# Supporting Information

## Nowack and Grossman 10.1073/pnas.1118800109

### SI Materials and Methods

**Generation and Affinity Purification of Peptide Antibodies.** Synthetic peptides, RGDKVRINRPESYWNNE and LNSTQFAESELSAP, corresponding to sequences at the N- and C-termini, respectively, of PsaE from *Paulinella chromatophora*, were synthesized and used for immunization of rabbits (Davids Biotechnologie). For affinity purification, 50 μg of the synthetic peptides were spotted on nitrocellulose membranes and the peptides were fixed to the membrane for 10 min with 0.5% glutaraldehyde. Polypeptides not tightly bound to the membranes were removed by treatment with 100 mM glycine-HCl, pH 2.5, for 5 min. Unspecific binding sites were blocked with 3% BSA in Tris-buffered saline (TBS) solution for 1 h, and membranes were washed in TBS. Antibodies in 1 mL crude antiserum were incubated with the prepared membranes overnight at 4 °C to allow for antibody binding. The antibodies were then eluted from the membranes with glycine-HCl, pH 2.5. The solution was brought to pH 7.0 with 1 M Tris-HCl, pH 8.0, and then sodium azide and BSA were added to 5 mM and 1 mg/mL, respectively, to stabilize the antibodies. For all experiments, biochemically purified α-PsaE$_{pepN}$ or affinity-purified α-PsaE$_{pepC}$ antibodies were used.

**Postembedding Immunogold EM.** *P. chromatophora* cells were harvested in late logarithmic phase and fixed in culture medium on ice for 45 min, containing 2% paraformaldehyde (EM grade, from 16% stock; Polysciences), 0.5% glutaraldehyde (EM grade, from 50% stock; Polysciences), or 0.1% $OsO_4$ (from 4% stock; Electron Microscopy Sciences). Samples were washed three times with culture medium, resuspended in approximately 5 μL culture medium, and embedded in a small block of 6% low melting point agarose (Gibco). Cell pellets encased in the agarose were dehydrated in increasing concentrations of ethanol: 15%, 30%, 40%, 50% (on ice), and then 65%, 80%, 90%, and 100% (at −20 °C). Incubation time per step was at least 30 min, and the last step was overnight. Finally, the encased pellets were incubated in fresh 100% ethanol for 1 h at room temperature. Dehydrated cells were infiltrated with increasing concentrations of LR White resin (Polysciences) in ethanol over a 2-d period with gentle agitation at room temperature. LR White was polymerized at 50 °C for 3 d. Ultrathin sections (approximately 60 nm) were cut with a diamond knife on a microtome and mounted onto Formvar-coated nickel grids.

All steps of the following postembedding Immunogold labeling were performed at room temperature. Sections were rehydrated three times for 5 min in PBS solution containing 0.05% Tween20 (PBS-T) and then blocked for 25 min in blocking buffer [0.5% wt/vol ovalbumin (Sigma), 0.5% wt/vol BSA (Sigma) in PBS-T]. The blocked sections were incubated for 1 h with affinity purified α-PsaE$_{pepC}$ antibody (1:5 in blocking buffer) or, as a control, preimmune serum (1:500 in blocking buffer; Fig. S4 provides justification of this dilution). Sections were washed three times for 5 min in PBS-T and then incubated for 1 h with secondary antibody (goat α-rabbit conjugated with 15 nm gold particles; 1:50 in blocking buffer; Ted Pella). Sections were washed as before and then antigen/antibody complexes were cross-linked for 15 min in 8% glutaraldehyde. Glutaraldehyde was washed out in a stream of $dH_2O$, and grids were air-dried. Samples were contrasted consecutively with uranyl acetate and lead citrate as described earlier (1). Micrographs were taken with a Jeol JEM-1400 transmission electron microscope.

**Statistical Analysis of Gold Particle Distribution.** For each treatment (thin sectioned cells immunoreacted with α-PsaE$_{pepC}$ antibodies or preimmune serum), six cells were imaged. To guarantee a random selection of cells, the cells to be imaged were selected at a magnification of 500×, a magnification at which gold particles are not clearly visible. Selection criteria were visibility of chromatophore (CR) and Golgi and absence or low abundance of intracellular silica scale (SSs). Areas representing CR, Golgi, nucleus, theca, and internal SSs were excised from the montage images by using Photoshop CS4 (Adobe); excised compartments and areas representing remaining cells were saved as separate files. Gold particles over CRs, Golgi, and the remaining cell area were separated from the background image by creating a threshhold signal by using ImageJ. To separate gold particles with overlapping outlines, we used the Watershed segmentation algorithm implemented in ImageJ. Finally, particles were automatically counted by using the Analyze Particles command in ImageJ. Gold particles over the nucleus were counted manually because electron-dense patches in the nuclei could not effectively be distinguished from gold particles by using the threshold function. Areas representing cell theca and internal SSs were excluded from analysis as a result of their dark coloration in EM images. Gold particle density over various compartments was calculated as particles per $10^6$ pixels. Mean particle densities over different compartments (CR, Golgi, remaining cell) were compared independently for all treatments by using a one-way repeated-measures ANOVA; if statistically significant differences were found, mean particle densities were compared pairwise between compartments in post-hoc Holm–Sidak tests with Sigma Plot version 12.

**Isolation of Photosystem I.** Trimeric photosystem I (PSI) was isolated from *P. chromatophora* cells in the late logarithmic phase of growth. All steps were performed at 4 °C. *P. chromatophora* cells were washed 3 × in 50 mM Tris-HCl, pH 8.0, resuspended in 2 mL 50 mM Tris-HCl, pH 8.0, and disrupted in a bead beater by using three cycles of 20 s at 5,000 rpm. Cell debris was pelleted by centrifugation at 3,000 × *g* for 4 min. Thylakoids (and probably other membranes) were pelleted from the supernatant by ultracentrifugation at 50,000 × *g* for 45 min (Ti 70 rotor; Beckmann). The membrane fraction was washed by resuspending the pellets in 50 mM Tris-HCl, pH 8.0, and pelleted again as described earlier. Finally, thylakoid membranes were resuspended in 1 mL 50 mM Tris-HCl, pH 8.0, containing 1% (wt/vol) of n-dodecyl β-D-maltoside (DM) and then subjected to gentle agitation for 2 h to solubilize the membranes. Material that was not solubilized was pelleted by centrifugation at 16,000 × *g* for 20 min, and the supernatant was loaded onto a continuous 5% to 20% sucrose gradient in 50 mM Tris-HCl, pH 8.0, containing 0.03% DM. Centrifugation was for 14 to 16 h at 140,000 × *g* (SW41 rotor; Beckman), and PSI complexes were collected from the lower green band and dialyzed two times for at least 1 h against 1 L of 50 mM Tris-HCl, pH 8.0. The dialyzed PSI samples were diluted 1:1 with 50 mM Tris-HCl, pH 8.0, 0.03% DM, concentrated by ultrafiltration in Vivaspin 20-columns MWCO 50,000 (GE Healthcare), and then loaded onto and resolved on a second sucrose gradient (the gradient and centrifugation parameters were identical to those used to initially resolve PSI particles, except that no detergent was included in the sucrose). After the second centrifugation, the pellet, which contained most of the PSI, was resuspended in 50 to 150 μL of 50 mM Tris-HCl, pH 8.0, 0.03% DM. The protein concentration was determined in triplicate by

using the BCA assay (BioRad), and 4× SDS PAGE loading buffer [150 mM Tris-HCl, pH 7.0, 12% (wt/vol) SDS, 30% (vol/vol) glycerol, 6% (vol/vol) β-mercaptoethanol, 0.05% Coomassie blue G-250 (Serva)] was added to each sample before it was flash-frozen in liquid nitrogen and stored at −80 °C until further use.

**Western Blots.** Proteins were transferred from the polyacrylamide gel to PVDF membranes (Immun-Blot; BioRad) using a semidry transfer method. Transferred polypeptides were fixed to PVDF membranes by glutaraldehyde treatment as previously described (2). Blots were blocked in 5% milk in TBS containing 0.1% Tween 20 for at least 2 h before incubation with the primary antibodies in TBS containing 0.1% Tween 20/2% milk for 60 min. Dilutions of primary antibodies used for immunoblot analyses were as follows: α-PsaC, 1:50,000; α-PsaD, 1:100,000; α-PsaE$_{pepN}$, 1:5,000; α-PsaF, 1:4,000; α-PsaL, 1:4,000; α-PsbA, 1:60,000; and α-PB, 1:10,000.

**Mass Mapping.** The sections of the polyacrylamide gel containing the polypeptides of interest (polypeptides were stained with SYPRO Ruby) were excised and incubated in 45 mM DTT/10 mM ammonium bicarbonate, pH 7.8, for 90 min at 55 °C, and then in 100 mM iodoacetamide for 60 min at room temperature in the dark. The gel sections were then washed in 10 mM ammonium bicarbonate/50% acetonitrile for 30 min, dried to completion at room temperature, rehydrated in 10 mM ammonium bicarbonate containing 1 to 5 pmol trypsin, and incubated at 37 °C overnight. Aliquots were withdrawn from the liquid phase and analyzed by MALDI-MS using reflector mode to obtain monoisotopic peptide masses, followed by MS/MS from selected peptides to obtain fragment information. A protein database was constructed containing all amino acid sequences encoded on the *P. chromatophora* chromatophore genome. This database and public protein and genomic databases were searched by using Mascot software (3).

**In Vivo Radiolabeling.** *P. chromatophora* cells were grown to midlogarithmic phase, harvested by centrifugation (300 × *g* for 2 min), washed three times in sterile Waris-H culture medium supplemented with 1.5 mM $Na_2SiO_3$ adjusted to pH 7.3 (not supplemented with soil extract), and resuspended to 5 μg/mL chlorophyll A, and 30-mL aliquots were used for the labeling experiments. For inhibition of translation, chloramphenicol (100 μg/mL) and/or cycloheximide (1 μg/mL) were used. Cells were preincubated with or without inhibitors at growth conditions for 30 min, carrier-free $NaH^{14}CO_3$ (Perkin-Elmer) was added to a final concentration of 5 μCi/mL, and the flasks containing the cultures were sealed airtight. Over a time period of 6 h, flasks were opened every 2 h for aeration and repeated addition of 5 μCi/mL $NaH^{14}CO_3$. Finally, cells were washed three times in 50 mM Tris-HCl, pH 8.0, to remove unincorporated label, labeled PSI complexes were isolated as described earlier, the polypeptides of the complex were resolved by SDS/PAGE, and radiolabel in the resolved proteins was visualized by exposing the dry gel to a low-energy/high-sensitivity storage phosphor plate (Amersham Pharmacia) and read out by using a Typhoon scanner (Amersham Biosciences).


1. Reynolds ES (1963) The use of lead citrate at high pH as an electron-opaque stain in electron microscopy. *J Cell Biol* 17:208–212.
2. Karey KP, Sirbasku DA (1989) Glutaraldehyde fixation increases retention of low molecular weight proteins (growth factors) transferred to nylon membranes for western blot analysis. *Anal Biochem* 178:255–259.
3. Perkins DN, Pappin DJC, Creasy DM, Cottrell JS (1999) Probability-based protein identification by searching sequence databases using mass spectrometry data. *Electrophoresis* 20:3551–3567.


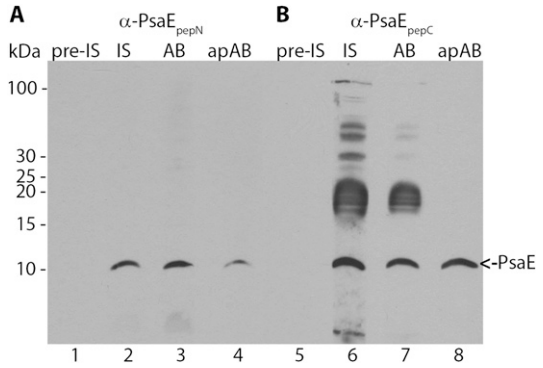


**Fig. S1.** Western blot of *P. chromatophora* total proteins with antibodies raised to (*A*) N-terminal and (*B*) C-terminal polypeptides of the PsaE protein. Proteins (25 μg total protein) from lysed *P. chromatophora* cells were resolved by SDS/PAGE on a 16% polyacrylamide Schägger gel (1). The proteins were then transferred to PVDF membranes, and Western blots were performed to identify the PsaE protein band on the gel. The lanes show the results with preimmune serum (pre-IS; lanes 1 and 5), crude immune serum (IS; lanes 2 and 6), antibodies purified from the immune serum (AB; lanes 3 and 7), and affinity-purified antibodies (apAB; lanes 4 and 8). Dilutions were as follows: preimmune and crude immune sera, 1:5,000; biochemically purified α-PsaE$_{pepN}$ and α-PsaE$_{pepC}$, 1:5,000 and 1:50,000, respectively; and affinity-purified α-PsaE$_{pepN}$ and α-PsaE$_{pepC}$, 1:100 and 1:300, respectively. Exposures: lanes 1–4, 45 s; lanes 5–8, 20 s.


1. Schägger H (2006) Tricine-SDS-PAGE. *Nat Protoc* 1:16–22.

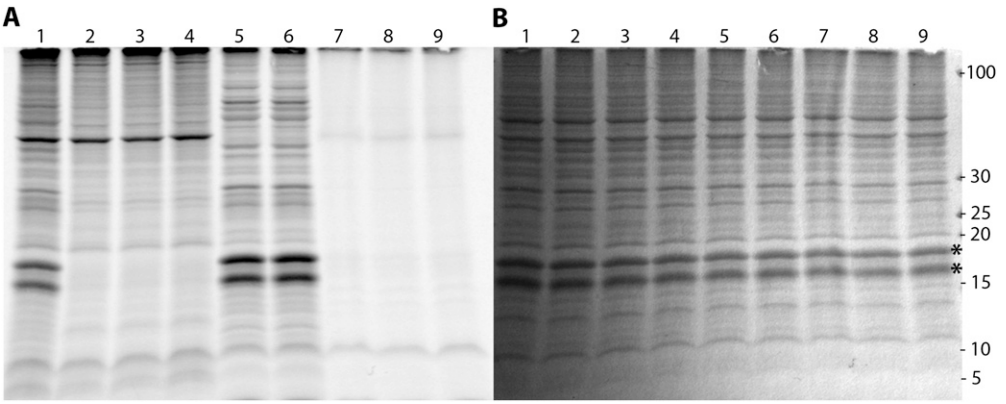


**Fig. S2.** Effects of various concentrations of chloramphenicol and cycloheximide on protein biosynthesis in the amoeba *P. chromatophora*. Newly synthesized proteins of *P. chromatophora* were radiolabeled, under standard growth conditions over a 6-h period by using 4 μCi $ml^{-1}$ of $NaH^{14}CO_3$, which was added to the cell suspension at 2-h intervals (three times over the course of the experiment), either without addition of inhibitor (lanes 1) or in the presence of chloramphenicol at concentrations of 300 μg/mL, 200 μg/mL, and 100 μg/mL (lanes 2, 3, and 4, respectively), cycloheximide at concentrations of 1 μg/mL and 0.75 μg/mL (lanes 5 and 6, respectively), and both inhibitors at concentrations of chloramphenicol/cycloheximide 300/1 μg/mL, 200/0.75 μg/mL, and 100/0.75 μg/mL (lanes 7, 8, and 9, respectively). Radioactivity was added after 30 min of preincubation with the respective inhibitors. Equal amounts of total proteins (50 μg per lane) were resolved by SDS/PAGE (Schägger buffer system) on a 16% polyacrylamide gel. The incorporated radioactivity was visualized using a PhosphorImager (*A*). The Coomassie stained gel is depicted in *B*. The addition of 300 to 100 μg/mL chloramphenicol appears to inhibit protein synthesis in the CR as indicated by a lack of incorporation of label in phycobiliproteins (CR-encoded proteins that resolve as dominant bands at 15–20 kDa; highlighted with asterisks). The addition of cycloheximide leads to a complementary inhibition pattern. The simultaneous addition of both inhibitors blocks incorporation of nearly all radioactivity into protein. Based on these results we assume that chloramphenicol leads to the inhibition of 70S ribosomes and cycloheximide to the inhibition of 80S ribosomes in *P. chromatophora* as has been described for numerous other organisms.

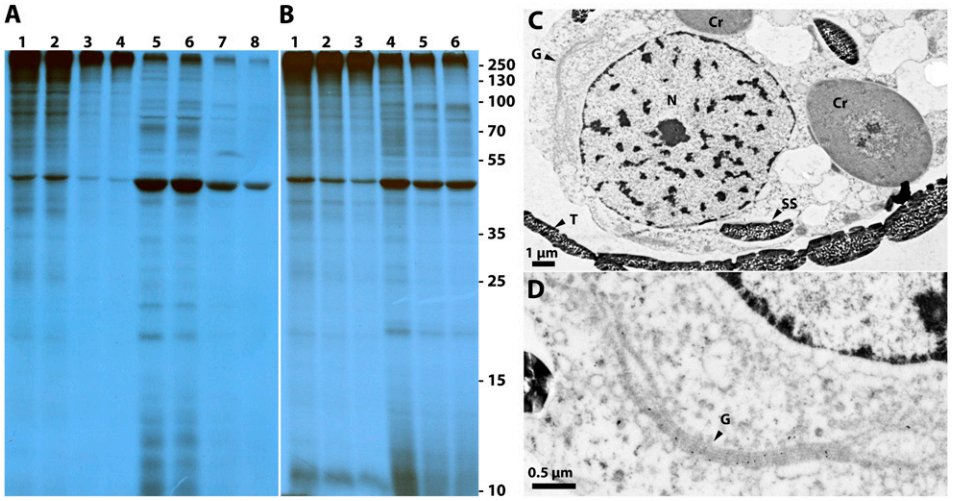


**Fig. S3.** Effects of Brefeldin A (BFA) on *P. chromatophora* cells. (*A* and *B*) Impact of BFA on cytoplasmic protein synthesis. Cytoplasmically synthesized proteins of *P. chromatophora* were radiolabeled under standard growth conditions over a 12-h period by using 10 μCi/mL $Na_2{}^{35}SO_4$; labeling was in the presence of chloramphenicol and various concentration of BFA. BFA was added every 3 h (concentrations given later) from a 1-mg/mL stock in methanol. Proteins were extracted, separated into soluble and insoluble fractions and 50 μg protein per lane was resolved by SDS/PAGE (Laemmli buffer system) on 11% polyacrylamide gels. Incorporation of radioactivity was visualized by exposure of the dry gel to X-ray film. (*A*) Impact of high concentrations of BFA on protein synthesis. Lanes 1 to 4, insoluble proteins; lanes 5 to 8, soluble proteins. Lanes 1 and 5, no BFA; lanes 2 and 6, mock treatment with only methanol at a volume corresponding to the highest BFA concentration added; lanes 3 and 7, 5 μg $mL^{-1}$ 3 $h^{-1}$ BFA; lanes 4 and 8, 10 μg $mL^{-1}$ 3 $h^{-1}$ BFA. (*B*) Impact of low concentrations of BFA on protein synthesis. Lanes 1 to 3, insoluble proteins; lanes 4 to 6, soluble proteins. Lanes 1 and 4, no BFA; lanes 2 and 5, 0.25 μg $mL^{-1}$ 3 $h^{-1}$ BFA; lanes 3 and 6, 1 μg $ml^{-1}$ 3 $h^{-1}$ BFA. Note, even at 0.25 μg $mL^{-1}$ 3 $h^{-1}$ BFA, there was visibly inhibition of overall translation (based on incorporation of $^{35}SO_4$ into protein). (*C* and *D*) Impact of BFA on Golgi morphology. *P. chromatophora* cells were incubated for 1 h under standard conditions but in the presence of 0.5 μg/mL BFA. Following the BFA treatment, cells were fixed, embedded, sectioned, and analyzed by Immunogold EM using α-$PsaE_{pepC}$ and α-rabbit-IgG-15 nm gold. (*C*) Micrograph of sectioned, BFA-treated cell. (*D*) Close-up of the Golgi in *C*. Note that the Golgi morphology and PsaE distribution (visible as crisp black gold particles) appear to be undisturbed (compare with Fig. 4, which shows Golgi morphology in an untreated cell).

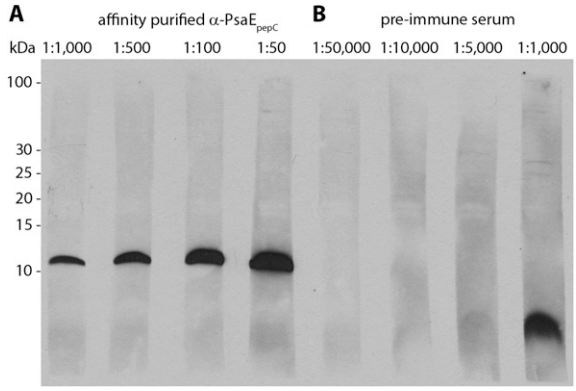


**Fig. S4.** Background signals resulting from various concentrations of (*A*) affinity purified α-PsaE$_{pepC}$ and (*B*) various concentrations of the corresponding preimmune serum. Immunoblot of a 16% polyacrylamide Schägger gel with 25 μg *P. chromatophora* total protein extract loaded per lane, using affinity purified α-PsaE$_{pepC}$ at dilutions of 1:1,000 to 1:50; the corresponding preimmune serum was used at dilutions of 1:50,000 to 1:1,000. The blot was exposed to X-ray film for 15 min (which resulted in overexposure of the film) to visualize background signals. Note that background signals of 1:50 and 1:100 dilutions of the affinity purified α-PsaE$_{pepC}$ correspond approximately to 1:5,000 and 1:10,000 diluted preimmune serum, respectively.

**Table S1. Mass mapping results for the presumed PsaA/PsaB band that was excised from a polyacrylamide gel**

| | | Difference | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Calculated mass | Observed mass | In Da | In ppm | Start seq. | End seq. | Sequence | Ion score | CI, % | Modification |
| Rank: 1* | | | | | | | | | |
| 1,126.6367 | 1,126.6135 | −0.0232 | −21 | 688 | 697 | TPLANLVGWR | — | — | — |
| 1,126.6367 | 1,126.6135 | −0.0232 | −21 | 688 | 697 | TPLANLVGWR | 8 | 98.44 | |
| 1,151.6360 | 1,151.6105 | −0.0255 | −22 | 161 | 169 | FRPSLAWFK | — | — | — |
| 1,474.6056 | 1,474.5707 | −0.0349 | −24 | 555 | 567 | DFGYSFPCDGPGR | 16 | 99.76 | Carbamidomethyl (C) [8] |
| 1,474.6056 | 1,474.5707 | −0.0349 | −24 | 555 | 567 | DFGYSFPCDGPGR | — | — | — |
| 1,618.7820 | 1,618.7744 | −0.0076 | −5 | 400 | 413 | DYDPEANKNNVLAR | — | — | — |
| 1,651.8075 | 1,651.7743 | −0.0332 | −20 | 5 | 19 | FPSFSQGLAQDPTTR | — | — | — |
| 1,807.9086 | 1,807.8596 | −0.0490 | −27 | 5 | 20 | FPSFSQGLAQDPTTRR | — | — | — |
| 1,951.9872 | 1,951.9381 | −0.0491 | −25 | 2 | 19 | ATKFPSFSQGLAQDPTTR | 62 | 100 | |
| 1,951.9872 | 1,951.9381 | −0.0491 | −25 | 2 | 19 | ATKFPSFSQGLAQDPTTR | — | — | — |
| Rank: 2† | | | | | | | | | |
| 1,229.7001 | 1,229.6761 | −0.0240 | −20 | 577 | 587 | LVPDKANLGFR | — | — | — |
| 1,563.7484 | 1,563.7117 | −0.0367 | −23 | 582 | 595 | ANLGFRFPCDGPGR | — | — | Carbamidomethyl (C) [9] |
| 2,009.0239 | 2,008.9591 | −0.0648 | −32 | 19 | 36 | NPVPVDFDVLGKPGHFDR | — | — | — |
| 2,009.0239 | 2,008.9591 | −0.0648 | −32 | 19 | 36 | NPVPVDFDVLGKPGHFDR | — | — | — |
| 2,578.3162 | 2,578.2617 | −0.0545 | −21 | 14 | 36 | AQVDRNPVPVDFDVLGKPGHFDR | — | — | — |
| 2,578.3162 | 2,578.2617 | −0.0545 | −21 | 14 | 36 | AQVDRNPVPVDFDVLGKPGHFDR | 16 | 99.78 | — |

Mapping the observed spectra against a database containing sequences of all proteins encoded on the CR genome using Mascot software (with a cutoff of 40 ppm in difference between observed and calculated peptide mass) yielded the proteins PsaB and PsaA, which were ranked 1 and 2, respectively. For both proteins, the calculated mass, the observed mass, and difference between these values in Daltons and in parts per million are listed for each peptide observed (columns 1–4); the beginning and end of the peptide relative to the complete protein sequence and the amino acid sequence are also listed (columns 5–7). Furthermore ion scores, percent confidence intervals (CIs) and modifications, if any, are provided (columns 8–10). (Fig. 2*A* provides further information).
*Protein name, PsaB; protein molecular weight, 81933.4; peptide count, 7; protein score, 100; total ion score, 86; total ion CI, 100%.
†Protein name, PsaA; protein molecular weight, 83570.8; peptide count, 4; protein score, 24; total ion score, 16; total ion CI, 99.78%.