Immunochemical Characterization of *Porphyridium cruentum* B-Phycoerythrin: Proof of Cross-Reaction between Chromophore-Free Apoprotein and Holoprotein-Specific Antibodies

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Antibodies against phycobilisomes of *Porphyridium cruentum* immunoreact with B-phycoerythrin from the same organism. A strong antigen-antibody complex is not only formed with the native B-phycoerythrin but also when the chromophore is chemically reduced, split or even degraded with chromic acid. Characteristics of precipitations and dissolutions in the presence of the chaotropic reagents urea and potassium rhodanide are discussed for the immunocomplex with native B-phycoerythrin. A sandwich-type immunoadsorbent column appropriate for antigen-isolation is described; its binding properties are investigated biochemically and by electron microscopy.

Introduction

Biliproteins are bile pigment-chromophore complexes with thioether-linked chromophores [1–5]. According to their occurence and spectral properties they have been divided into C-, R-, Cryptomonad phycocyanin and B-, R-, Allo-, Cryptomonad phycoerythrin (for classification, see refs. [6–9]). They are part of the light-harvesting system of Cryptophytae and the so called phycobilisomes of cyanobacteria and red algae [12–31]. Two types of phycobilisomes are described: the *Porphyridium* type found in *Porphyridium cruentum* [20–27, 31] and the *Rhodella* type of *Rhodella violacea* [28–30] which is typical for many cyanobacteria too, *e.g.* for *Synechococcus* 6301 [18]. The phycobilisomes of *Porphyridium* have the geometry of the half of compressed rotational ellipsoids [31]. The *Rhodella* type phycobilisomes are disk-shaped and consist of rods of different lenghts. For phycobilisomes of *Porphyridium cruentum*, (B+b)-PE is the major constituent biliprotein (84%) [32] besides R-PC and allo-PC.

In addition to spectroscopic investigations characterizing the chromophores and chromophore-protein interactions of biliprotins (*e.g.* [33–36], for review see [37], immunological methods have been applied on a number of biliproteins from various organisms [10, 26, 27, 38, 39]. In some cases, immunological cross-reactions have been reported; for example, antisera against C-PC of a red alga reacts with C-PC of a cyanobacterium [10]. Cross-reaction between different classes of biliproteins was not observed, *e.g.* antibodies against B-PE do not recognice C-PC [10]. Till now investigations have been carried out exclusively with native biliproteins or their subunits. It is unknown thus far whether specific antisera against the holoprotein cross-react with apoprotein containing chemically altered chromophore(s) or with the chromophore-free apoprotein. This is mainly due to the difficulties of apoprotein-preparation which usually results in denaturation. In the special case of cytochrome c from *Neurospora crassa*, practically no cross-reactivity was found between apo- and holoprotein [40]. In this communication we describe that holo- and apoprotein of B-PE appear strongly related immunochemically and that their cross-reactivity is little affected by alterations in the chromophore region. Various methods available for the modification or cleavage of the chromophore make the B-PE-system suitable for this type of study. In addition, we report experiments to design an immunoadsorbent column appropriate for antigen isolation.

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Abbreviations: B-PE, B-phycoerythrin from Porphyridium cruentum; PC, phycocyanin; PBS, phosphat buffered saline.

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Materials and Methods

Phycobilisomes of Porphyridium cruentum

Phycobilisomes of *Porphyridium cruentum* were prepared using a saccharose density gradient as described by Gantt and Lipschultz 1972 [22]. The phycobilisome fraction was dialyzed against 0.75 M/l sodium phosphate buffer, pH 6.8 at 4 °C and the dialysate applied again on top of a saccharose density gradient. The phycobilisomes reached the equilibrium density after 16 h at 40000 × *g*.

Antiserum preparation

1 ml of the phycobilisome fraction ($A_{555\,nm} = 0.3$) was emulgated with 1.5 ml complete Freund's adjuvant (Difco). The emulsion was injected subcutaneously into rabbits (600 µl per rabbit). After 25 days, a booster-injection was given. Serum was obtained from blood drawn from the ear vein 52 resp. 63 days after the initial injection. The presence of antibodies was demonstrated by the double diffusion method of Ouchterlony with electrophoretically pure B-PE from *Porphyridium cruentum* [3]. Ouchterlony plates were prepared as described [41].

Kinetics of immunoprecipitation

Increasing volumes (2–10 µl) of a solution (1 mg B-PE/ml) of electrophoretically pure B-PE were diluted to 1 ml with PBS. The fluorescence of the dilutions recorded at 585 nm ($\lambda_{exc.} = 380$ nm, light path 1 cm) was used as reference. For precipitation experiments, antiserum (100 µl, 150 µl, 200 µl) was mixed with B-PE solution (10 µl, 10 mg/ml B-PE) and made up to 1.000 ml with PBS (mixing = starting time = zero). After 10, 20, 30, 40 min, the precipitate was removed by centrifugation (10500 × *g*; 5 min) and the fluorescence of the supernatant immediately determined. The amount of B-PE still present was calculated from the standard fluorescence curve and corrected for fluorescence due to antiserum components.

Dissolution and formation of immunoprecipitates in solutions containing chaotropic reagents

Immunoprecipitates from 200 µl antiserum/50 µl B-PE solution (10 mg/ml), washed twice with PBS, were used throughout the experiments. The precipitate was resuspended in 1 ml of potassium rhodanide solution (0, 0.5, 0.75, 1, 1.5, 2, 2.5, 3 M/l) and kept at 20 °C with occasional agitating. The residual precipitate was pelleted by centrifugation and washed twice with PBS. An analogous experiment was conducted in the presence of urea (0–9 M/l). The pellets were dissolved either in 200 µl of 96% formic acid and immediately diluted to 1 ml with PBS, or in 1 M/l acetic acid. The absorption of the resulting solution was measured at 555 nm. The amount of B-PE was determined using a standard reference curve for B-PE recorded under the same conditions.

In precipitation experiments, 200 µl of antiserum and 50 µl of B-PE solution (10 mg/ml) were mixed with freshly prepared KSCN or urea solution (final concentration 0–3 M/l and 0–9 M/l respectively) and incubated at 20 °C (incubation time: 1 h for KSCN; 2 h and 16 h for urea). After centrifugation the pellets were analyzed for B-PE as above.

Determination of standard precipitation

The standard is defined as the amount of B-PE contained in an immunoprecipitate formed from 50 µl B-PE solution (10 mg/ml) and 200 µl of the resp. antiserum in a final volume of 1 ml.

Determination of standard dissolution

The standard is defined as the percentage of precipitate that dissolves in a parallel control experiment using PBS without chaotropic reagent (amount dissolved = 0%; residual pellet = 100%).

Immunoprecipitation of B-phycoerythrin with modified chromophore: preparation of B-PE with reduced chromophore [35]

Solutions: a) urea-PBS: 48.0 g urea with PBS to 100 ml; b) 10% sodium dithionite in urea-PBS. 10 mg of B-PE were dissolved in buffer a. Buffer b was added dropwise until the red color had completely dissappeared (ca. 0.1 ml). Urea and salts were removed by chromatography on a Sephadex G 10 column (5 cm × 20 cm) and the nearly colorless protein fraction was used for precipitation experiments.

Phycoerythrin with completely degraded chromophore

10 mg B-PE were dissolved in 1 ml PBS containing 6 M/l urea. 100 µl chromic acid (2 g CrO_3,

100 ml 2 N H_2SO_4) were added. Excess chromium (VI) was reduced by passing a stream of SO_2 through the reaction mixture and subsequent adjustment of the pH to 7.8 with 1 M NaOH. Chromium (III) formed was removed by dialysis for 16 h against 6 M/l urea-PBS or chromatography on Sephadex G10 (developed with 6 M/l urea-PBS). Small precipitates formed during the work-up were removed by centrifugation. Precipitation experiments were carried out with antiserum containing 6 M/l urea.

Preparation of immunoadsorbents

a) Direct technique with polyspecific IgG

IgG was enriched by fractionated precipitation with ammonium sulphate (25% and 35% saturation) and fixed to CNBr-activated Sepharose 4B (fixation procedure as given by Pharmacia, Sweden). 1 ml of a solution of minimal size chromopeptides (Cys-(Val, Leu)-chromophore (1) and 1 ml B-PE solution, each with an absorbance of 0.5 at 555 nm (= 0.15 mg/ml Cys-(Val, Leu)-chromophore resp. 3 mg/ml B-PE) were applied to the resp. column. Free material was removed by washing with PBS.

b) Sandwich-technique

10 mg of electrophoretically pure B-PE (3) from *Porphyridium cruentum* were bound to 1 g of CNBr-activated Sepharose 4B as described above. The column (LKB elution column for flat bed electrophoresis, Bromma, Sweden) was rinsed with 8 M/l urea-borate buffer (0.1 M/l borate, 1 M/l NaCl, 8 M/l urea, pH 8.0) and the gel linked B-PE subjected to the diazo cleavage procedure of Kufer [42]. The red column was treated with 0.57 M/l sodium dithionite in urea-borate buffer, producing colorless hydrazo products. The column was washed with 30 ml PBS; 3 ml of rabbit anti-B-PE antiserum were applied to the column which then was closed and slowly rotated end over end for 16 h at 4 °C. Unspecific bound protein was removed by washing with PBS. The remaining specifically bound antibodies were cross-linked to the antigen by incubating the gel beads with 1.5% glutardialdehyde in PBS (20 min, 25 °C); excessive reagent was removed with 50 ml PBS and residual aldehyde groups were neutralized with 1% glycin-1% glutamate. To prepare a control column, the same steps were carried out, replacing IgG by a mixture of egg-white proteins.

Immunofixation of whole phycobilisomes

The described sandwich-column was equilibrated with 0.75 M/l potassium phosphate buffer, pH 6.8 and incubated with a phycobilisome solution in the same buffer. Free phycobilisomes were removed by extensive washing with equilibration buffer.

Immunofixation of B-phycoerythrin

The sandwich-column was equilibrated with PBS. 0.5 ml of a 0.1% B-PE solution were applied and the unbound material was removed by several rinses with PBS. The desorption was carried out with 8 M/l urea-borate buffer (pH 8) or 8 M/l urea-PBS. The amount of immunofixed B-PE was determined by subtracting the amount of B-PE recovered from the washings. The column was regenerated by rinsing with PBS (50 ml) and was then ready for a new immunofixation cycle. All steps (except immunofixation at 20 °C) were carried out at 4 °C. After the 6th regeneration with PBS the gel beads were freeze-dried. After 2 days they were suspended in PBS and the immunofixation carried out as above.

Electron microscopy

Whole cells: Porphyridium cruentum cells were prepared for electron microscopy as described earlier [31].

Phycobilisomes and phycoerythrin

Buffer solutions containing either phycobilisomes ($A_{555\,nm} = 0.3$) or B-PE (1 mg/ml) were fixed with 1% glutardialdehyde in 0.25 M/l tricine buffer (pH 7.5). Drops of these solutions were placed on carbon coated grids (phycobilisomes) or graphite oxide covered holey carbon films (B-PE) as described by Formanek 1979 [43]. Negative staining of phycobilisomes and phycoerythrin was achieved with 0.5 to 2% solutions of potassium phosphotungstate pH 6.8. Gel beads with immunofixed phycobilisomes or B-PE were fixed for 90 min at 20 °C with 3% glutardialdehyde in 0.75 M/l potassium phosphate buffer, pH 6.8. After several rinses with 0.1 M/l Tris buffer (rinsing buffer) pH 6.8 the gel was postfixed for 90 min with 2% osmium tetroxide. After several washings with rinsing buffer, the samples were stained "en bloc" with 0.5% uranyl magnesium acetate for 30 min, dehydrated in graded series of

ethanol and embedded in Spurr's low viscosity resin [44]. 50 nm sections were cut with a diamond knife on a LKB Ultrotome II and mounted on uncoated copper grids. Pictures were taken with a Siemens Elmiskop 101 electron microscope at 80 kV. The magnification was calibrated with a cross line grating replica. Measurements were made with a semi-automatic measuring device (Kontron MOPAM O2).

Results and Discussion

The aim of the present study was to investigate the immunochemical relationship between B-PE holo- and apoprotein and to develop an immunoadsorbent technique suitable for antigen isolation. Antibodies against B-PE were elicited in rabbits by immunization with purified phycobilisomes. This is reasonable because the surface of phycobilisomes is mainly made up of (B+b)-PE, representing about 84% of the total biliprotein content of a phycobilisome [32]. The antisera obtained were characterized by the Ouchterlony double diffusion technique [41] as well as by the precipitin reaction and its kinetics (Fig. 1). All antisera precipitate more than 92% of the B-PE already after 10 min of incubation. After 40 min, the precipitation is nearly complete (Fig. 1). The precipitation behaviour in the presence of the chaotropic reagents urea and KSCN as well as the inverse experiment of dissolution of an already formed precipitate is depicted in Figs. 2 and 3. If precipitations are carried out in the presence of urea (Fig.3) the formation of precipitate is basically finished within 2 h. A small additional precipitate however is formed within 16 h post-incubation. Although the absolute amount decreases, the percentage of this additional precipitate increases with increasing urea molarity. For example, in 8 M/l urea no significant precipitate is obtained after 2 h;

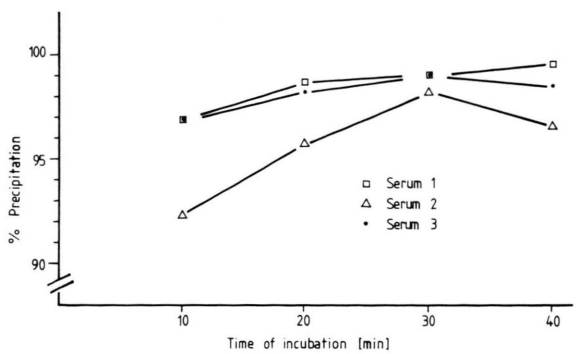


Fig. 1. Kinetics of immunoprecipitation of B-Phycoerythrin (B-PE). B-PE (10 µg/ml final concentration) is incubated with sera from 3 different rabbits at optimal antigen/antibody proportion (serum 1: 200 µl, serum 2: 100 µl, serum 3: 150 µl). More than 90% of the biliprotein is precipitated within 10 min.

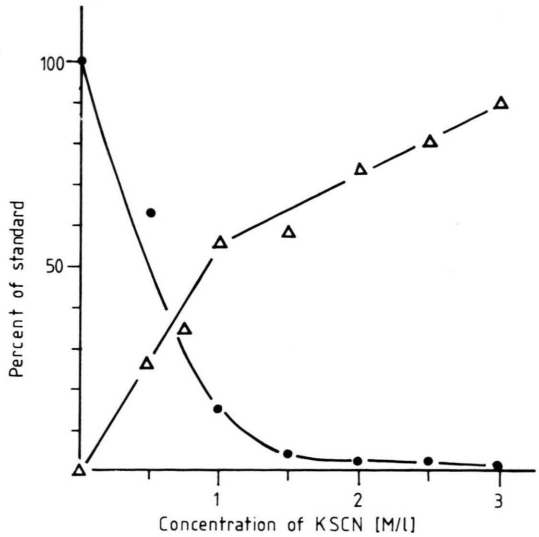


Fig. 2. Characteristics of immunoprecipitation of B-PE in the presence of potassium rhodanide at increasing molarities (●–●) and dissolution of an already formed precipitate (△–△).

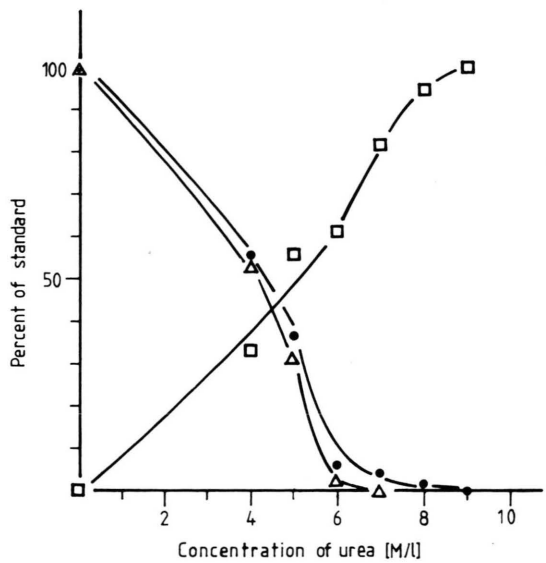


Fig. 3. Immunoprecipitation of B-PE in the presence of increasing urea molarities (incubation time: 2 h △–△ and 16 h ●–● resp.) resp. dissolution (□–□). The immunoprecipitate is dissolved completely in 9 M/l urea. Some precipitate is formed at a urea concentration of 8 M/l.

some precipitate however is formed after 16 h (Fig. 3). Although the amount of precipitate decreases rapidly with increasing rhodanide or urea concentrations, a certain amount of precipitate forms even in the presence of 2 M/l KSCN or 7 M/l urea. The amount of chaotropic reagent needed for dissolution of immunoprecipitates (3 M/l KSCN, 9 M/l urea) (Figs. 2, 3) is shifted to higher molarities as compared to the precipitation experiment. Studies of circular dichroism (CD) of C-PE lead to the assumption that most of the peptide chain is in the random coil state in 8 M/l urea solutions [45]. The unfolding of the protein chain(s) is accompagnied by a decrease of fluorescence emissions in the presence of 8 M/l urea [45] resp. 3 M/l KSCN; the chromophore changes from the extended to a cyclic conformation. In spite of the probably totally "unfolded" protein and the changed chromophore conformation a small immunoprecipitate is still observed in the presence of 8 M/l urea. This may be caused by reaction of antibodies with sequential determinants, whose antigenic reactivity is not impaired by conformational changes of the polypeptide chain. In analogy to the enhanced stability of enzymes against heat denaturation in the presence of specific antibodies [46, 47], a certain amount of conformation could be preserved in the presence of chaotropic reagents as well. The decrease of antigenicity in chaotropic solutions may be either due to conformational changes of the polypeptide chain and/or the chromophore(s).

The phycoerythrobilin chromophores of B-PE are tightly linked to the apoprotein by a thioether linkage [1 – 5] and a second more labile as yet uncharacterized linkage [48] (Fig. 4). This second linkage fixes at least 30% [48] of the chromophores present at one of the middle rings (B resp. C). Such a firm linkage will put a considerable strain on the apoprotein. For another biliprotein [41] it has been demonstrated that 50% of the folding energy of the protein is used for imposing on the chromophore the extended conformation required for photosynthetic functionality (*i.e.* energy transfer according to the Förster mechanism [49]). In a reciprocal manner, strain is also exerted on the protein. Upon chromophore splitting this strain is relieved and a conformational change of the apoprotein may result. The ability of antibodies to recognize the altered protein might therefore be impaired. For cytochrome c from *Neurospora crassa*, Korb and Neupert [40] found almost complete absence of cross-reactivity between apo- and holoprotein. This might be explained by the remarkable sensitivity of cytochrom c resulting from its weak immunogenicity and low antigenic valency [50]. B-PE from *Porphyridium cruentum* however, is a very strong immunogen, especially if applied in the form of phycobilisomes. In addition to the considerable size of those particles, which often occur as aggregates (see below) this may be due to the large number of identical subunits on the phycobilisome surface, comparable to the coat protein assembly of viruses. Further, our experiments show that denaturation of the antigen with denaturing agents such as urea and KSCN do not inhibit the precipitation at lower concentrations. We therefore conclude that modification or removal of the chromophore can not inhibit the antigenicity of the B-PE apoprotein.

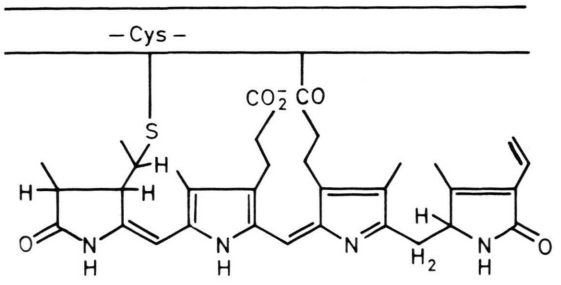


Fig. 4. Structure of B-phycoerythrin chromophore (phycoerythrobilin) with bilin-apoprotein linkages.

Immunoprecipitation of B-PE with chemically modified chromophore(s)

The following experiments show that the "B-PE-apoprotein" reacts with antibodies against the native holoprotein. The middle methin bridge of the phycoerythrobilin chromophore forms a sulfinate adduct when treated with sodium dithionite [35]. Most likely, this altered chromophore will no longer be recognized by antibodies. The modified B-PE however is precipitated equally well by antiserum as the biliprotein with native chromophore(s). This excludes a major role of the chromophore(s) in the precipitin reaction.

Chromic acid degradation

Treatment with chromic acid degrades the tetrapyrrole chromophore to single (oxidized) pyrrole

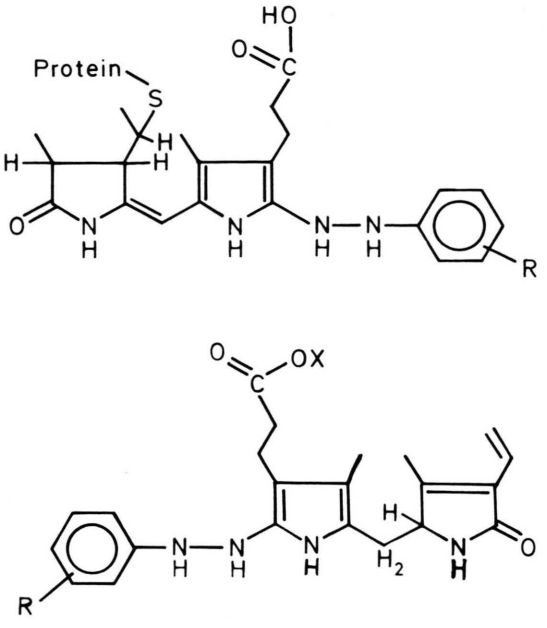


Fig. 5. Structures of reduced diazotized dipyrroles (hydrazo compounds) deriving from B-PE. The colorless compounds are covalently linked to the apoprotein which is bound to Sepharose 4B. X = −H or -apoprotein.

nuclei (imides) [51 − 53]; the sulfur (linking chromophore and apoprotein) is oxidized to a sulfone [54]. After reduction with sulfur dioxide to remove excess chromium (VI) (and removal of the resulting chromium (III) which is a protein denaturing agent) the chemically formed "B-PE-apoprotein" is precipitated by antisera, even in the presence of 6 M/l urea. As a control, antiserum was subjected to chromic acid degradation; no precipitation was found. Obviously, a chromophore is not necessary for immunoprecipitation. Remarkable is the remaining antigenicity of the protein, even though chromic acid converts sulfhydryl groups to sulfonic acid groups and thioethers to sulfones (*e.g.* methionine to methionine sulfone) and splitting to large peptides occurs [48].

Immunoreactivity of B-PE-chromopeptides

To test whether any immunoreactivity is contributed by the chromophore, chromopeptides prepared by digestion with pepsin [1, 3] were used. These chromopeptides are of low molecular weight (*e.g.* Cys-(Val, Leu)-chromophore, MW 1200) [1]. They

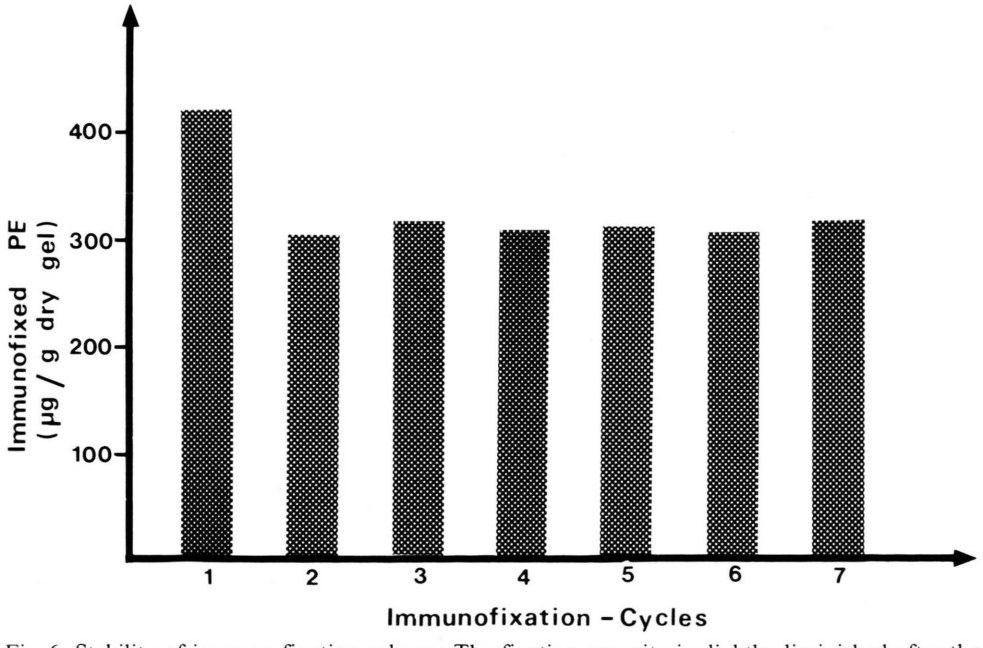


Fig. 6. Stability of immuno fixation column. The fixation capacity is slightly diminished after the first run and remains stable thereafter. The column can be reused many cycles (cycle 2−5) without loss of capacity, stored for 10 days at 4 °C (cycle 6) and even freeze dried and reswollen (cycle 7).

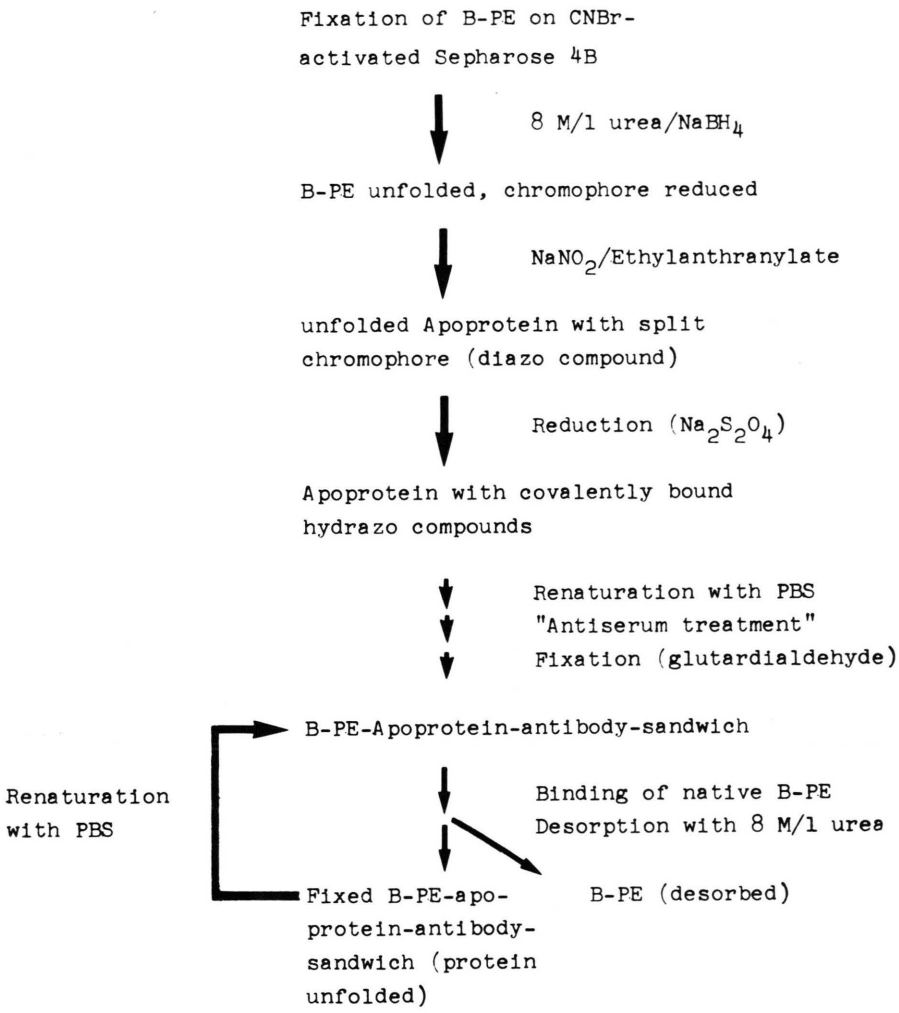
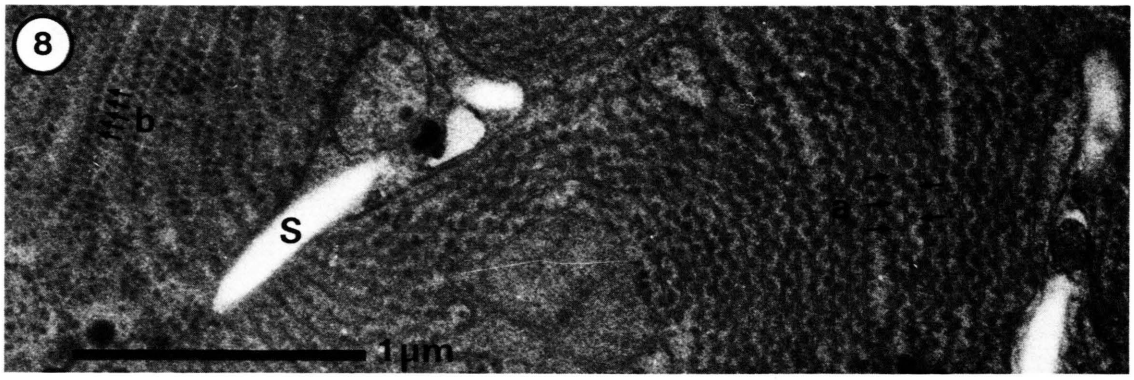


Fig. 7. Preparation of immunoadsorbent.

give a much better approximation to the in situ chromophore than isolated bile pigments. Their small size however excludes a precipitation with chromophore-specific antibodies. To circumvent this problem, we fixed enriched IgG to CNBr-activated Sepharose 4B which was incubated either with native B-PE or the mixture of small chromopeptides. Whereas native B-PE is bound in considerable amounts, chromopeptides are not adsorbed. We therefore exclude the presence of antibodies against this chromophore in the antiserum used.

Preparation of a highly specific immunoadsorbent and recovery of the immunofixed antigen

Because of the very strong antigen-antibody interaction, the usually applied methods to isolate monospecific antibodies, *e.g.* by common affinity chromatography were unsuccessfull. We therefore prepared a specific immunoadsorbent by a "sandwich-technique". In the first step, electrophoretically pure B-PE was fixed to CNBr-activated Sepharose 4B. To produce a colorless matrix we splitted the chromophore using the diazo reaction [42] and reduced the resulting diazo compounds to their colorless hydrazo products (Fig. 5). After incubation with the polyspecific antisera the immuno complexes formed were covalently fixed by crosslinking antigen and antibodies using glutardialdehyde. In this way the second antigen binding site is still intact and immunoadsorbs B-PE. Unspecific protein binding is excluded by a control experiment: If instead of antiserum a mixture of egg-white proteins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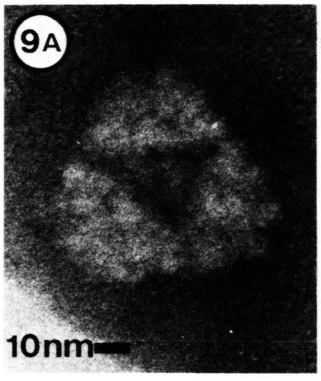
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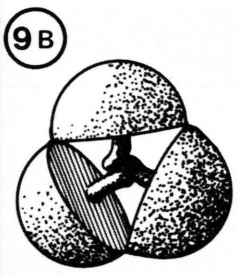
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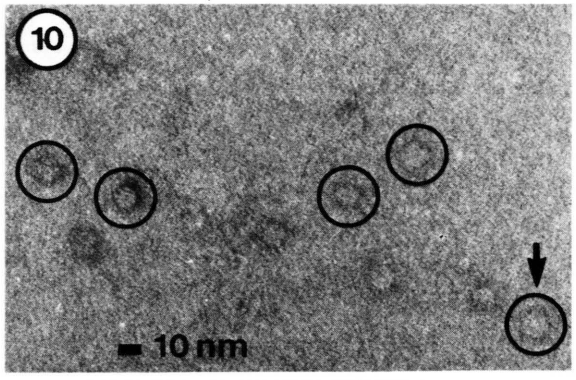
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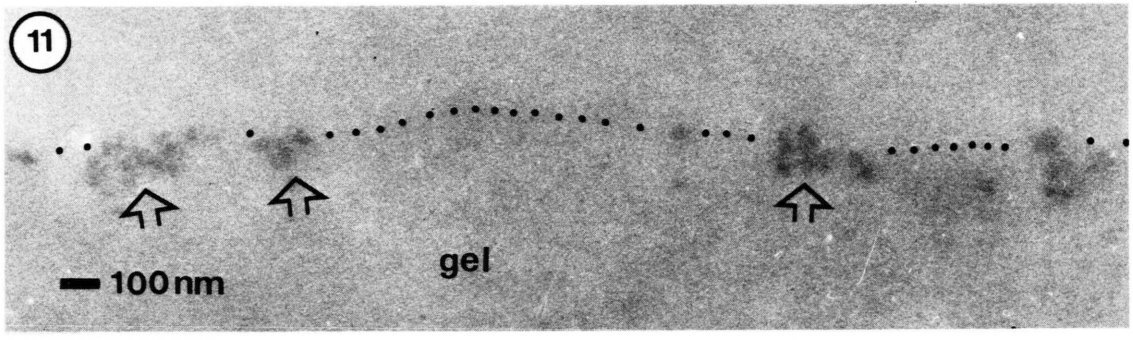
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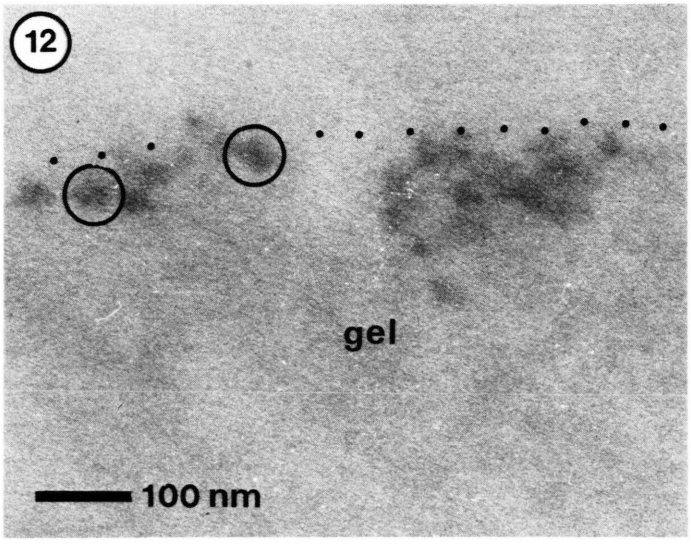
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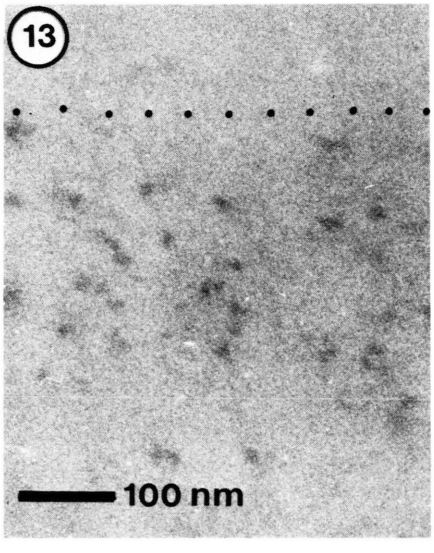
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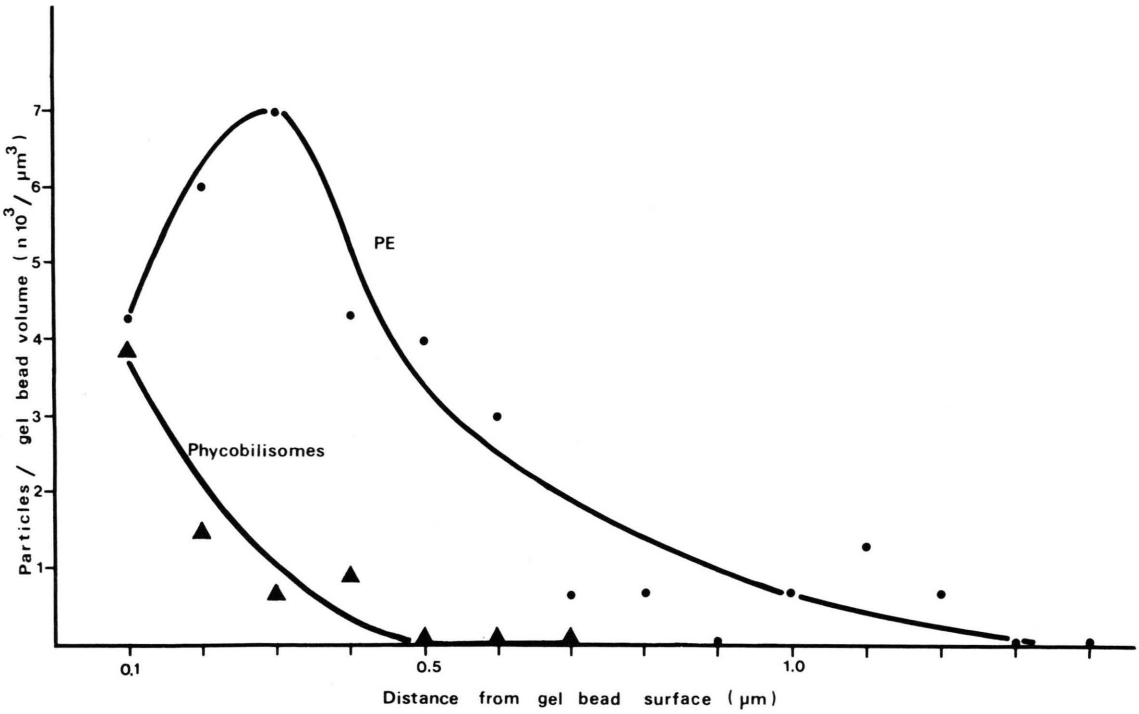

Fig. 14. Distribution of phycobilisomes and phycoerythrin (mono- and oligomers) as a function of the migration distance.

is used, B-PE does not bind to such a matrix. – The biliprotein is recovered by dissociating the immunocomplex with 8 M/l urea. After removal of the urea by simple washing with PBS, it is possible to repeat the immunoadsorption-desorption cycle at least 7 times without loss of activity (Fig. 6). The column capacity, except for the first cycle, remains constant. We interpret the loss of binding capacity after the first cycle as the formation of immunocomplexes stable in 8 M/l urea (compare Fig. 3); we rather exclude loss of activity due to irreversible denaturation of antibodies. The immunoadsorbent is remarkably stable; it can be stored at least 10 days in PBS at 4 °C and even freeze-dried without loss in activity. The column preparation and immunofixation procedure is summarized in Fig. 7.

Electron microscopy

Phycobilisomes of *Porphyridium cruentum* are attached to the thylakoid membranes in high density and typical array [31] (Fig. 8). After isolation and enrichment by twofold density gradient centrifugation they are of uniform size and shape. Preferentially they aggregat to di- tri- and tetramers caused by hydrophobic interaction of a foot-like structure

Fig. 8. Electron micrograph of *P. cruentum* thylakoid membrane with attached phycobilisomes (arrows) seen from the broad side (a) and the small side (b).

Figs. 9, 10. Isolated negatively stained phycobilisomes and B-PE Phycobilisomes often aggregates to trimers (9A) due to the hydrophobic interaction of a food structure (scheme Fig. 9B). Single B-PE molecules are disc-shaped with subunit structure (Fig. 10, arrows).

Fig.11. Thin section of gel bead with immuno fixed phycobilisomes which are often aggregated to oligomers (arrows). Dotted line marks bead surface.

Figs. 12 and 13. Penetration of gel bead depends on particle size. Phycobilisome oligo- and monomers are located near the surface (Fig. 12, circle) wheras phycoerythrin molecules (mono- and oligomers) are distributed over a greater depth.

[31, 55, 56] (Fig. 9A, 9B). The solution of electrophoretically pure B-PE yields single disks with a diameter of about 10 nm upon negative staining on graphite oxide. Occasionally, subunits are visible (Fig. 10), which correspond to the $(\alpha\beta)_6\gamma$-structure described by Glazer [57]. After preparation for electron microscopy, gel beads remained unchanged in diameter and retain their spherical shape. The contrast of the gel matrix to the resin is extremly low but the osmium/uranyl stained phycobilisomes are visible without poststaining. Immunofixed phycobilisomes are restricted to the periphery of the gel beads (penetration depth = $0.2 - 0.4\,\mu m$) (Fig. 14). Mainly aggregates are observed (Fig. 11, 12). These oligomers with an estimated particle weight of $10^7 - 3\times10^7$ (for $2 - 6$ phycobilisomes) are at the upper limit of the fractionation range given by the supplier ($6\times10^4 - 2\times10^7$ daltons).

Occasionally disaggregated immunofixed phycobilisome are observed. Concluding from their size (≈ 10 nm) they should represent phycoerythrin mono- and oligomers. These fragments penetrate up to 1.2 µm into the gel (Figs. 13, 14). This is expected from the lower molecular weight: phycobilisomes $\triangleq 5\times10^6$ daltons, phycoerythrin monomer: 2.36×10^5 which is well within the fractionation range. A similar distribution is observed for the immunofixed B-PE "sandwich", but the image contrast is so low that no adequate prints can be published. In the control gel the covalently fixed B-PE (chromophore free) could not be detected. Obviously a minimum aggregate size (PE – antibody – PE) and/or the presence of the bile pigment chromophore is necessary for sufficiant contrast in the sections. Based on the volume density and distribution of phycobilisomes and phycoerythrin (oligo- and monomers) (compare Fig. 14), the gel capacity is calculated: The mean bead radius (swollen gel) is $36.4 \pm 9.6\,\mu m$ (dry gel: $25 \pm 8\,\mu m$), corresponding to a volume of $3\times10^{-5}\,\mu m^3$. With a mean density of phycoerythrin (regarded as monomer) $2.7\times10^3/\mu m^3$ and a penetration depth of 1.2 µm the total number of immunofixed PE molecules per bead is 5.2×10^7 corresponding to 260 µg B-PE per dry gel. This is in good agreement with the value determined biochemically (= 400 µg PE/g dry gel).

In the present work we have demonstrated that holo- and a chemically prepared apoprotein of *Porphyridium cruentum* B-PE are immunochemically strongly related and show cross-reactivity. This finding may serve as a basis for further immunochemical investigation of B-PE apoprotein biosynthesis. As a sensitive tool, an immunoadsorbent column has been designed for a apoprotein isolation. The biochemically determined capacity of the column is in good agreement with data determined morphometrically from electron micrographs. Since most of the phycobilisomes and phycoerythrin molecules are bound at the surface of the gel beads (Sepharose 4B), the binding capacity (15% of the bead volume) could be improved by increasing the ratio surface/volume either by using smaller beads or by changing the geometry *e.g.* to disk-shape.

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