RESEARCH NOTE

RECONSTITUTION OF ALLOPHYCOCYANIN FROM *Mastigocladus laminosus* WITH ISOLATED LINKER POLYPEPTIDE

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Abstract—The core linker polypeptide $L_c^{8.9}$ was isolated from *Mastigocladus laminosus* and purified on a preparative scale. A method for the reconstitution of allophycocyanin (AP)–linker complexes from isolated polypeptides was developed. The complex $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$ was reconstituted and compared to $(\alpha^{AP}\beta^{AP})$ and $(\alpha^{AP}\beta^{AP})_3$ by sucrose density gradient ultracentrifugation, absorption, fluorescence emission and circular dichroism spectroscopy. Differences in the spectra of reconstituted and of directly isolated AP complexes are discussed.

INTRODUCTION

Phycobilisomes, the light-harvesting antennae of cyanobacteria, red algae and cyanelles, are supramolecular, highly structured protein complexes located on the thylakoid surface.[1,2] They are composed of chromophore-bearing phycobiliproteins[3–5] absorbing light over a wide spectral range and of linker polypeptides that regulate and stabilize the phycobilisome structure but also modify the light-absorption properties of the phycobiliproteins.[6–15]

In cyanobacterial phycobilisomes, an array of rods is linked by one or more rod-core linker polypeptides to the phycobilisome core. The rods are composed of the peripheral biliproteins phycocyanin (PC)† and (if present) phycoerythrin (PE) or phycoerythrocyanin (PEC) and of specific rod linker polypeptides. The bi- or tricylindrical core substructures are formed by allophycocyanin (AP), two minor biliproteins structurally related to AP, and the two linkers L_c and L_{cm}, the latter carrying a chromophore and anchoring the core to the membrane.[16] This allows for an efficient excitation energy transmission[4,17–19] from the short-wavelength-absorbing complexes at the peripheral ends of the rods to the supposed terminal emitters allophycocyanin B (APB) and L_{cm} in the core and ultimately to the chlorophylls of photosystem II (and possibly photosystem I[20,21]).

The key role of the linker polypeptides in the aggregate formation and the fine-tuning of the absorption characteristics of the phycobiliproteins has been extensively studied

but is still only poorly understood. Primary sequences of the different linkers of various organisms have been determined directly[22–24] or deduced from the sequences of their genes.[25–31] Conspicuous homologies indicate the presence of binding motifs for the different biliproteins. Much of the function of individual linkers has been deduced from the construction of deletion mutants.[26,32] Large subcomplexes believed to be originally present in the phycobilisomes and containing specific linkers have been isolated after partial dissociation of phycobilisomes.[20,33] Smaller complexes have been purified after complete dissociation in low molarity buffers and partial reassociation[11,13,14,34–36] or after partial proteolytic digestion of phycobilisomes or isolated subcomplexes[10,12,37] and have been reconstituted to larger complexes.[7,9] In some cases, complete phycobilisomes have been reconstituted from isolated subcomplexes.[38,39]

An alternative approach expected to give insight into the details of linker–biliprotein interactions are controlled reconstitution experiments of their complexes from the isolated components or fragments thereof. Due to the well-defined conditions, this is also important for satisfying the high demands of sensitive spectroscopic techniques on sample purity and concentration. However, due to problems with linker isolation and purification, relatively few studies of this kind have been undertaken.[9,11,15]

Here, we present a method for the isolation of pure biliproteins and linker polypeptides under mildly denaturing conditions and an optimized procedure to reconstitute functional biliprotein–linker complexes. The method was used to characterize AP complexes of the thermophilic cyanobacterium *Mastigocladus laminosus* with or without the core linker $L_c^{8.9}$ with regard to absorption, fluorescence, circular dichroism (CD) and aggregate size. It is also shown that reconstitution can provide an assay for other polypeptides that can influence the absorption spectra of biliproteins.

MATERIALS AND METHODS

Absorption measurements were made using a Lambda 2 UV/vis spectrophotometer (Perkin-Elmer). Fluorescence emission was mea-

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†*Abbreviations:* α, β, biliprotein subunits; AP, allophycocyanin; APB, allophycocyanin B; CD, circular dichroism; DA/A_{max}, ratio of CD signal height to absorbance value at λ_{max}; HPLC, high-performance liquid chromatography; L, linker polypeptide (subscripts denote origin and function in phycobilisomes: r = rod, c = core, rc = rod core, cm = core membrane; superscripts denote molecular weight); OD, optical density; p/s, peak (λ_{max})-to-shoulder (600 nm) absorbance ratio; PC, phycocyanin; PE, phycoerythrin; PEC, phycoerythrocyanin; PTH, phenylthiohydantoin; RCARS, resonance-enhanced coherent anti-Stokes Raman spectroscopy; SDS-PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis.

sured on an F-2000 fluorescence spectrophotometer (Hitachi) at an optical density of 0.1 at the absorption maximum in 1 cm cells; excitation was at 580 nm. The CD measurements were made with a CD 6 Dichrograph (I.S.A. Jobin Yvon) at an optical density (OD) of 0.35–0.75 in 0.5 cm cuvettes.

Allophycocyanin was isolated from *M. laminosus* by the method previously described for PC.[15] Allophycocyanin was purified from PC contaminations on a hydroxylapatite (Bio-Gel HT, Bio-Rad) column (10–200 mM potassium phosphate buffer, pH 7). Purification was checked by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and absorption spectroscopy. Trimeric AP fractions containing linker polypeptides were pooled.

Linker polypeptides were isolated from the latter on S-Sepharose Fast Flow (Pharmacia) in 10 mM Tris/HCl containing 3.5 M urea (pH 8.9, measured at room temperature). Allophycocyanin was eluted with the application buffer. Colorless polypeptides were fractionated with a 0–300 mM KCl gradient in the application buffer and pooled according to SDS-PAGE and preliminary reconstitution experiments.

Linker-free AP was prepared on Fractogel TSK DEAE-650(2) (Merck) in 5 mM potassium phosphate buffer, pH 7, containing 3.5 M urea. Colorless polypeptides were eluted with application buffer, and pure AP fractions were eluted with a gradient of 0–400 mM KCl in the application buffer or a gradient of 5–200 mM potassium phosphate in 3.5 M urea.

For concentration of larger volumes of protein solutions 70% ammonium sulfate precipitation followed by dialysis against the desired buffer was used routinely. Small samples were concentrated with Centricon-10 microconcentrators (Amicon).

The aggregation state was determined by 3–12% sucrose density gradient ultracentrifugation in 100 mM or 700 mM potassium phosphate buffer (pH 7.5) with trimeric PC samples (λ_{max} 618–620 nm) as markers (RPS40T rotor, 270 000 g, 19 h, 4°C). Size exclusion column chromatography was done in 100 mM potassium phosphate buffer at pH 7 (Sephadex G-75, Pharmacia).

Reconstitution experiments. Linker-free AP (approximately 30 nmol, concentrated solution from the TSK-DEAE column, generally ≈0.2 mM) was mixed with isolated $L_c^{8.9}$ (≥30 nmol, concentrated solution from the S-Sepharose FF column, generally ≈40 μM). Tris/HCl buffer (10 mM, pH 8.9) containing 3.5 M urea was added to make the final volume 1.5 mL. The samples were dialyzed first for 3 h against 3.5 M urea in potassium phosphate buffer (pH 7.5, 100 mM or 700 mM), then overnight against the same buffer without urea. Both steps were done at room temperature in the dark. Samples were purified from precipitated protein by short centrifugation (6000 g, 20 min) and analyzed by absorption spectroscopy and SDS-PAGE. The complexes were further purified by the same sucrose density gradient ultracentrifugation as was used for the analytical runs (see above). The isolated bands were analyzed by absorption, fluorescence and CD spectroscopy and by SDS-PAGE.

Monomerization of AP was done by addition of solid KSCN (1 M final concentration).[40]

N-terminal amino acid sequences were determined as described by Eckerskorn *et al.*[41] The protein sample was separated by micropreparative SDS-PAGE and electroblotted onto a siliconized glass-fiber membrane. Bands were stained with Coomassie brilliant blue (Serva), excised, destained and sequenced in a gas-phase sequenator (Applied Biosystems model 470A). The phenylthiohydantoin (PTH) amino acids were analyzed using an on-line isocratic HPLC system.[42]

The SDS-PAGE was done according to Lämmli,[43] using a 4% stacking gel and a 15% separation gel on a Mini Protean II Dual Slab Cell (Bio-Rad).

RESULTS

The separation of crude biliprotein extracts of *M. laminosus* by anion-exchange chromatography yields generally three to four distinct fractions. Allophycocyanin (λ_{max} 653 nm) is contained in the second one. It was purified from other biliproteins on hydroxylapatite. Analysis by SDS-PAGE shows that it is a complex mixture of AP subunits with linker polypeptides, their fragments and other unspecified protein
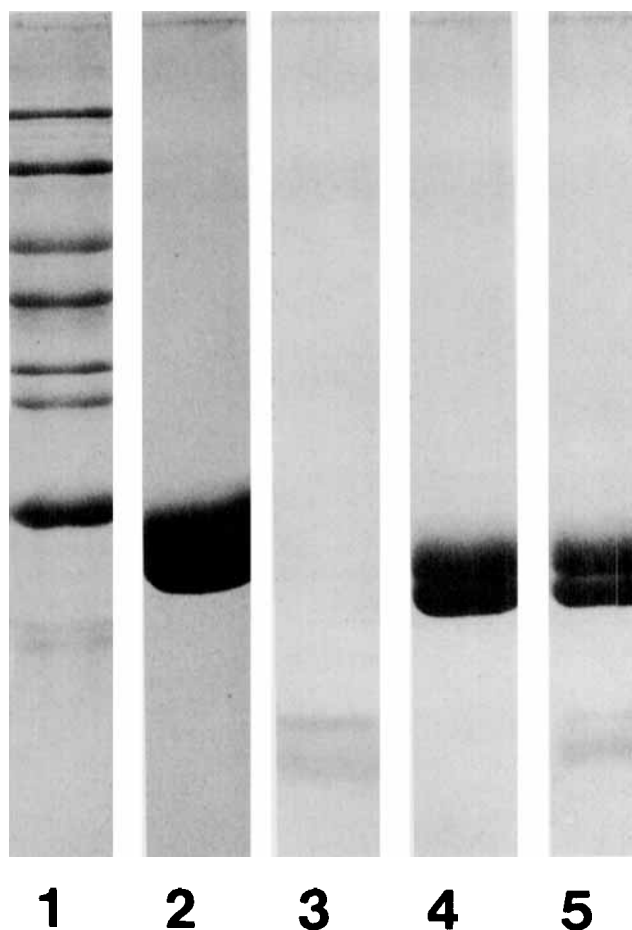

Figure 1. Sodium dodecyl sulfate PAGE of isolated polypeptides and reconstituted complexes. Lane 1, molecular weight markers (Sigma SDS VII-L marker kit: 14.2, 20.1, 24, 29, 36, 45, 66 kDa; plus β-galactosidase: 116 kDa); lane 2, AP as obtained from the TSK-DEAE column; lane 3, $L_c^{8.9}$ as obtained from the S-Sepharose FF column; lane 4, reconstituted $(\alpha^{AP}\beta^{AP})_3$ complex; lane 5, reconstituted $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$ complex.

contaminations. By comparison with phycobilisomes, the most readily identifiable intact linkers are $L_c^{8.9}$ and $L_{rc}^{29.5}$ (not shown). Sucrose density gradient ultracentrifugation shows that most of the pigment is present as trimers. Crude AP fractions have characteristic absorption spectra (not shown) with a maximum at 653 nm and a shoulder of varying height at about 600–625 nm. The peak-to-shoulder ratio (p/s; defined in this work as peak absorption to 600 nm absorption) increases roughly with an increasing amount of the $L_c^{8.9}$ polypeptide. Samples with maximum p/s (≈2.4) show absorptions as reported for purified $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$ aggregates.[13] However, other linkers and their fragmentation products can modify the spectrum of AP in a similar way.

Allophycocyanin was purified from all other proteins by variation of a method developed for the purification of PC under mildly denaturing conditions.[15] Anion exchange chromatography on TSK-DEAE in the presence of 3.5 M urea removed the linker polypeptides, which do not bind to the column, whereas AP eluted with a salt gradient (potassium phosphate or KCl) in highly concentrated fractions (Fig. 1, lane 2). An alternative to purify AP was by way of cation-
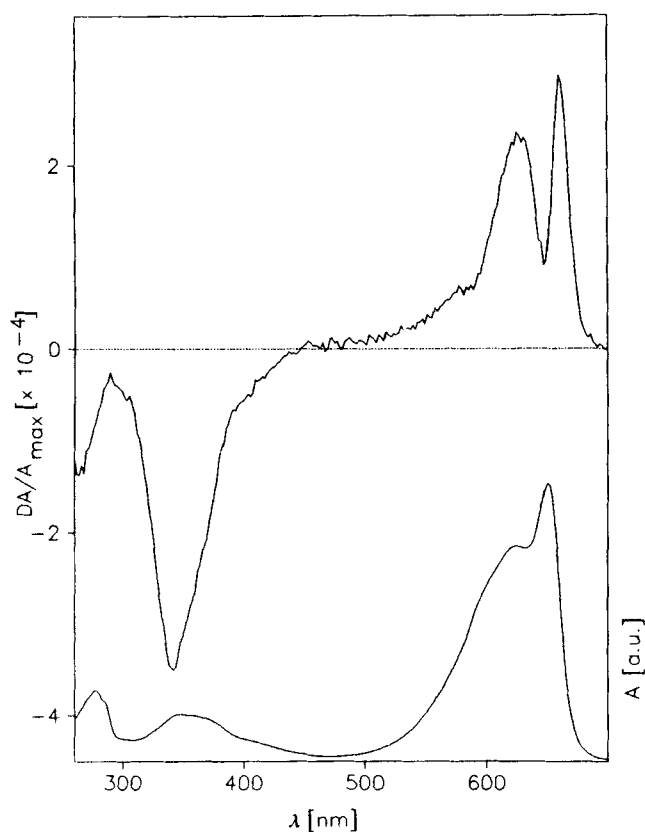
Figure 2. Absorption (bottom) and CD (top) spectra of reconstituted $(\alpha^{AP}\beta^{Ar})_3$ complex. Solvent was 700 mM potassium phosphate buffer (pH 7.5). $A_{max}^{1cm} \approx 1.5$, CD pathlength = 0.5 cm. The DA/A_{max} gives the CD normalized to the maximum absorption in the red spectral region.

exchange chromatography, as used for the isolation of linker polypeptides (see below). The AP eluting with the application buffer was free of known linker polypeptides but contained small amounts of a large, colorless polypeptide ($\approx$70 kDa) already identifiable in the crude AP preparation (not shown). This polypeptide was not characterized further, but it is possibly a proteolytic fragment of L_{cm}[89]. The impurity disappeared during subsequent steps of the reconstitution procedure and, by comparison with experiments using pure AP (see below), did not influence the results.

Allophycocyanin isolated by either method in the presence of 3.5 M urea is present in a trimer–monomer equilibrium even at high protein concentrations ($A_{max} \leq 15$ cm^{-1}). In the absence of urea, AP should dissociate into monomers only at protein concentrations corresponding to peak absorbances <0.1 cm^{-1} in 10 mM phosphate buffer[44] and is even more stable in buffers of higher ionic strength.[45] After dialysis against 100 mM potassium phosphate buffer (pH 7) free of urea, the absorption maximum was at 651 nm, indicating trimeric AP without linkers.[13] However, the spectra showed an unusually high short-wavelength absorption rising to a second peak at 621–623 nm (Fig. 2). This feature (p/s $\approx$ 1.5–1.6) is indicative of a trimer–monomer mixture. However, it was stable in the main high molecular weight fraction eluting first during size-exclusion chromatography on Sephadex G-75. The p/s ratio was only partly enhanced after sucrose density gradient ul-

tracentrifugation, which removed some more monomeric AP but showed the bulk of the material to sediment as far as the trimeric AP and PC markers. When this AP was treated as a control under optimum reconstitution conditions for the formation of AP–linker complexes (see below), the p/s was somewhat variable ($\approx$1.5–1.7; Fig. 2: p/s = 1.5) with different isolates. Fractions with the maximum value had absorptions as published for purely trimeric AP without linkers.[13]

The purity of linker-free AP was confirmed by SDS-PAGE (Fig. 1, lane 2) and N-terminal amino acid sequence determination. On the gel, three overlapping colored biliprotein bands could be distinguished (see Discussion). The upper two bands were both sequenced to the 21st amino acid and showed the identical sequence of α^{AP}; the lower band was sequenced to the 10th amino acid and showed the sequence of β^{AP} as published by Sidler et al.[46] No indication of contamination by other biliprotein subunits was found. The staining intensities of the combined α^{AP} bands compared to that of the β^{AP} band appeared to be consistent with a 1:1 stoichiometry of the subunits, but a detailed analysis has not been undertaken.

Cation-exchange chromatography on S-Sepharose FF of AP eluted from the hydroxylapatite column (see above) was very effective in fractionating linker polypeptides.‡ In order to identify polypeptides inducing spectral changes, all column fractions were scanned by SDS-PAGE and selected fractions were screened by reconstitution with AP. The $L_c^{8.9}$ was found to elute in a very broad and dilute peak at the end of the gradient and was pooled.|| Contamination by other polypeptides was very small and disappeared completely during subsequent reconstitution and purification procedures. Because M. laminosus also contains another linker polypeptide of similar molecular weight ($L_{rc}^{8.9}$),[23] the 8.9 kDa band on the gel was N-terminally sequenced to the 10th amino acid and identified as pure $L_c^{8.9}$ with cysteine at position nine.[22]

Allophycocyanin–linker complexes with $L_c^{8.9}$ (Fig. 1, lane 5; Fig. 3) have been repeatedly reconstituted and examined. During these experiments, the optimal conditions for reconstitution were developed by comparison of various param-

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‡The fraction of colorless polypeptides eluting from TSK-DEAE in the application buffer contained about 50% $L_c^{8.9}$ and effectively reconstituted APC–linker complexes (p/s = 2.4). Attempts to further purify this material from higher molecular weight polypeptides by size-exclusion chromatography with Bio-Gel P6DG, 10DG, P10, P60 or Sephadex G-75 were unsuccessful. Centricon-10 or Centricon-30 microconcentrators did not differentiate the proteins, either. This could be due to the high polarity of the linker proteins and their tendency to form unspecific aggregates even in solutions containing urea.

||Additionally, a pool of three unidentified polypeptide bands at apparent molecular weights of about 21–23 kDa eluting together at about 100 mM KCl was found to reconstitute an $(\alpha^{AP}\beta^{AP})_3$·21–23 kDa polypeptide complex: absorption λ_{max} 651.5 nm, p/s 2.2–2.4, fluorescence emission λ_{max} 662 nm, CD $DA/A_{max} = 5.0$–5.4 $\times$ 10^{-4}. It appears that these two fractions constitute the bulk of colorless polypeptides in crude AP preparations, which can effectively interact with AP alone to form spectrally distinct stable complexes. The origin of the newly discovered 21–23 kDa polypeptides is unclear. Because they are absent in isolated phycobilisomes, they are probably proteolytic fragments of one or more larger linkers.

eters.§ Because trimeric AP complexes with and without linkers could not be separated quantitatively by sucrose density gradient ultracentrifugation, the linkers were always added in a stoichiometric excess (about 1–10 copies per AP monomer) to ensure complete reconstitution. An exact quantification was not attempted; the amount of linker polypeptide solution to be added for best results was found empirically for each batch. Typical AP concentrations in the reconstitution mix were 20–30 μM, the yield of linker complexes was 80–90%. In aqueous solutions without urea, the linkers are not soluble and are irreversibly lost by precipitation, degradation and/or adsorption to the dialysis tubing when they are not protected by formation of stable complexes with biliproteins.[9] They are also extremely susceptible to proteolysis. Therefore, during the reconstitution involving the removal of urea from the sample, the loss of linker polypeptides was by far higher than the loss of biliproteins and depended on the procedure and on the quality and age of the samples.

After removal of precipitated protein by short centrifugation, the aggregation state of reconstituted AP–linker complexes was determined by sucrose density gradient ultracentrifugation with trimeric AP and PC as markers and was found to be predominantly (80–100% in different experiments) trimeric independent of the buffer concentration, although the bands tended to be broader in 700 mM buffer. Small amounts of monomeric AP were separated from linker-free AP that had been used as a control of the reconstitutions (Fig. 1, lane 4; Fig. 2). Traces of farther sedimenting biliproteins could be found in some cases, but these were not different from trimeric AP when examined by spectroscopy and SDS-PAGE. If there is an equilibrium in the samples between trimeric and hexameric (or higher) aggregates, then it is strongly on the trimeric side.

The reconstituted AP-trimer with $L_c^{8.9}$ shows an absorption maximum at 653.5 nm (Fig. 3), the p/s is 2.2–2.4. Linker-free trimeric AP treated under the reconstitution conditions has λ_{max} at 651 nm and a p/s of 1.5–1.7 (Fig. 2). The fluorescence emission maxima (spectra not shown) were found to be 662 nm for $(\alpha^{AP}\beta^{AP})_3$ and 664 nm for $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$. No contamination by α^{APB} or L_{cm}^{89} was detectable.

The CD spectrum of $(\alpha^{AP}\beta^{AP})_3$ shows one broad and one narrow positive band in the red range with a narrow minimum (Fig. 2), whereas the spectrum of the AP–linker complex (Fig. 3) shows a set of positive and negative bands. The positive long wavelength signal at about 656–660 nm is more intense (DA/A_{max} = 3 × 10^{-4}) in the $(\alpha^{AP}\beta^{AP})_3$ complex than in $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$ (DA/A_{max} = 1.5 × 10^{-4}) at the same concentration (A_{max} ≈ 0.7). In $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$, the negative band at 588–594 nm is more pronounced than that at 640–642 nm. All CD spectra can be rationalized by assuming two contributing species or subpopulations. One has a positive band in the region of 620–630 nm, only this band is present
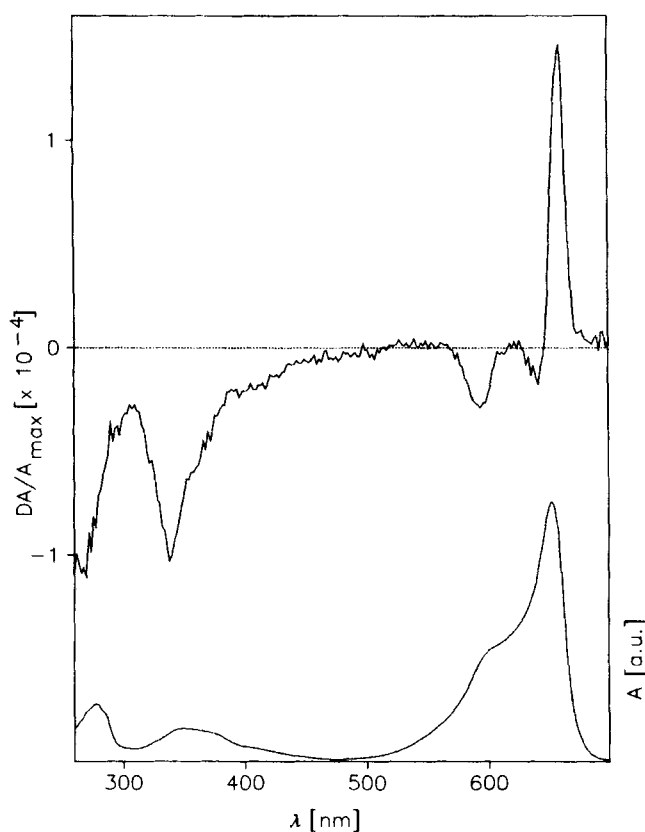

Figure 3. Absorption (bottom) and CD (top) spectra of reconstituted $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$ complex (details as in Fig. 2).

in the $(\alpha^{AP}\beta^{AP})$monomer (absorption λ_{max} 617 nm, CD λ_{max} 621 nm; Fig. 4). The other has a narrow positive band at 656–660 nm, and a broad negative band in the 620–630 nm region. The latter species is dominant in $(\alpha^{AP}\beta^{AP})_3 \cdot$ linker complexes. The negative UV band shows a similar structure throughout, but its intensity varies and shows no obvious relation to that of the red bands. Because there seems to be an overlap of a broad negative band with the positive signal at 620–630 nm, the positive peak is reduced and possibly shifted corresponding to the intensity and position of the negative band. The spectrum of $(\alpha^{AP}\beta^{AP})_3$ (Fig. 2) shows only a shoulder at ≈590 nm, which we take as an indication of the broad negative band.

DISCUSSION

Some sensitive spectral techniques like spectral hole burning or resonance-enhanced coherent anti-Stokes Raman spectroscopy (RCARS), which have only recently been applied to study the biophysics of biliproteins, demand high concentrations and/or purity of samples. Biliprotein–linker complexes are difficult to isolate in intact form and with sufficient purity. Furthermore, scrambling of the components cannot be excluded, in particular, if low-ionic strength conditions are used transiently. An example for the latter process is the subunit exchange among AP and APB trimers.[47] The alternative approach, viz. reconstitution of such complexes from the isolated components, gives principally access to well-defined complexes *in vitro*, and also allows more detailed

§Dialysis first against 3.5 M urea in the desired potassium phosphate buffer, then against urea-free buffer, gave far better results than dialysis against buffer without urea in both steps. Dialysis at room temperature was better than doing one or both steps at 4°C, but preheating the sample for 15 min at 42°C prior to dialysis had no effect. The yield was slightly higher in 700 mM buffer than in 100 mM, and higher at pH 7.5 than at pH 7.0. Complete denaturation of the sample with 8 M urea or monomerization with 1 M KSCN prior to dialysis had a pronounced negative effect.
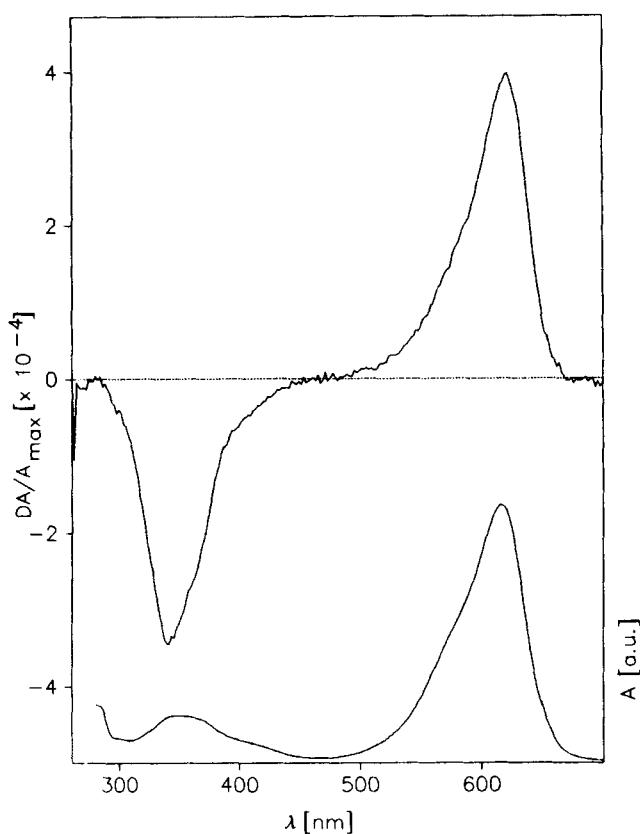
Figure 4. Absorption (bottom) and CD (top) spectra of monomeric AP. Same sample as shown in Fig. 2 but after addition of solid KSCN (1 M; further details as in Fig. 2).

investigations on the specificities of the interactions. To our knowledge, there is only one report in the literature[11] in which this technique has been explored for AP complexes. The approach requires isolation of pure components and optimized reconstitution conditions, which are the focus of this study.

Linkers have only been purified on a small scale under strongly denaturing conditions.[9,11,22] We have developed an efficient method for the isolation of linker polypeptides from phycobilisomes and biliprotein complexes on a preparative scale. The key is the use of intermediate urea concentrations[15,45] and cation-exchange chromatography at high pH near the isoelectric points of the strongly basic[22,23] linker polypeptides.

A critical point for the reconstitution experiments is the purity of AP. Using absorption and fluorescence emission spectroscopy, SDS-PAGE and N-terminal sequence determination, we have confirmed that there are only AP subunits in the sample. The split of α^{AP} into two overlapping bands on the gels (Fig. 1) may have one or more reasons. It could be an artefact of SDS-PAGE. The chromophore, which is exposed to the solvent, can be readily modified, resulting in a changed apparent molecular weight. Such a process has been suggested previously for PC[45] from the different chromophore-to-protein ratios. In the case of AP, however, the weaker upper band seemed to be colored in unstained gels as well. There also might be a proteolytic degradation at the C-terminal end. Reuter and Wehrmeyer[20,36] have postulated a microheterogeneity of all subunits of AP and PC from their

results of SDS-PAGE. They discuss a possible posttranslational modification of some amino acids within the polypeptide chains. N^4-methylasparagine has been identified in β^{AP}, β^{PC} and $\beta^{16.2}$ of M. laminosus[48] but not in the α subunits, and no heterogeneity was discussed. The microheterogeneity of bands on SDS-PAGE is confirmed by our results, but there is still no clear evidence to indicate whether this is also present in phycobilisomes or is an artefact.

Pure AP in an aggregating buffer (100–700 mM) is characterized by a low and somewhat variable p/s ratio (Fig. 2). While such a variability is expected for varying trimer–monomer ratios, there was no evidence for this by size-exclusion chromatography and sucrose density gradient ultracentrifugation. The spectrum of AP changes considerably with variations of protein concentration or of the physicochemical environment,[44,49] but, to our knowledge, no systematic aggregation study of linker-free AP of M. laminosus has been published. On the other hand, there may be more than one trimer population present, involving, e.g. "open" and "closed" rings. Also, complexes from which one subunit is missing would not be easily separable from intact trimers $(\alpha\beta)_3$. Linkers are generally thought to stabilize the biliprotein aggregates, so completely linker-free trimers might not be as stable. A part of the chromophores might have been bleached, resulting in aggregates with one or more colorless subunits. From the results of picosecond fluorescence spectroscopy studies of directly isolated AP complexes, Holzwarth et al.[50] have deduced a manifestation of some chromophore and protein heterogeneity caused by higher flexibility and deviations from native conformations after removal of the linkers. This might be even more pronounced after partial dissociation and reassociation under the influence of urea. However, reconstitution of AP–linker complexes from various isolated AP preparations yielded consistently the same results, which argues against a chemical modification. It should be noted that p/s ratios for a linker-free trimer as low as the ones found here have not been reported previously. On the other hand, because the influence of various linker polypeptides and fragments is so pronounced, the effect of trace contaminations in directly isolated complexes might have been underestimated previously.

Considerable attention was given to optimizing the reconstitution. The gentle reconstitution method for trimeric AP–linker complexes of M. laminosus gives high yields ($\leq 90\%$ with respect to the amount of AP used), with no contamination by linker-free trimers. The complexes are stable when kept on ice for a few days. The only comparable experiment with AP was done by Lundell and Glazer[11] on the $(\alpha^{AP}\beta^{AP})_3 \cdot$ 10.5 kDa complex of Synechococcus 6301 strain AN112, using lyophilized proteins that had to be dissolved in acidic 8 M urea, and achieving only 20% yield and 70% purity.

The absorption (Figs. 2 and 3) and fluorescence emission spectra of the reconstituted complexes $(\alpha^{AP}\beta^{AP})_3$ and $(\alpha^{AP}\beta^{AP})_3 \cdot$ L$_c^{8.9}$ are generally in agreement with the spectra published for M. laminosus[13,36,50] and the analogous complexes in Synechococcus 6301 strain AN112.[10,11] Small variations (≤ 1.5 nm for absorption and ≤ 3 nm for emission maxima) are explainable by different instrumentations. There is one exception: Reuter and Wehrmeyer[36] have measured 662 and 664 nm as the emission maxima of $(\alpha^{AP}\beta^{AP})_3$ and $(\alpha^{AP}\beta^{AP})_3 \cdot$ L$_c^{8.9}$, respectively, whereas Holzwarth et al.[50] have published the values 660 and 665 nm. Since the complexes have been

isolated in the same laboratory, this seems to emphasize our point that the composition of isolated complexes may not be very well defined.

Our CD spectrum of linker-free $(\alpha^{AP}\beta^{AP})_3$ (Fig. 2) differs considerably from those of Füglistaller et al.[13] and Holzwarth et al.[50] The negative signal at 584 nm is lacking in our samples, and the positive 656–660 nm band is reduced with respect to the one at 627 nm. If this is compared to the relatively low absorption at 651 nm in our samples, the positive 656–660 and negative 584 nm CD signals may be related to the same subpopulation. It is again conceivable that only a cyclic trimer gives rise to these spectral features or that contaminations with linkers or their fragments in previously published spectra are the cause for the deviations. The $(\alpha^{AP}\beta^{AP})$ monomer (Fig. 4) shows only one positive CD signal at 621 nm, whereas the trimer signal is at 627 nm. This may be a further argument that the reconstituted $(\alpha^{AP}\beta^{AP})_3$ does not contain a significant amount of monomers. The CD spectrum of $(\alpha^{AP}\beta^{AP})_3$ is reminiscent of that of PC trimers, albeit of smaller amplitude.[51] There, it had been rationalized by a superposition of a broad band due to an inherent dissymmetry of the individual chromophores, with a red-shifted exciton couplet. A moderate exciton coupling has been suggested[52,53] but cannot be proven by CD spectroscopy alone. However, recent time-resolved polarized spectroscopy data for AP are compatible with a Förster transfer mechanism, which argues at least against strong (>200 cm^{-1}) excitonic coupling.[54]

The differences in the spectra of $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$ are even more apparent. Our spectrum (Fig. 3) shows small negative signals at 594 and 642 nm, whereas the corresponding signals at 591–595 and 633–636 nm of Füglistaller et al.[13] and Holzwarth et al.[50] are very pronounced. Their spectra were made of isolated complexes. Füglistaller et al.[13] concede that their sample was contaminated by the complex $(\alpha^{AP}{}_2\beta^{AP}{}_2\beta^{16.2}L_{cm}{}^{89})$, but do not quantify this impurity. This involves two additional chromophores and multiplies the theoretical possibilities for chromophore–chromophore interactions. The CD of the latter complex (contaminated by $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$) shows pronounced negative signals at 591 and 634 nm, and the 634 nm signal was attributed to $(\alpha^{AP}{}_2\beta^{AP}{}_2\beta^{16.2}L_{cm}{}^{89})$. We suggest that the main part of the 591 nm signal could be caused by it as well (and/or possibly by $(\alpha^{APB}\alpha^{AP}{}_2\beta^{AP}{}_3) \cdot L_c^{8.9}$, another complex with a pronounced negative signal at 591–598 nm[13,50]), and that our spectrum of $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{8.9}$ with small negative signals represents the pure complex. It is also in reasonable agreement with the spectrum published by Lundell and Glazer[12] for the analogous complex $(\alpha^{AP}\beta^{AP})_3 \cdot L_c^{10.5}$ from Synechococcus 6301 strain AN112.

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