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pH-induced reversible changes in the absorption spectrum and photoactivity of bacteriochlorophyll in photosynthetic bacteria chromatophores

Bacteriochlorophyll in *Rhodospirillum rubrum* exhibits a pronounced absorption maximum at about 880 nm (B880) and a weak one at about 800 nm (B800). THOMAS *et al.*[1] observed that at low pH, B800 is strongly reduced, while B880 is only slightly affected. Previous work[2] in this laboratory showed that B800 is converted to bacteriopheophytin absorbing at 755 nm (Bph755) probably *via* bacteriochlorophyll intermediates which absorb at 770–790 nm (B770–790). The Bph755 is subsequently transformed to a form absorbing at 850 nm (Bph850). B880 appeared to be very slowly pheophytinized directly to Bph850.

The accumulation of the above-mentioned B770–790 intermediates prior to the formation of bacteriopheophytin was shown by GHOSH *et al.*[3,4] in their bacteriochlorophyll–protein complex exposed to low pH. This was attributed to a conformational change of the protein. B770–790 were also observed when B880 was pheophytinized to Bph755 in the presence of Triton X-100 (ref. 2). This communication demonstrates that under appropriate conditions these intermediate forms of bacteriochlorophyll can be converted back to B800 upon increasing the pH. This is more clearly manifested in *Rhodopseudomonas spheroides* chromatophores which have a pronounced absorption maximum at about 800 nm.

The majority of the bacteriochlorophyll molecules (B880 or B800) serves as a light-harvesting pigment. Photosynthetic reaction centers are composed of a small fraction of bacteriochlorophyll, namely specialized photoactive bacteriochlorophylls (P800–P870). P870 undergoes photobleaching attributed to its photooxidation. This change is accompanied by a concomitant blue-shift of P800. KE *et al.*[5] investigated the effect of pH on the photooxidation of the photoactive bacteriochlorophyll in a photochemically active subchromatophore particle derived from *Chromatium*. When the pH was lowered from 4 to 3, the reaction rapidly deteriorated. The present investigation reveals that the photoactivity is temporarily lost at pH 2–2.5 but partially regenerated by increasing the pH within a certain time limit.

Fig. 1A shows the disappearance and reappearance of B800 in *R. rubrum* chromatophores kept at pH 2.5 for 30 min and subsequently changed to pH 10.5. Inactivation and partial regeneration of the photoactivity by the same acid–base transition are depicted in Fig. 1B. The photoactivity was measured by observation of the blue-shift of P800 with an Aminco-Chance dual wavelength spectrophotometer. A Kodak 88A filter was placed in front of an Amperex XP-1003 photomultiplier. A Corning CS 4-96 filter was used with the side illumination set up. The pH was lowered by adding small amounts of 1 M HCl to chromatophore suspensions in distilled water and raised by subsequent addition of 1 M NaOH. The pH's were measured with an Instrumentation Laboratory, Model 205 pH meter.

The reversible spectral and photoactivity changes obtained at pH 2.5 were not found at pH 1.6 even upon immediate neutralization. This indicates the completion of pheophytinization of B800 or the permanent damage of the photoactive bacteriochlorophyll complex (P800–P870) within a short period. B880 was not immediately affected even at this pH.

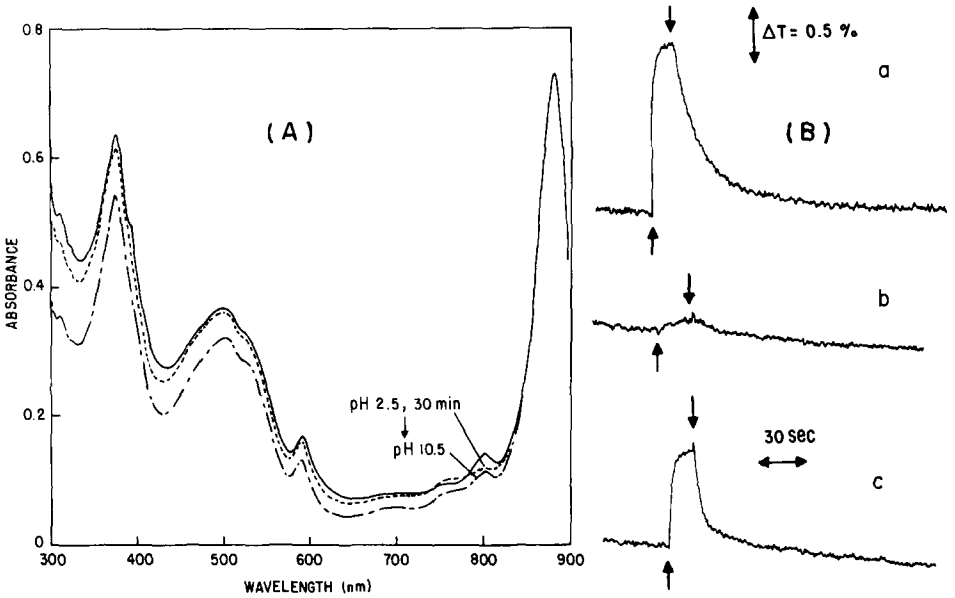

Fig. 1. (A) Absorption spectra of *R. rubrum* chromatophores. ———, in distilled water; – – – –, at pH 2.5 for 30 min; — — —, after subsequent change to pH 10.5. (B) Time courses of light-induced transmission changes (ΔT) in *R. rubrum* chromatophores. a, in distilled water; b, at pH 2.5 for 30 min; c, after subsequent change to pH 10.5. The two wavelengths chosen were 803 and 797 nm. The on and off of the actinic light are indicated by up and downward pointing arrows, respectively.

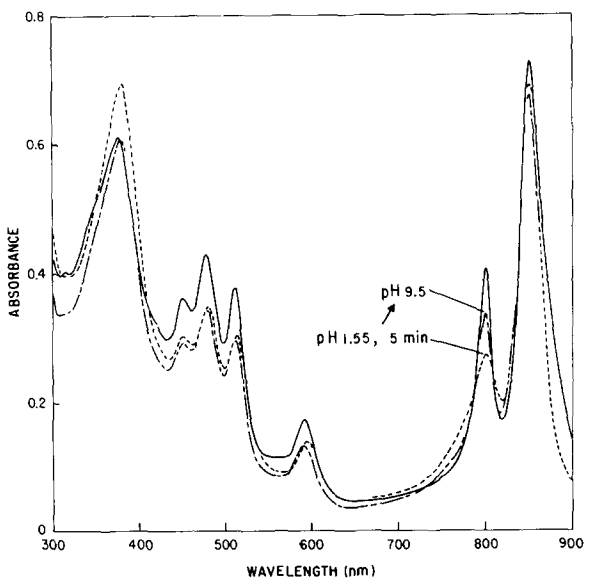

Fig. 2. Absorption spectra of *R. spheroides* chromatophores. ———, in distilled water; – – – –, at pH 1.55 for 5 min; —·—, after subsequent change to pH 9.5.

As shown in Fig. 2 in the case of *R. spheroides* chromatophores, the early stage (5 min) of the decrease of B800 at pH 1.55 was clearly accompanied by an increase

of absorbance around 750–870 nm. Upon raising to pH 9.5, B800 was partially regenerated at the expense of the 750–780 nm absorption. This suggests that the B770–790 intermediates are capable of being converted back to B800. Fig. 2 also shows that B880 disappears within 5 min at pH 1.55 and is not regenerated by changing the pH. Another pronounced absorption band at 850 nm (B850) was hardly affected. At this pH, the photoactivity underwent irreversible inactivation despite the partial regeneration of B800. *Chromatium* chromatophores behaved similarly, though the regeneration of B800 was less pronounced than with *R. spheroides*.

When *R. spheroides* and *Chromatium* chromatophores were kept at the pH range 2.0–2.4, B800 was gradually reduced but B880 was not strongly affected. By bringing back the pH to neutral or alkaline (pH 6–11), the photoactivity was regenerated in the same way as shown for *R. rubrum* (Fig. 1B). The acid-induced spectral changes reported here are generally in agreement with the results by THOMAS *et al.*[1], except that they observed little change of B800 in the case of *Chromatium*. The reason for this discrepancy is not clear.

The pH-induced modification and regeneration of the photoreactive bacteriochlorophyll complex (P800–P870) and B800, reported in this communication, indicate that these species differ considerably from other forms of bacteriochlorophyll (B850 and B880). B880 and B850 are more resistant to low pH, but once affected, the changes are almost irreversible. B800 and P800–P870, on the other hand, are apparently in specific and intimate association with proteins. Their reversible response to acid–base transition suggests the occurence of pH-dependent reversible conformational changes in their protein environments.

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Chlorophyll–pheophytin interactions*

Long wavelength forms of chlorophyll occur in green plants[1] and are considered important in photosynthesis[2]. The origin of these red-shifted spectral transitions and the chlorophyll species responsible for them are still obscure. Long wavelength forms

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