CROSSLINKING OF PHOTOSYNTHETIC MEMBRANE POLYPEPTIDES OF *RHODOPSEUDOMONAS CAPSULATA*

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1. Introduction

In *Rhodopseudomonas capsulata*, the reaction center (RC) and light-harvesting (LH) complexes are the major pigment–protein constituents of the photosynthetic membrane [1,2]. The LH complexes serve to gather light energy and funnel it to the RCs for conversion to chemical potential energy. Two LH complexes are recognized:
(i) LH I, absorbing light maximally at 870 nm (B870);
(ii) LH II, absorbing at 800 and 850 nm (B800–850) [1].
Polypeptides associated with these complexes have been identified [2,3]. Models of how these complexes and their polypeptides are topographically related for efficient energy transfer have been proposed [1,5,6]. However, direct biochemical elucidation of their structural associations in the membrane is lacking.

Here, we describe an approach to investigating the topographical, near-neighbor relationships among these polypeptides in the membrane using a reversible, bifunctional crosslinking reagent in combination with immunochemical techniques. With this approach, we have observed specific crosslinking of an RC polypeptide (H) to other polypeptides of the RC and LH complexes.

2. Materials and methods

2.1. *Cell cultivation and membrane purification*

Cells of *R. capsulata* strain St Louis (ATCC 23782) were grown anaerobically in malate-based medium [7] containing 0.5% casamino acids (Difco) in 100 ml bottles at 30°C with light (20 000 lux). Radiolabel L-[^{35}S]methionine (Amersham) was added (500 μCi/100 ml) during logarithmic growth ($A^{1\,cm}_{680\,nm} = 0.1$) and harvested ~5 h later. The cells were sonicated (10 s bursts) and pigmented membranes were purified on sucrose gradient [8]. Membranes were suspended in triethanolamine buffer (50 mM, pH 8.3) at 1 mg protein (Lowry)/ml and stored at 4°C.

2.2. *RC and immunoglobulin G (IgG) preparation*

RCs were purified from *R. capsulata* strain A1a$^+$ as in [9]. Rabbit antisera directed against RCs were obtained as in [10]. IgG fractions were prepared by ammonium sulfate fractionation and stored at 4°C in 10 mM sodium phosphate (pH 8.0) and 0.02% sodium azide.

2.3. *Crosslinking and immunofractionation*

Solutions of dithio-bis-(succinimidylpropionate) (DTSP) (Pierce) were freshly prepared in dimethylsulfoxide (3.3 mg/ml), and 4 aliquots of 0.5 μl each were added at 15 min intervals to 60 μl (60 μg protein) of membrane suspension at room temperature. After 15 min from the last addition, Tris–HCl (pH 8.8) *N*-ethylmaleimide and phenylmethylsulfonylfluoride were added to final conc. 50 mM, 10 mM and 1 mM, respectively. SDS (10%) was added (0.25% final) and the mixture heated for 5 min at 60°C. A 10-fold volume of 2% Triton X-100 in TA buffer (160 mM Tris, 80 mM Na-acetate, 2 mM EDTA (pH 8.8)) was added and incubation continued for 15 min at room temperature. IgG (10 μl) was added and the mixture incubated for 1 h at 4°C. Swollen beads of protein A–Sepharose CL-4B (Pharmacia) (60 μl) were added and the solution gently stirred for 16 h at 4°C. The beads were washed by repeated suspension and centrifugation twice with TA buffer containing 2% Triton X-100 and 3 times with TA buffer alone.

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2.4. *SDS solubilization and 2-dimensional polyacrylamide gel electrophoresis*

The pelleted protein A–Sepharose beads were treated with 50 μl of 3% SDS, 9 M urea, 0.3 M Tris–HCl (pH 6.8), 20% glycerin and heated at 60°C for 15 min. The beads were centrifuged and a supernatant aliquot containing ~2 × 10^4 cpm was removed for electrophoresis. Two-dimensional gel electrophoresis (diagonal mapping) was conducted basically as in [11] using Laemmli gel buffers [12], linear gradient slab gels (12–16.5%), and dithioerythritol (15 mg/ml) in the upper agarose layer of the second dimension gel. Gel autoradiography was performed using fluorographic methods [13]. Polypeptide M_r-values were estimated by comparison of migration distances with polypeptides of known M_r.

3. Results

Two-dimensional diagonal mapping following immunofractionation of DTSP-treated membranes revealed specific polypeptide crosslinking involving RC polypeptide H (fig.1a,b). Polypeptides covalently

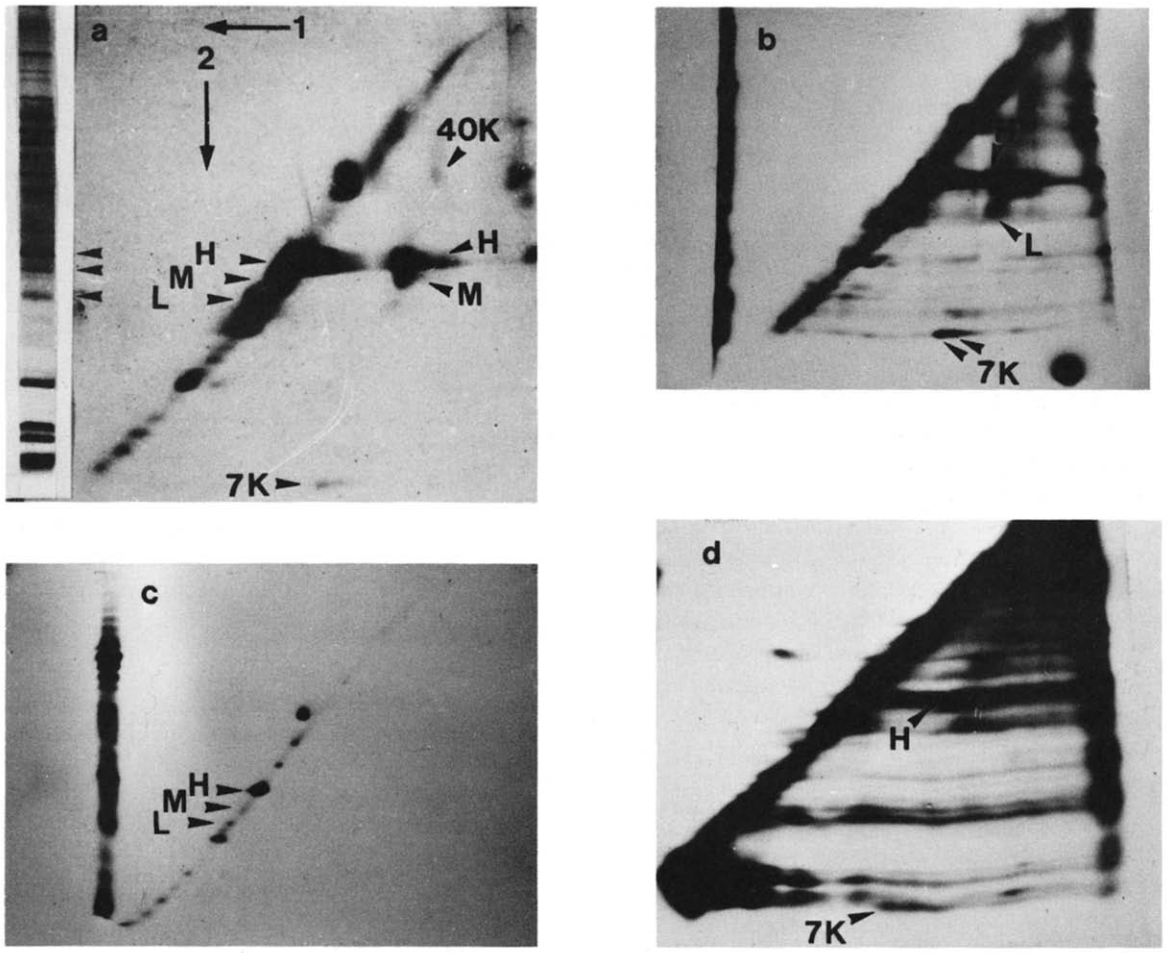

Fig.1. Autoradiograms of two-dimensional SDS–polyacrylamide gels (diagonal maps) of immunofractionated (a–c) and unfractionated (d) ^{35}S-labeled membranes. Membranes were (a,b,d) or were not (c) treated with DTSP. Gels were exposed for 3 days (a,c) or 18 days (b,d; overexposed to reveal cross-linked polypeptides). Variability in the amounts of polypeptides M and L fractionated are revealed in a and c. H, M and L were identified by comparisons to Coomassie blue-stained (inset, a) or ^{35}S-labeled one-dimensional gel track patterns of membranes incorporated in the second dimension gel. Direction of electrophoresis in the first (1) and second (2) dimensions are given.

linked to H via DTSP were identified by their migration to positions below and above spots of polypeptide H located below a diagonal line resulting from thiol cleavage during electrophoresis in the second dimension. The diagonal line was formed by non-crosslinking polypeptides and included the 3 RC polypeptides designated H (M_r 28 000), M (M_r 24 000) and L (M_r 20 500) [9]. Most evident were H crosslinking to M, to a 7000 M_r polypeptide, and to a 40 000 M_r polypeptide. Large ($M_r > 120\,000$), perhaps multipolypeptide, products were also observed which did not penetrate the first dimension gel. Crosslinking of H to L and other but more obscure relationships were seen after long-term autoradiographic exposure (fig.1b).

Crosslinking relationships were also implied from correlations of the summed M_r-values of the individual polypeptides and the relative migration distances (and thus M_r-values) of the uncleaved products in the first dimension gel. The products H · M, H · L and H · 40 000 M_r polypeptide correlated with apparent M_r-values of 57 000, 53 000 and 71 000, respectively, indicating 1:1 polypeptide stoichiometries in these products. At least 2 different products of H and the 7000 M_r polypeptide were evident indicating 1:1 (M_r 34 000) and 1:2 (M_r 40 000) stoichiometries between these species (fig.1b).

Autoradiograms of immunofractionated but untreated membranes revealed no equivalent crosslinked products showing the dependence of these patterns on DTSP (fig.1c).

DTSP-treated membranes analyzed directly without immunofractionation showed highly complex patterns (fig.1d). Crosslinking of the lower M_r LH polypeptides [3] was prominent. Nevertheless, diagonal map patterns observed after immunofractionation (fig.1a,b) were superimposable on these latter maps. Because of the complexity, assignments of polypeptides to particular crosslinked products could not be deduced from these maps alone.

4. Discussion

Membrane immunofractionation with anti-RC IgG permitted identification of crosslinked products which could not be deduced with unfractionated membranes. The crosslinking of H to several other polypeptides suggests an important structural role for this polypeptide in the *R. capsulata* photosynthetic membrane. Chemical crosslinking of H to L was reported in detergent-treated RCs of *R. sphaeroides*, a closely related organism [14].

Crosslinking of M to L as suggested for the photosynthetic bacterium *Rhodospirillum rubrum* [15] was not apparent here, despite immunofractionation of these polypeptides with H.

The rôle of the 40 000 M_r polypeptide in *R. capsulata* is unknown. We speculate that the small 7000 M_r polypeptide is associated with LH I for the following reasons:

(i) A similar size polypeptide is implicated in the *R. sphaeroides* LH I complex [16];
(ii) *R. capsulata* mutant, A1a$^+$, which lacks LH II but possesses LH I has an equivalent polypeptide [2];
(iii) RC is expected to be physically closer to LH I than to LH II because of energy transfer considerations [4,5] and thus more likely to crosslink to LH I.

Crosslinking with DTSP reflects distances of 11 Å between available primary amino groups of neighboring polypeptides presumably having long-term stable associations [17]. Here, crosslinking resulting from random, short-term (collisional) associations in the membrane appeared minimal since products containing only 2 polypeptide species in single copies were predominant.

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