *Chromatium vinosuim* [11,17]. The type II B800-850 complex is eluted before the B800-830 complex; however, their separation is not very clear and care is required to obtain good resolution between them. Again with the iolated strain 7050 complexes their absorption maxima closely correspond to those seen in the intact whole cells, and the whole cell absorption spectrum is clearly adequately accounted for in terms of the antenna types isolated.

There is some degree of variability in the position of the long-wavelength absorption band of

both the type II B800-850 and the B800-830 antenna complexes (just as was experienced with the corresponding light-harvesting complexes from *C. vinosum* [11,17]). This spectral variability (815–830 nm for the B800-830 and 835–850 nm for the type II B800-850) seems to depend upon such factors as the concentration of salts and detergent in the solution. The relationship between these two antenna types is not yet clear. Both are clearly present in the intact photosynthetic membrane (see Fig. 2) and so are not just generated during our preparative producers. However, we show below that they appear to have an identical polypeptide composition. It is therefore possible that these two antenna types may represent altered forms of the same basic structural unit.

We have attempted to characterise the polypeptide composition of the isolated antenna complexes by SDS gradient polyacrylamide gel electrophoresis. In this characterisation we have sought to demonstrate two major points. Firstly, that the polypeptides identified actually bind the light-harvesting pigments, and secondly that these polypeptides were also present in the intact photosynthetic membranes prior to their fractionation (i.e., that they were not artefactually created). When the B800-850 antenna complexes from either *Rps. sphaeroides* [14] or *Rps. capsulata* [16] are run on SDS-polyacrylamide gels, if the antenna samples are not boiled, to denature them prior to the electrophoresis, then they migrate as intact, pigmented bands. If the same sample is progressively denatured by boiling for increasing periods of time then the complex breaks down revealing its individual, polypeptide components. The pigmented band is then replaced by the set of bands representing the constituent, polypeptides and the pigments, now stripped off the complex, run at the ion front. This technique clearly reveals which polypeptides are actually involved in binding the pigments and allows contaminating polypeptides, which do not usually co-migrate with the intact pigmented complex, to be identified.

Fig. 4 shows the results of such an experiment run with the type I B800-850 antenna complex from strain 7750. The undenatured sample runs as a pigmented band with an apparent molecular weight of greater than 100000. Electrophoresis of the denatured type I B800-850 complex reveals the presence of three low molecular weight polypeptides in the 5000-9000 region. However, some of the highest molecular weight component is

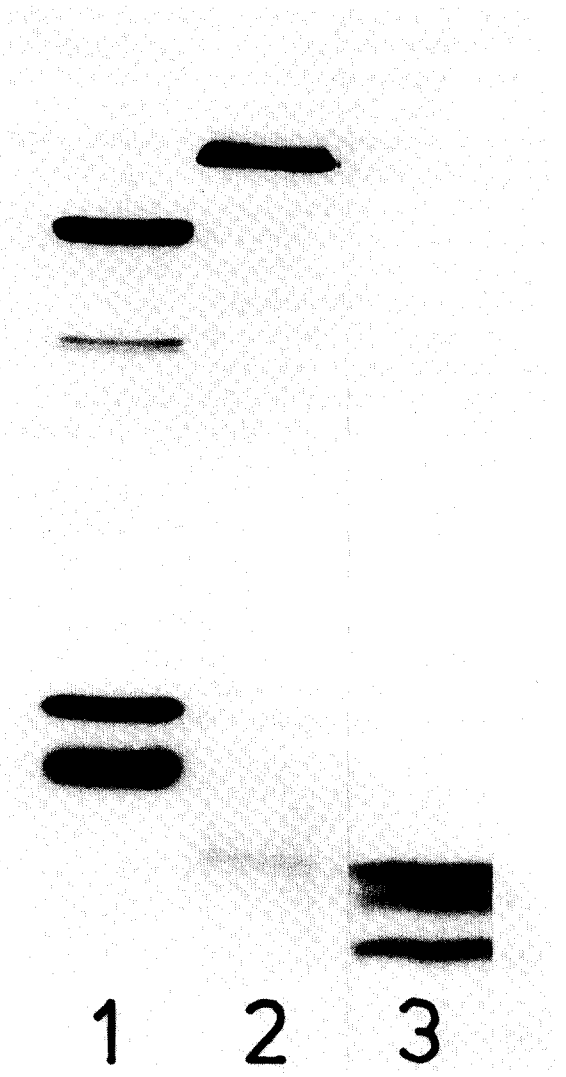


Fig. 4. An SDS-polyacrylamide gradient gel (11.5–16.5% acrylamide) of the type I B800-850 light-harvesting complex from *Rps. acidophila* strain 7750, under non-denaturing and denaturing conditions. (1) Standard proteins: bovine serum albumin 68 000 $M_r$; alcohol dehydrogenase 41 000 $M_r$; myoglobin 17200 $M_r$ and cytochrome *c* 12200 $M_r$. (2) Type I B800-850 non-denatured (i.e., not boiled prior to the electrophoresis). (3) Type I B800-850 denatured by boiling for 2 min prior to the electrophoresis. In track 2 the densely staining high molecular weight band ran as a pigmented band and retained its 'native' absorption spectrum.

clearly removed from the pigmented complex by SDS, even without denaturing by boiling. Indeed, on some occasions nearly all of this component is removed without denaturing. Therefore, it seems most probable that the pigments are bound to the two lower molecular weight polypeptides and that the third polypeptide, if it is a true membrane of the complex, is unpigmented (a similar unpigmented polypeptide is found in the B800-850 antenna complex from *Rps. capsulata* [16]).

Fig. 5 shows a gel where we have compared the polypeptide composition of each of the antenna complexes isolated, under denaturing conditions. In each case the low molecular weight polypeptides are the antenna components. B880 shows only one major polypeptide (8000-9000), a few minor contaminating polypeptides and the presumed reaction centre polypeptides. Type I B800-850 shows the three polypeptides described above. The highest molecular weight one of these three appears to have a molecular weight very similar to that of the B880 polypeptide. The type II B800-850 and the B800-820 both have a pair of low molecular weight polypeptides, which just on the basis of size appear to be very similar. We are currently continuing with this study attempting to see whether the polypeptides from these two complexes are really identical by seeing whether they will co-migrate on isoelectric gels.

Fig. 6 shows an SDS gel of whole membranes from strain 7750 grown at 2000 lx and strain 7050 grown at 400 lx. The polypeptide composition in the low molecular weight region clearly shows the presence of all the polypeptides which we have been able to identify as coming from the antenna complexes. We, therefore, have some confidence that the polypeptides we have identified as coming from the antenna complexes are not just artefacts of our preparative procedures, but do indeed reflect the in vivo situation.

Table II presents the results of an analysis of the pigment content of the isolated antenna complexes. The B880 complex is the least pure preparation as it also contains reaction centres. The two light-harvesting complexes from strain 7050 show a high BChl *a*/protein ratio, however, an accurate knowledge of the molecular weights of their constituent polypeptides wiil be required before this number can be converted into the number

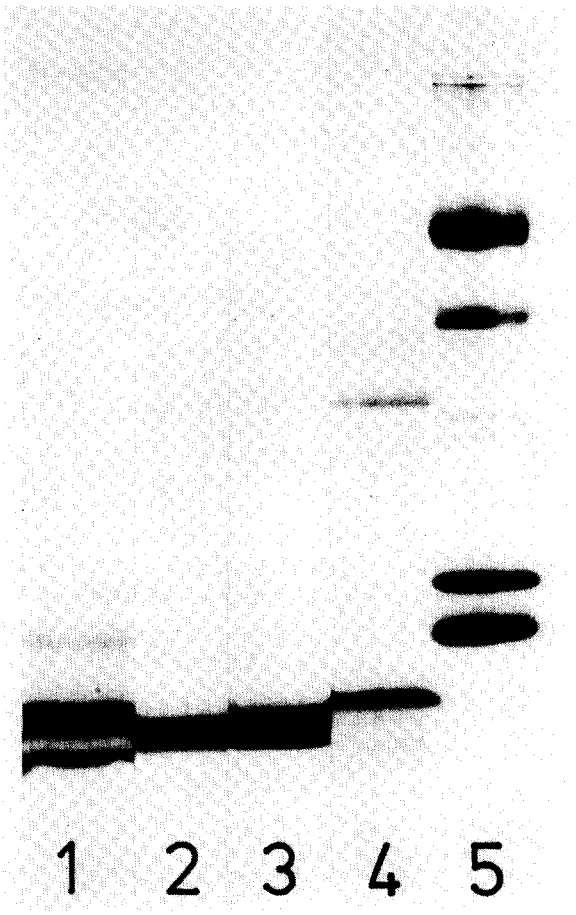


Fig. 5. A comparison of the constituent polypeptides of the various light-harvesting pigment-protein complexes from *Rps. acidophila* on an SDS-polyacrylamide gradient gel (11.5–16.5% acrylamide). (1) Type I B800-850 from strain 7750, (2) type II B800-850 from strain 7050, (3) B800-830 from strain 7050, (4) B880 from strain 7750, (5) standard proteins as in Fig. 4. In each case the samples were denatured by boiling for 2 min prior to the electrophoresis. All the tracks are taken from a single slab gel, but for convenience only the relevant tracks have been cut out and presented here.

of BChl *a* molecules present per polypeptide. The BChl *a*/carotenoid ratio in both these complexes is nearly 3 : 1. These values are very similar to those obtained from the analogous light-harvesting complexes from *C. vinosum* [11,17].

The type I B800-850 antenna complex, with its colourless polypeptide, has a lower BChl *a*/protein content than the type II complex whilst, just as in the case of the analogous B800-850 com-

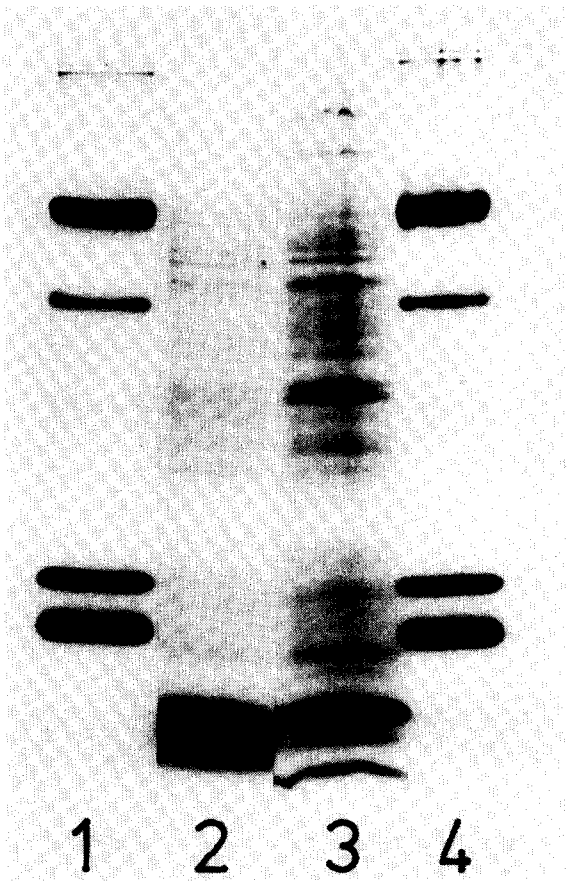


Fig. 6. A comparison of the polypeptides of whole membranes of the two strains of *Rps. acidophila* on an SDS-polyacrylamide gradient gel (11.5–16.5% acrylamide). (1 and 4) Standard proteins, as in Fig. 4; (2) membranes from *Rps. acidophila* strain 7050 grown at 400 lx; (3) membranes from *Rps. acidophila* strain 7750 grown at 200 lx. As in Fig. 5 all these tracks were taken from a single slab gel and only four representative tracks are presented. The various light-harvesting polypeptides are clearly visible in tracks 2 and 3 in the low molecular weight region.

plexes from either *Rps. sphaeroides* [6] or *Rps. capsulata* [11], the type I B800-850 complex has a BChl *a*/carotenoid ratio of nearly 3 : 1. The different carotenoid compositions of the *Rps. acidophila* antenna complexes together with their functional signficance will be presented elsewhere (Cogdell, Hipkins and Schmidt, unpublished data).

It is interesting that some strains of *Rps. acidophila*, such as strain 7050, can contain both type I and type II B800-850 light-harvesting complexes. A similar situation has also been reported

TABLE II

THE PIGMENT CONTENT OF THE LIGHT-HARVESTING PIGMENT-PROTEIN COMPLEXES FROM *RPS. ACIDOPHILA*

Values of [BChl *a*]/[Protein] are based upon four separate preparations. The carotenoid composition of these complexes has been determined and will be reported elsewhere (Cogdell, Hipkins and Schmidt, unpublished data). Based upon these data we calculated average extinction coefficients of the carotenoid mixtures at their peak wavelengths. The pigments were then extracted into 7:2 acetone/methanol. [BChl *a*] was determined from the absorbance at 772 nm [9]. [Carotenoid] was then calculated from the absorption spectrum of the 7:2 acetone/methanol extract when the absorbance of the BChl *a* at the carotenoid wavelength was removed. This was accomplished by placing a solution of purified BChl *a* of equal absorbance at 772 nm to the extract in the position of the blank cuvette. The results shown are the average of two such determinations.

| Sample | | [BChl *a*]/[Protein] (%, w/w) | [Carotenoid]/[BChl *a*] (mol/mol) |
|---|---|---|---|
| Strain | Complex | | |
| 7750 | B880 | 5–6 | 1 : 1.9 |
| 7750 | Type I | | |
| | B800-850 | 15–16 | 1 : 2.8 |
| 7750 | Type II | | |
| | B800-850 | 20–22 | 1 : 3.1 |
| | B800-830 | 20–22 | 1 : 3.2 |

in *Rps. palustris* and *C. vinosum* [18,19]. We are currently undertaking a detailed spectral comparison of these antenna complexes to try and see how similar they really are and to assess whether the general group of B800-850 antenna complexes might not be better viewed as being composed of two major types (i.e., type I and type II).

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## References

1 Pfennig, N. (1969) J. Bacterial. 99, 597–602
2 Schmidt, K. (1971) Arch. Mikrobiol. 77, 231–236
3 Cogdell, R.J. and Thornber, J.P. (1980) FEBS Lett. 112, 1–8

4 Tonn, S.J., Gogel, G.E. and Loach, P.A. (1977) Biochemistry 16, 877–885
5 Clayton, R.K. and Clayton, B.J. (1972) Biochim. Biophys. Acta 283, 492–504
6 Cogdell, R.J. and Crofts, A.R. (1978) Biochim. Biophys. Acta 502, 409–416
7 Cogdell, R.J., Valentine, J., Lindsay, J.G. and Schmidt, K. (1982) Biochem. Soc. Trans. 10, 334–335
8 Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J. (1951) J. Biol. Chem. 193, 265–275
9 Mejbaum-Katzenellenbogen, S. and Drobryszycka, W.J. (1959) Clin. Chim. Acta 4, 515–522
10 Clayton, R.K. (1963) in Bacterial Photosynthesis (Gest, H., San Pietro, A. and Vernon, L.P., eds.), pp. 495–500, Antioch Press, Yellow Springs, OH
11 Cogdell, R.J. and Thornber, J.P. (1979) Ciba Found. Symp. 61 (New series), 61–73
12 Cogdell, R.J., Parson, W.W. and Kerr, M.A. (1976) Biochim. Biophys. Acta 430, 89–93
13 Laemmli, U.K. (1970) Nature 227, 680–685
14 Cogdell, R.J., Lindsay, J.G., Reid, G.P. and Webster, G.D. (1980) Biochim. Biophys. Acta 591, 312–320
15 Heinemeyer, E.-A. and Schmidt, K. (1978) Abstr. 5th, Int. Symp. Carotenoids, p. 21
16 Feick, R. and Drews, G. (1978) Biochim. Biophys. Acta 501, 499–513
17 Thornber, J.P. (1970) Biochemistry 9, 2688–2698
18 Hayashi, H. and Morita, S. (1980) J. Biochem. 88, 1251–1258
19 Hayashi, H., Miyao, M. and Morita, S. (1982) J. Biochem. 91, 1017–1027