Functional bacterial photoreaction centres with only one type of protein

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A preparation of purified *Rhodospirillum rubrum* photoreaction centres was subjected to dialysis under mild denaturing conditions. Sodium dodecyl sulfate–polyacrylamide gel electrophoresis showed that the resulting prepartation contained only one peptidic constituent, apparently the lighter one. The dialyzed centres retained the characteristic absorption spectrum in the visible–near infrared range and actinic light elicited the spectral changes which reflect primary change separation. Thus, it seems that the light protein of the photoreaction centre contains all the binding sites for the primary reactants of bacterial photosynthesis.

Photosynthesis, bacterial *Pigment–protein complex* *Reaction centre, bacterial* *Reaction centre proteins* Rhodospirillum rubrum

1. INTRODUCTION

The photosynthetic membrane contains two pigment populations which, in association with specific proteins, perform distinct functions in the primary events of the photosynthetic process. Only a minor fraction of the pigments belongs to the photoreaction centre and is responsible for primary photochemistry, while the major pigment population is a constituent of the light harvesting antenna that collects photons and transfers excitation energy to the photoreaction centre [1]. So far solubilized antenna-free photoreaction centres have been obtained only from photosynthetic bacteria [1]. These bacterial centres have been shown to contain bacteriochlorophyll, bacteriopheophytin, a carotenoid, a quinone, non-heme iron and 3 proteins of different M_r-value [2]. In [3] the heaviest protein constituent was removed without loss of primary photochemistry and thus the simplest isolated particle capable of photosynthetic activity was obtained. Now, we describe a photoreaction centre preparation which, according to all appearances, contains only one type of protein and retains all the characteristic pigments as well as photochemical activity.

2. METHODS

Photoreaction centres were prepared from photoanaerobically grown cells [4] of *Rhodospirillum rubrum*, wild-type strain S1, by a procedure which included solubilization with dodecylmethylamine *N*-oxide, fractionation with $(NH_4)_2SO_4$ and chromatography on diethylaminoethyl-cellulose [5]. Dialysis of this preparation under mild denaturing conditions was performed at room temperature in an Amicon diafiltration cell (model 202) equipped with an XM50 Diaflo membrane which allowed free diffusion of solutes of $M_r \lesssim 50000$. The sample (5 ml) with 21 ng equiv. primary electron donor was dialyzed against 2 l of 4 mg sodium deoxycholate/ml, 10 mM Tris–HCl (pH 8.0) at 50–100 ml/h a flow rate. Electrophoresis under dissociating conditions was done as in [6] with minor modifications. The gels contained 120 mg acrylamide/ml, 3.2 mg methylene-bisacrylamide/ml, 2 mM dithioerythritol and 0.36 M Tris–HCl, (pH 8.9) and were polymerized in 65 × 6 mm tubes. The electrode buffer was a solution of 37.5 mM glycine and 10 mg sodium dodecyl sulfate/ml which had been brought to pH 8.7 with Tris. Prior to electrophoresis, the samples were

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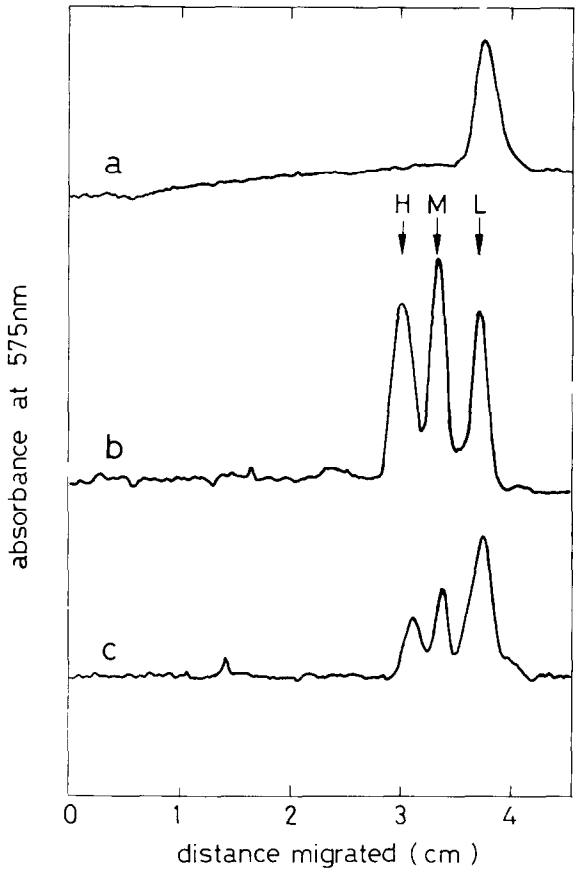

Fig. 1. Electrophoretograms of photoreaction centre preparations: dialyzed centres (a); undialyzed centres (b); a 1:1 mixture of dialyzed and undialyzed centres (c). H, M and L indicate the heavy, medium and light proteins, respectively.

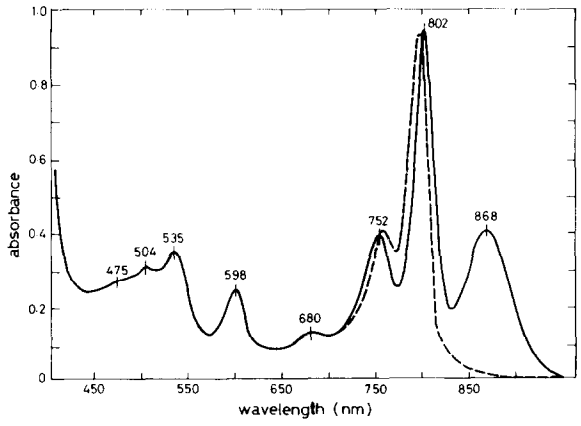

Fig. 2. Absorption spectra of the dialyzed photoreaction centre preparation. Numbers indicate the location of the maxima in the dark spectrum (——), which was obtained with a Hitachi 330 spectrophotometer. To record the light spectrum (---), the sample was cross-illuminated in the secondary compartment of a Hitachi 356 spectrophotometer as in [4].

mixed in a 1:1 ratio with a solution of 70 mg sodium dodecyl sulfate/ml, 170 mM dithioerythritol and 2.25 M glycerol in 0.21 M Tris–HCl, (pH 6.8). After 5 min at 42°C, 30 μl of this mixture, which contained 30 pg equiv. primary electron donor, were applied to the surface of the gel. The optical absorption of the bands in the gels was measured at 575 nm after staining with Coomassie blue, using a Gilford spectrophotometer (model 2400) with a slit of 0.05 mm, at a scanning rate of 0.5 cm/min. The output of the spectrophotometer was connected to a microprocessor-controlled recorder (Bascom-Turner 8120) which allowed estimation of the areas of the absorption bands.

3. RESULTS

When a preparation of photoreaction centres was subjected to prolonged dialysis under mild denaturing conditions, the protein of the resulting suspension was resolved as a single band by SDS–polyacrylamide gel electrophoresis (fig. 1a), which contrasts with the three-band pattern which is characteristic of the undialyzed preparation (shown in fig. 1b for comparison). The protein which remained after dialysis migrated the same distance as the light polypeptide of the original preparation, as it was observed when dialyzed and undialyzed centres were coelectrophoresed (fig. 1c).

Contrary to what happened to the peptidic constituents, the photoreaction centre pigments did not seem to be altered by the dialysis treatment. Thus, the visible–near infrared spectrum of the dialyzed centres (fig. 2) exhibited several bands, the location and the relative intensity of which were in good agreement with preparations that show three bands on electrophoresis gels [2,5]. Furthermore, the recovery of pigments after dialysis was almost total (95–100%). Since the ab-

sorption spectra of the photoreaction centre pigments are largely different in the free and in the native, protein-bound states [7], we may conclude that pigment–protein interaction was not significantly disturbed by the dialysis treatment.

The dialyzed photoreaction centres retained photochemical activity as demonstrated by the ability of light to induce in such preparation the spectral changes which reflect primary charge separation [2]. The broken line spectrum of fig. 2 illustrates some of those changes: the red shift of the bacteriopheophytin band at 752 nm, the blue shift of the bacteriochlorophyll band at 802 nm and the bleaching of the other bacteriochlorophyll band at 868 nm. For technical reasons we could not investigate whether light also induced the appearance of two spectroscopic features which are attributed to the oxidized form of the primary electron donor, i.e., an optical absorption band at 1245 nm and an ESR signal with a g-value of 2.0025 and a peak-to-peak first-derivative linewidth of 9.5 G [2]. However, fig. 3 shows that both the near infrared band and the ESR signal were elicited in the dialyzed centres by the addition of an oxidant in the dark. Thus, it appears that the primary reactants of bacterial photosynthesis are unaltered and fully functional in our photoreaction centre preparation.

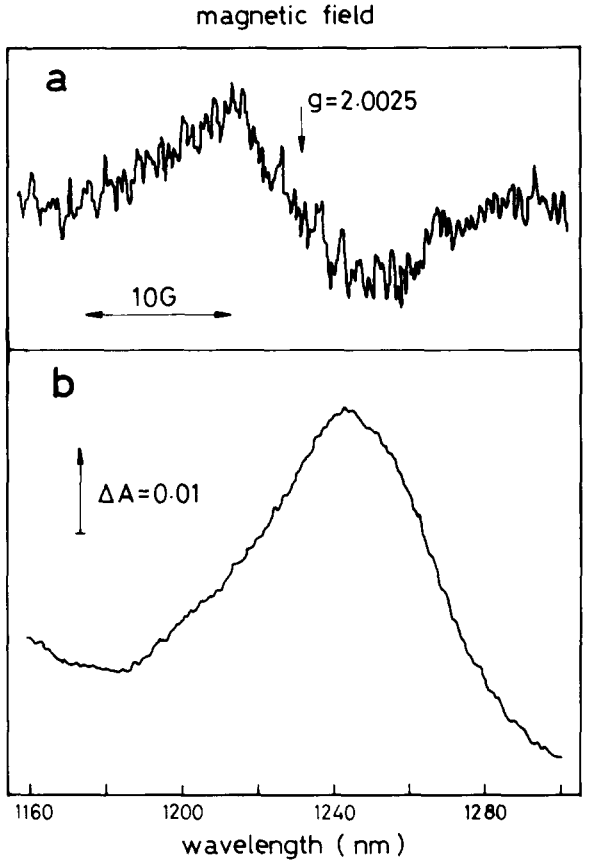

Fig. 3. Spectroscopic changes elicited by 0.33 mM $K_3(CN)_6Fe$ in the dialyzed preparation of photoreaction centres. The ESR spectrum (a) was obtained as in [11]; the near infrared spectrum (b) was obtained with a Hitachi 330 Spectrophotometer. The sample, in a 1-cm optical path cell, contained 3.1 ng equiv. primary donor/ml.

4. DISCUSSION

The migration properties of the single band in the electrophoresis gels of the dialyzed preparation suggests that only the light peptidic constituent of the photoreaction centre is present in such preparation. An alternative interpretation is that all three proteins are present but that some modification, brought about by the dialysis treatment, prevents their resolution by electrophoresis under our conditions. However, this latter interpretation would require that the area of the single band in fig. 1a were ~3-times larger than of the L-band in fig. 1b. Such is not the case because both areas differ by $< 7\%$. Then, it seems that the two heavier proteins are selectively removed by the dialysis treatment. The mechanism of the removal is under current investigation and might involve preferential degradation by traces of contaminant proteases. Elucidation of such mechanism is not needed to conclude from these data that only one of the proteins, apparently the lighter one, contains the binding sites for all the photoreaction centre pigments. Furthermore, since the induction of spectral changes by moderately intense steady light requires the presence of the ubiquinone which acts as the metastable primary electron acceptor [8], it appears that such redox carrier is also bound to the light protein (cf. [9]). From amino acid analysis M_r 25000 was estimated for the light protein of *Rhodospirillum rubrum* [5]. Since it is thought [5,10] that there is one peptide of this kind per prosthetic group (consisting at least of 7 pigment molecues), a minimal M_r 31000 results for the photoreaction centre in the dialyzed preparation. Particles of that size

would be expected to diffuse freely through the dialysis membrane used in the preparative procedure. Therefore, their occurrence as oligomers seems likely.

These data suggest that the two heavier protein components of the photoreaction centre play no apparent role in primary photochemistry. However, this tentative conclusion may be unsustained by further detailed analysis which might reveal some subtle functional differences between the preparations with one and with three proteins.

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