Biochimica et Biophysica Acta, 681 (1982) 440–450
Elsevier Biomedical Press

BBA 41137

PROTEIN COMPOSITION AND ARCHITECTURE OF THE PHOTOSYNTHETIC MEMBRANES FROM THE CYANOBACTERIUM, *ANACYSTIS NIDULANS* R2

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(Received January 4th, 1982)
(Revised manuscript received April 23rd, 1982)

Key words: Photosynthetic membrane; Membrane protein; Bacterial photosynthesis; (A. nidulans)

The protein composition of the photosynthetic membrane from the cyanobacterium, *Anacystis nidulans* R2, was analyzed by acrylamide gel electrophoresis following solubilization with lithium dodecyl sulfate. Autoradiograms of ^{35}S-labelled membranes revealed over 90 bands by this procedure. The effect of solubilization conditions on protein resolution was analyzed by modifying temperature and sulfhydryl concentrations. Labelling cells with ^{59}Fe yielded nine iron-containing bands on these gels. Three of these bands, at 33, 19, and 14 kDa, were also heme proteins as determined by tetramethylbenzidine staining, and represent cytochromes *f*, b_6 and *c*-552, respectively. The remaining iron proteins are highly sensitive to solubilization conditions, especially the presence of 2-mercaptoethanol, and we suggest that these bands may be Fe-S proteins. Lactoperoxidase-catalyzed iodination of the membranes indicated that at least 41 proteins have surface-exposed domains. Some of the known proteins with external surfaces include cytochrome *c*-552 and the chlorophyll-binding proteins of Photosystems I and II. Neither cytochrome *f* nor b_6 appear to be accessible to external labelling. When this structural information was combined with the isolation of functional submembrane complexes, we constructed a topological model of the membrane. Using this model we have discussed the protein architecture of the cyanobacterial membrane.

Introduction

The functioning of energy-transducing membranes depends upon a precise structural arrangement of the various components. Thus, a great deal of effort has gone into analyzing the composition and organization of the proteins within these membranes. The ultimate goal of these studies is to detect all of the membrane proteins, describe how they are arranged into functional complexes, and to understand their topology with respect to the lipid bilayer. A topological model of certain functional complexes in the chloroplast membrane has been proposed recently by Von Wettstein [1] based on work from many laboratories [2–10]. It is the aim of the present study to construct a related model for the photosynthetic lamellae of the cyanobacterium *Anacystis nidulans* R2.

We are using this organism for a wide range of experiments aimed at understanding the photosynthetic mechanism. *A. nidulans* R2 is ideal for mutant [11–13] and molecular genetic studies because it contains plasmids and can be transformed with high frequency by exogenous DNA [14,15]. Therefore, we have extended our previous work that was performed on the nontransformable

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Abbreviations: PS, photosystem; DCIP, 2,6-dichlorophenolindophenol; DCMU, 3-(3,4-dichlorophenyl)-1,1-dimethylurea; CP, chlorophyll-protein complex; LDS, lithium dodecyl sulfate.

cyanobacterium *Synechococcus cedrorum* [16]. We report the development of a high-resolution gel system capable of detecting over 90 membrane proteins. This gel system was used instead of a two-dimensional system because it permits analysis of intact chlorophyll-protein complexes; in addition, heme-catalyzed peroxidase activity of intact cytochromes can be detected on this gel system by treating the gel with H_2O_2 and tetramethylbenzidine [17]. Three different radioactive labelling procedures were used to identify specific proteins and to determine functional and topological relationships. Labelling with ^{35}S provides an estimate of the total protein composition. The second technique, lactoperoxidase-mediated iodination, labels only those proteins having susceptible residues (e.g., tyrosines) exposed on the membrane surface [18]. Finally, incorporation of ^{59}Fe into the cell allows an analysis of the iron-containing membrane proteins. By combining these techniques with the isolation of submembrane complexes, we can relate the structural entities to specific functions. Of the 90 membrane proteins detectable by our gel electrophoresis procedure, over 40 appear to possess surface-exposed domains and nine contain iron. By correlating these bands with the heme-staining procedure [17], we conclude that three of these iron-containing proteins are cytochromes; the remainder have properties suggestive of Fe-S proteins. Finally, we have combined this information into a tentative topological model that is comparable to that of the chloroplast membrane [1].

Materials and Methods

Cell growth and membrane preparation

A. nidulans strain R2 was grown axenically in BG-11 medium as previously described [13] and was routinely monitored for bacterial contamination both microscopically and by growth on nutrient agar. This strain of *A. nidulans* was originally characterized by Grigorieva and Shestakov [19] and was kindly provided by G. van Arkel, University of Utrecht. Photosynthetic membranes were isolated by the procedure of Guikema and Sherman [17] and resuspended in a buffer containing 20 mM Tricine, 0.5 M mannitol, and 5 mM $NaKHPO_4$, pH 7.5. Toluenesulfonyl fluoride (Sigma) was added to this buffer at a concentration of 20 μg/ml to inhibit proteases.

Radioactive labelling and trypsin treatment

Membrane proteins were labelled with ^{35}S by resuspending logarithmically growing cells in BG-11 medium that contained one-tenth the normal quantity of sulfate. $Na_2^{35}SO_4$ (New England Nuclear) was added to a final concentration of 50–100 μCi/ml and labelling proceeded for 12 h. Labelling with [^{59}Fe]ferrous citrate (New England Nuclear) was performed in a similar fashion. Cells were grown for at least 20 generations in a medium that lacked exogenous iron, and were resuspended in the low-iron medium. The cells were concentrated and were grown in the presence of 4 μCi/ml ^{59}Fe for 20 h; labelled membranes were then prepared as above.

Lactoperoxidase-catalyzed iodination of tyrosine and other amino acids in membrane proteins was achieved according to modifications of the method of Hubbard and Cohn [20]. Membranes were resuspended in 25 mM Tricine, pH 7.2 (with 20 μg/ml protease inhibitor), at a final concentration of 2 mg/ml protein and 100 μg/ml chlorophyll; this protein-to-chlorophyll ratio of 20:1 is typical for *A. nidulans* membranes grown in BG-11 medium. The iodination mixture contained the following components: 5 mM glucose, 7.2 munits/ml glucose oxidase, 3.6 munits/ml lactoperoxidase, and 50 μCi/ml $Na^{125}I$ (New England Nuclear). Samples were incubated for 30 min at 20°C, washed three times with buffer containing 50 mM $Na^{127}I$, and resuspended in one-half vol. of 25 mM Tricine. Lactoperoxidase controls were performed as previously described [16]. Under the conditions detailed above, the omission of lactoperoxidase resulted in incorporation of less than 1% of the ^{125}I level obtained in the presence of the enzyme.

For the trypsin experiments, the labelled membranes were treated with 1 μg trypsin/ml for 1, 2 and 10 min; the trypsin-to-protein ratio was thus 1:2000. The protease reaction was stopped by the addition of 100 μg/ml trypsin inhibitor (Sigma) and washed as above.

Electrophoresis and autoradiography

Acrylamide gel electrophoresis of the mem-

brane proteins was performed by the LDS procedure discussed previously [3,17]. The key variables that must be considered in order to obtain the resolution described in this work include: (i) Solubilization conditions. Membranes were resuspended to 4 mg of protein/ml in 10 mM Tricine plus 6% sucrose. LDS (Gallard-Schlesinger) was added to a final concentration of 1% and, in most experiments, either 30 mM dithiothreitol or 6.0% 2-mercaptoethanol was also included. The samples were either held on ice or were heated to 70°C for 10 min in a water bath; (ii) Electrophoresis conditions. Gels were run at constant power (2.5–2.75 W using an E-C Corp. power supply) for 16 h in the cold (approx. 4°C); (iii) Acrylamide concentration. All the gels shown below contained acrylamide gradients of 10–20%, although for specific purposes other concentration gradients were used. (iv) Gel length. The resolution was also improved by utilizing 15-cm gels.

The best resolution was obtained when samples containing about 80 μg protein were loaded onto the gel. In most experiments, this corresponded to approx. 400000 cpm ^{125}I and ^{35}S or 40000 cpm ^{59}Fe. The molecular masses of the proteins were obtained by coelectrophoresis of the following unlabelled and ^{14}C-labelled (Bethesda Research Labs) standard proteins with each gel: myosin (200 kDa), phosphorylase *a* (97 kDa), bovine serum albumin (67 kDa), ovalbumin (45 kDa), chymotrypsinogen (25 kDa) and cytochrome *c* (12 kDa). Despite the high-resolution system, it was not always possible to correlate a band on a ^{125}I or ^{59}Fe gel to a precise band on the ^{35}S gel. When such ambiguities existed, the two closest bands were considered as one. Thus, band 67/68 implies that we have not yet determined whether the iron-containing band in Fig. 3 is band 67 or 68.

After electrophoresis, the gels containing ^{35}S or ^{125}I were first stained for heme and then for protein [17] and prepared for autoradiography [16]. However the gels containing ^{59}Fe were treated somewhat differently. The samples were loaded so that each half of the gel contained identical patterns. One-half of the gel was treated as above, but the other half was immediately dried and used for autoradiography. This was mandatory, since gels which were pretreated by staining or destaining retained only ^{59}Fe which comigrated with cytochrome bands. Presumptive Fe-S bands, however, were labile to the acidic pretreatment. Exposures were typically 2–7 days for either ^{35}S- or ^{125}I-labelled proteins, but were as long as 6 weeks with ^{59}Fe gels.

Preparation of submembrane fractions and assays

Digitonin-fractionated sub-membrane particles were prepared as described previously (21; see also 16, 17). Photosystem assays were performed according to Guikema and Sherman [13]. Protein was analyzed by the method of Lowry et al. [22] and chlorophyll by the procedure of Arnon [23].

Chloroform/methanol extraction

Membranes were lipid extracted by treating with 10 vol. of a 2:1 (v/v) mixture of chloroform/methanol. After vortex mixing and centrifugation, the pellet was dried under air and resuspended in sample buffer.

Results

Gel electrophoretic patterns of A. nidulans membranes

An autoradiogram of ^{35}S-labelled proteins from *A. nidulans* R2 membranes is shown in Fig. 1. This gel portrays three different types of solubilization: lanes a and b depict membranes solubilized at 0 and 70°C, respectively, whereas lane c represents membranes solubilized at 70°C after chloroform/methanol extraction. The solubilization conditions of Fig. 1b were optimal for membrane disruption and therefore were taken as standard. Protein bands were numbered using Fig. 1b as the prototype, since 70°C solubilization generates monomeric protein species. A total of 92 bands can be detected on this gel and they are numbered consecutively from high to low molecular mass; the band numbers of some of the major proteins are listed on the right of the figure.

The effect of solubilization temperature can be seen by comparing Fig. 1a and b. Solubilization at 0°C enables detection of the chlorophyll-protein complexes [3,17]. These complexes cannot migrate far into a 10–20% gradient gel and these high molecular mass complexes are seen above band 1 in Fig. 1a. Heating the membranes at 70°C results in the breakdown of these complexes and resultant

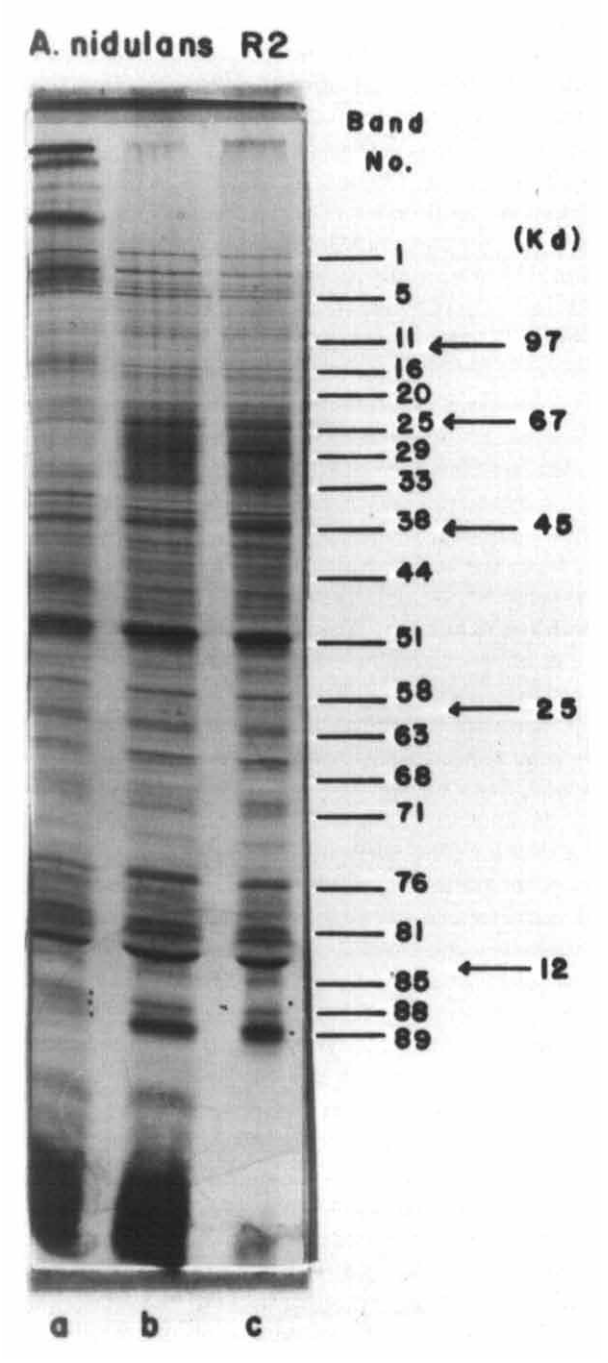

Fig. 1. Autoradiogram of ^{35}S-labelled membranes from *A. nidulans* R2. (a) Solubilized at 0°C; (b) solubilized at 70°C; (c) treated with chloroform/methanol and subsequently solubilized at 70°C. The band numbers and the molecular masses of the standard proteins are shown on the right (Kd, kDa). The acrylamide gel electrophoresis conditions for this and the following figures are stated in Materials and Methods.

quantitative changes in some of the other bands. In particular, bands 25, 29 (and other faint bands in this region), 64, 65, 68, 75, 88 and 89 increase in intensity after mild heating. One straightforward conclusion of these results would be that these proteins are part of the chlorophyll-protein complexes. Bands 88 and 89 have a high ^{35}S composition and most likely represent a large proportion of the radioactivity seen in the high molecular mass complexes in Fig. 1a. Some of the bands that change in intensity upon heating are not structural components of the chlorophyll-protein complexes. We first saw this phenomenon in regard to cytochrome *f* [17]. Using tetramethylbenzidine to localize heme-containing proteins, we discovered that cytochrome *f* (but not the other cytochromes) shifted from a molecular mass of 28 to 33 kDa after solubilization at 0 and 70°C, respectively. In this gel system, the above change corresponds to a shift from band 56 (0°C) to band 49 (70°C).

Membrane extraction with chloroform/methanol has little effect on the observed 70°C-solubilized protein pattern. Comparison of lanes b and c in Fig. 1 indicates that the main difference brought about by the extraction is the removal of the low molecular mass components migrating faster than band 89. Most of this material is near the dye front and might be expected to contain lipid. We have also found that chloroform/methanol treatment followed by solubilization at 0°C results in the same shift in cytochrome *f* discussed above (data not shown). It would appear, therefore, that either mild heating or chloroform/methanol treatment results in the removal of lipid from cytochrome *f*; the cytochrome can then migrate according to the size of the polypeptide alone. Based on these results, we conclude that solubilization at 70°C for 10 min yields patterns representative of uncomplexed, monomeric proteins.

Fig. 2 represents an autoradiogram (AR) and a protein stained profile (CB) of identical lanes on a single gel and shows two comparisons: (i) the protein pattern obtained by autoradiography vs. Coomassie blue staining, and (ii) solubilization at three temperatures (0, 70 and 100°C). It is obvious that there are major qualitative and quantitative differences between the ^{35}S label and Coomassie blue staining. For example, only about 70 bands are readily detectable on the stained gel instead of

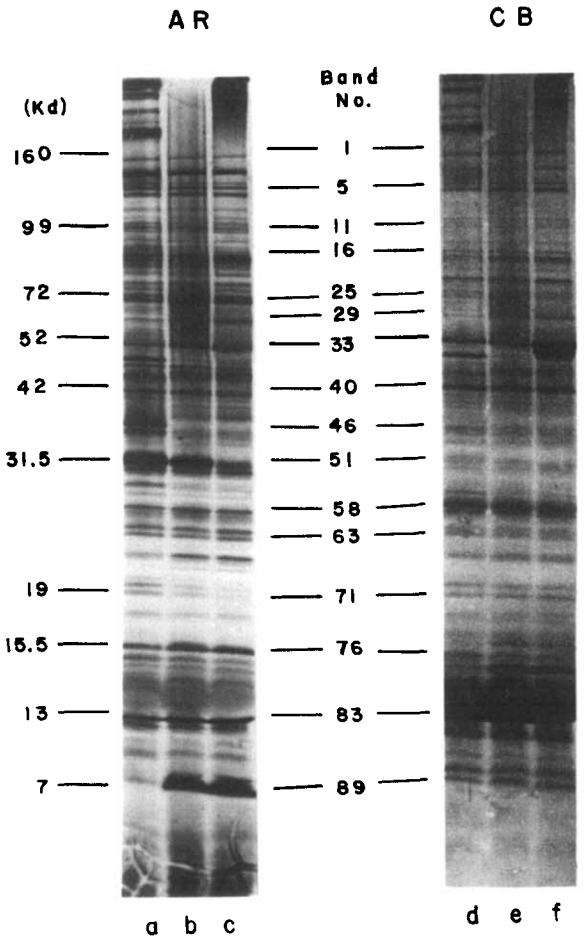

Fig. 2. Acrylamide gel pattern of *A. nidulans* membranes, comparing the ^{35}S-labelled bands (AR) to the bands obtained after Coomassie blue staining (CB). Solubilization temperatures for these samples were: lanes a and d, 0°C; lanes b and e, 70°C; lanes c and f, 100°C. The molecular masses of some membrane proteins are listed on the left (Kd, kDa). The heavy band that comigrates with band 83 is lysozyme which binds to the membranes in some preparations.

the 90 seen by autoradiography. Some of the proteins show much greater intensity after ^{35}S incorporation than after staining. These include bands 16, 25, 38, 51, 67, 75, 76, 88 and 89. The opposite relationship is also obtained. Thus, bands 40, 58 and 79/80 show relatively greater intensity after Coomassie blue staining than with ^{35}S.

The second experiment depicted in Fig. 2 again concerns solubilization temperature. In addition to 0 and 70°C, the samples were also prepared by boiling for 2 min (Fig. 2c and f). The same general relationship exists that was discussed for Fig. 1, but there are two additional points to be made. First, boiling produces a major artefact in the 50–55 kDa range (bands 33–35). A large band appears in the Coomassie blue pattern that is probably composed of two or three species. This is also seen in the autoradiogram as a smearing of band 33. Secondly, band 51, with a molecular mass of 31.5 kDa appears to be highly temperature labile. This band represents a major ^{35}S-labelled region after 0°C solubilization; however, the intensity decreases significantly after boiling. The same pattern can be detected after staining, but with much less clarity (Fig. 2d–f).

Iron-containing proteins

In order to detect the position of iron-containing proteins on acrylamide gels, cells were grown in the presence of [^{59}Fe]ferrous citrate. An autoradiogram prepared as described in Materials and Methods is shown in Fig. 3. The four lanes in this gel correlate solubilization temperature and the presence and absence of thiols during solubilization. Lane a shows ^{59}Fe incorporation into bands 56, 71 and 81; these three bands are also stained with tetramethylbenzidine and represent cytochromes *f*, b_6 and *c*-552, respectively [17]. Bands 71 and 81 are affected only slightly by changes in solubilization conditions (cf. Fig. 3a–d); this is the same response we reported previously with tetramethylbenzidine staining for heme [17]. Mild heating, however, converts the ^{59}Fe at band 56 stoichiometrically to migrate at band 49; once again this is consistent with our previous observations [17]. At a specific temperature, this protein (cytochrome *f*) is relatively insensitive to the presence or absence of thiols during solubilization.

There are several other positions on the gel where ^{59}Fe incorporation can be detected. In the presence of 2-mercaptoethanol (Fig. 3a), these include the chlorophyll-protein complexes, a band at 140 kDa, and band 89. The 140 kDa protein does not correspond to any specific band on our standard gel (Fig. 1). However, it does appear to comigrate with a faint band seen below band 2 in Fig. 2. Therefore, we designate this iron-containing species as band 2a. This species is somewhat sensitive to thiols in that the intensity is greater in both

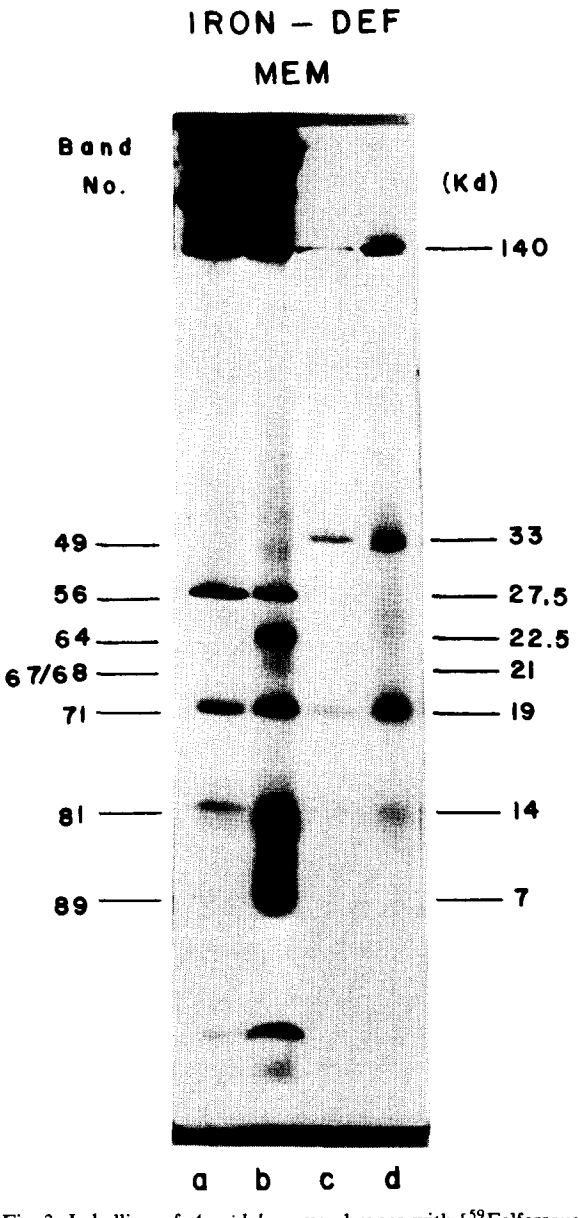

Fig. 3. Labelling of *A. nidulans* membranes with [^{59}Fe]ferrous citrate. Solubilization conditions were as follows: (a) 0°C plus 2-mercaptoethanol; (b) 0°C minus 2-mercaptoethanol; (c) 70°C plus 2-mercaptoethanol; (d) 70°C minus 2-mercaptoethanol.

Fig. 3b and d. In addition, the apparent molecular mass of this band varies depending upon the acrylamide concentration of the gel (data not shown). The iron incorporation at band 89 is sensitive to both temperature and thiols; the greatest intensity is seen after solubilization at 0°C without 2-mercaptoethanol (lane b).

Two other iron-containing species (bands 64 and 67/68) demonstrate their strongest intensities in lane b. These proteins are most sensitive to the presence of thiols (lanes a and c), and are somewhat sensitive to temperature (cf. lanes b and d). In addition, there are two other iron-containing proteins which appear primarily in the membrane supernatant and which comigrate with bands 85 and 88. The solubilization properties of these proteins are identical to those of bands 64, 67/68 and band 89. We consider it likely that all five bands belong to the same class of proteins. Therefore, we summarize the results of fig. 3 by placing the proteins into two discrete classes (Table I). The significance of this classification will be discussed below.

Disruption of ^{59}Fe-labelled chlorophyll-protein complex organization by extracting membranes with chloroform/methanol leads to an additional observation. Lane c of Fig. 1 shows such an extraction using ^{35}S-labelled membranes. Notice that radioactive material with mobility greater than that of band 90 was largely removed. An identical treatment of ^{59}Fe-labeled membranes, however, followed by solubilization at 0°C, yields an intensely labelled band at approx. 2.5 kDa (data not shown). This band is lost if these membranes are solubilized at 70°C after extraction.

Protein composition of photosystem complexes

We have reported the isolation of particles from cyanobacteria that are highly enriched in PS I and PS II activity [24]. This procedure utilized digitonin solubilization, sucrose density gradient centrifugation and DEAE-cellulose chromatography. The protein pattern of these particles depicted on the current gel system is summarized in Table II. These proteins represent the species most often seen with the active photosystems, though other proteins sometimes appeared.

Detection of membrane proteins with surface-exposed domains

The technique of lactoperoxidase-catalyzed iodination of external tyrosines was used to determine which proteins possessed surface-exposed domains. Fig. 4 is an autoradiogram that compares the iodinatable proteins with those proteins

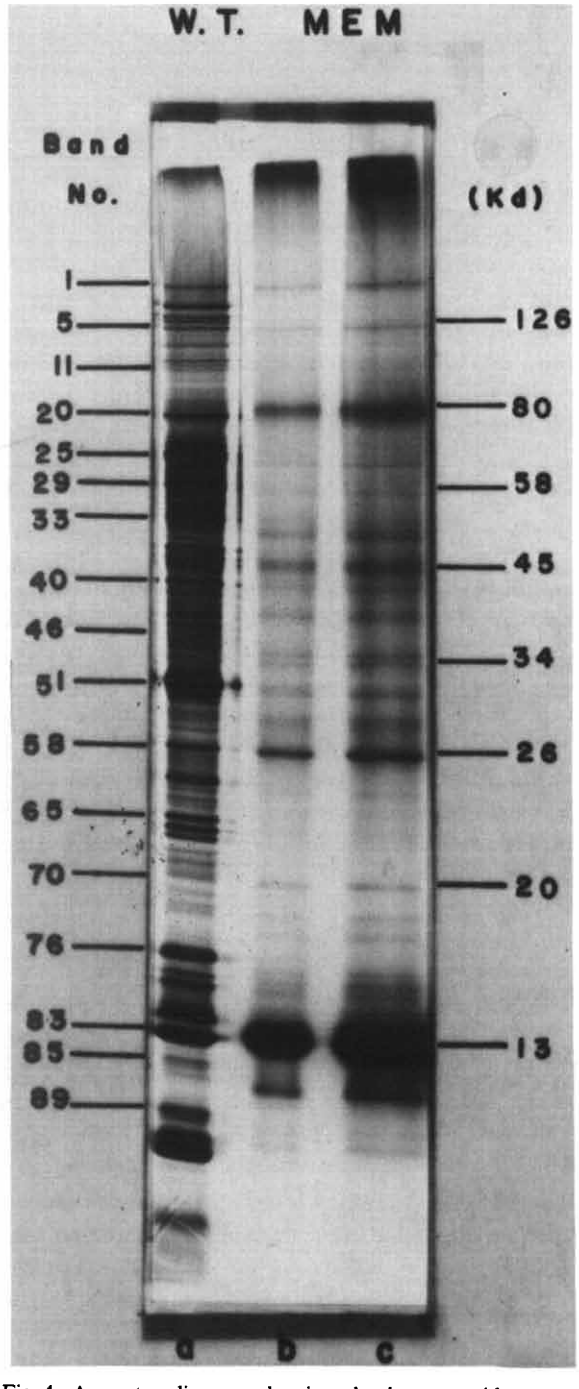

Fig. 4. An autoradiogram showing the lactoperoxidase-catalyzed iodination of *A. nidulans* membranes. (a) ^{35}S-labelled membranes run as a standard to indicate the relationship of the ^{125}I-labelled bands to the full complement of membrane proteins. The sample contained $9.5 \cdot 10^5$ cpm of labelled membranes. (b and c) ^{125}I-labelled membranes loaded with $4.2 \cdot 10^5$ and $5.8 \cdot 10^5$ cpm ^{125}I, respectively.

labelled with ^{35}S. A total of 41 bands are visible in lanes b and c, indicating that approx. 45% of the proteins have external domains (e.g., exposed tyrosines). The most intense label is seen at bands 18/19, 58 and 83, whereas the intensity of the remainder of the bands is similar. A rough estimate of the relative intensity of each band is given in Table III.

The ^{125}I-labelled protein pattern obtained using our previous SDS gel system [16] is shown in Fig. 5a. A comparison of Fig. 5a with our present results (Fig. 4b) documents the enhanced resolution obtained here. Fig. 5b and c shows the effect

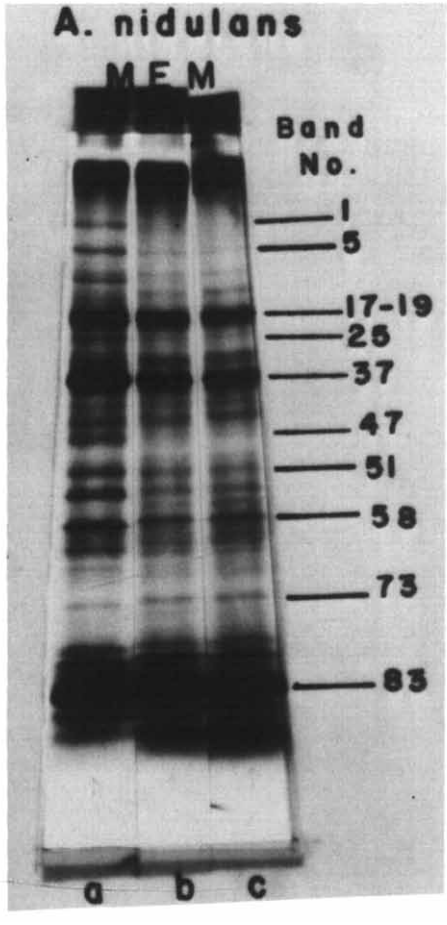

Fig. 5. Effect of trypsin on the lactoperoxidase-catalyzed iodination of *A. nidulans* membranes. Acrylamide gels were run according to the procedure of Sadewasser and Sherman [16]. (a) Control, no trypsin; (b and c) treatment with 1 μg/ml trypsin for 2 and 10 min, respectively. All lanes contain 80 μg protein and approx. 400000 cpm ^{125}I.

TABLE I

IRON-CONTAINING PROTEINS

	Band No.	Molecular mass (kDa)	Identity
Cytochromes	49 (at 70°C)	33	cytochrome *f*
	56 (at 0°C)	27.5	cytochrome *f*
	71	19.0	cytochrome b_6
	81	14.0	cytochrome *c*-552
Iron-sulfur proteins			
	2A	140	–
	64	22.5	[a]
	67/68	20.5	[a]
	85	11.5	ferredoxin soluble
	88	≤10.5	Fe-S soluble
	89	≤10.0	Fe-S bound

[a] Appear only in the absence of 2-mercaptoethanol

of brief trypsin treatment on the ^{125}I-labelling pattern. Incubation with 1 μg trypsin/ml for 2 and 10 min (Fig. 5b and c, respectively) immediately degrades certain proteins completely (e.g., bands 1, 11 and 47) and most of the other external proteins to some extent. A summary of the effect of trypsin treatment for 10 min is given in Table III, where the proteins are categorized as disappearing entirely or diminished by the treatment. It is obvious that most of the ^{125}I-labelled proteins are susceptible to this brief trypsin treatment, another indication that we are monitoring proteins with external domains. Those unaffected by trypsin may not possess external lysine and arginine side chains or these amino acids may be sterically shielded from the enzyme during this mild treatment.

TABLE II

PS I AND PS II PROTEINS

Photosystem particles were isolated by the digitonin procedure of Newman and Sherman [21]. PS II activity was measured as the photoreduction of ferricyanide with semicarbizide as donor. This preparation had an activity of 650 μmol/mg chlorophyll per h and 75% of this activity was sensitive to 50 μM DCMU. PS I activity was determined by monitoring O_2 consumed during light-driven electron transport from DCIP-H_2 to methyl viologen and the PS I particles had an activity of 380 μmol O_2 consumed/mg chlorophyll per h. The polypeptide composition was analyzed by the techniques described in Materials and Methods.

PS I		PS II	
Band No	Molecular mass (kDa)	Band No.	Molecular mass (kDa)
25	64	21	70
		36	48
70	20	38	45
75/76	15.5	40	42
85	11.5	47	35
89	≤10	72	18.5
		73	18

Discussion

In this communication we have described an acrylamide gel system capable of resolving over 90 proteins from the cyanobacterial photosynthetic membrane. By combining this technique with specific radioactive labelling, we have determined which proteins contain iron and which are exposed to the external membrane surface. Furthermore, by isolating functional submembrane complexes such as PS I and PS II and chlorophyll-protein complexes, we are now in a position to construct a tentative, topological model of the photosynthetic membrane (Fig. 6). We can only extend the structure-function relationship to approx. 25 proteins at present, or for about one-quarter of the detectable species. Nonetheless, the model presented in Fig. 6 represents a summary of part of the results described above and is a good point of departure for future research.

TABLE III

PROTEINS LABELLED BY LACTOPEROXIDASE-CATALYZED IODINATION

Lactoperoxidase-catalyzed iodination was performed as described in Materials and Methods. ^{125}I-labelled species were compared to the ^{35}S-labelled proteins as shown in Fig. 4 to obtain the protein band number. The protein identity is based on the results discussed in the text. The trypsin results were based on three experiments similar to those shown in Fig. 5. The keys for the two experiments are as follows: lactoperoxidase iodination (LPO): (+) light, (++) moderate, and (+++) heavy labelling; trypsin treatment: (x) band diminished by treatment; (xx) band disappeared entirely after treatment.

Protein band No.	Protein identity	LPO	Trypsin	Protein band No.	Protein identity	LPO	Trypsin
1		++	xx	51	PS II	++	x
5		++	x	54		++	x
7		+	xx	55		+	x
9		+		57		+	
10		+		58		+++	x
11		+	xx	61		+	x
14		+	xx	64	Fe-S	+	x
18/19		+++	x	65		+	
25	PS I (CP I–V)	++	x	70	PS I	++	x
29		+	x	73	PS II	++	x
33		+	xx	74	CP I, II, III	++	x
34		+		77		++	
35		++		78		++	
36	PS II (CP VI)	+		79	CP I, II, III	++	
38	PS II (CP VI)	++	x	81	cytochrome *c*-552	++	xx
40	PS II	+	x	82		++	xx
41		+		83		+++	
42		+		85	PS I (Fe-S)	++	xx
43		+	x	87		+	x
47	PS II	++	xx	89	PS I (Fe-S)	+	x
48		++	xx				

The most conclusive evidence concerns the identification and localization of the cytochromes. Heme staining with tetramethylbenzidine [17] and ^{59}Fe incorporation indicate that after 70°C solubilization, band 49 is cytochrome *f* (33 kDa), band 71 is cytochrome b_6 (19 kDa) and band 81 is cytochrome *c*-552 (14 kDa). Lactoperoxidase iodination of the membranes failed to label either cytochrome *f* or b_6 and we place these cytochromes within the membrane bilayer. However, band 81 is heavily labelled by ^{125}I, is trypsin digestible and is thus an external protein. Since cytochrome *c*-552 functionally replaces plastocyanin, this external localization is appropriate.

Our evidence concerning the identification of certain bands as iron-sulfur proteins is much more speculative. Our contention is based on the fact that the bands contain iron and possess a relatively high level of ^{35}S. In addition, the bands appear with much greater intensity in the absence of 2-mercaptoethanol. Though a firm designation cannot be made without additional evidence, it would appear that such a classification makes a valuable working hypothesis. The three lower molecular mass iron proteins are involved with PS I function, though their solubility properties differ. Band 85 (which comigrates with spinach ferredoxin) is membrane-bound but labelled with ^{125}I. Thus, it appears to be an external protein, and at times it can be found in the membrane supernatant. Band 88 is always found in the supernatant, while band 89 is always membrane bound and externally located. The topological relationship of these proteins is indicated in Fig. 6.

Bands 64 and 67/68 contain iron on the gels only when 2-mercaptoethanol is absent from the

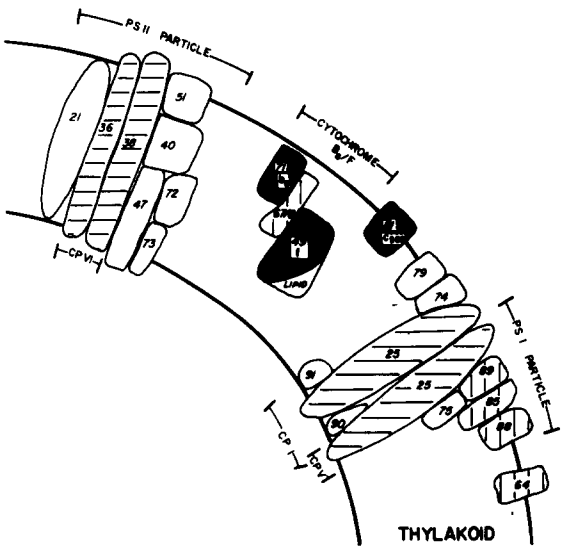

Fig. 6. A diagrammatic representation of the photosynthetic membrane of *A. nidulans*. This model depicts the topological relationship of the proteins to the lipid bilayer; those proteins protruding from the membrane were found to have external surfaces by lactoperoxidase-catalyzed iodination. The proteins are grouped according to functional relationships deduced from the isolation of submembrane particles and designated according to their band number obtained from Fig. 1. Cytochromes, cross-hatched; chlorophyll-binding proteins, horizontal lines; Fe-S proteins, vertical lines.

solubilization mixture. However, ^{35}S-labelled bands remain at these positions, implying that the polypeptides are still present. In Figs. 1 and 2, it is evident that both bands, but especially bands 67 and 68, are present in greater amounts after solubilization at 70°C than at 0°C. This may indicate that, like cytochrome *f*, these proteins are complexed tightly with lipid prior to electrophoresis. Neither band 67 nor 68 is labelled with ^{125}I, so that this iron protein does not have external tyrosines; however, band 64 is slightly labelled. In Fig. 6, cytochrome *f* and b_6 and band 67/68 are depicted as a complex without any external domains. This relationship is based on the recent isolation of a complex from spinach chloroplasts that contains cytochromes *f*, b_6 and a Rieske Fe-S center [24], a structure that is similar to the cytochrome *b-c* complex of mitochondria [25].

A hypothetical, topological organization of our digitonin-solubilized PS I and PS II particles is shown in Fig. 6. Our data indicate that the chlorophyll-binding proteins of PS II (bands 36 and 38) and PS I (band 25) have external domains. We have drawn all three as transmembrane proteins, though we have no specific evidence that two surfaces are exposed. In fact, band 38 appears to be more heavily labelled than band 36. It is equally possible that each of the PS II chlorophyll-binding proteins is exposed to one surface of the membrane. In any event, it would seem that a transmembrane configuration for such proteins would be reasonable based on the concept of charge separation. The remainder of the photosystem proteins are arranged in accordance with their ^{125}I labelling by lactoperoxidase. In PS II, bands 47 and 51 are both in the molecular mass range typical of the herbicide-binding protein that has been characterized in both chloroplasts and algae [4,26]. However, band 51 (31.5 kDa) has a particularly high intensity after labelling with ^{35}S. This may imply that this species is rapidly turned over, another characteristic that has been identified with the herbicide-binding protein [27].

Though we consider some of the details in Fig. 6 as tentative, it is the most complete structural analysis of the cyanobacterial membrane available. It should be compared with the model of the chloroplast membrane constructed by Von Wettstein [1]. This model shows the organization of proteins that comprise three important complexes: PS I, PS II, and CF_0-CF_1 (the H^+-translocating ATPase). The diagram summarizes a large quantity of contemporary research, but still contains many missing features, including precise topological relationships (e.g., from ^{125}I studies). Nonetheless, both models are very valuable for future research. Our model of the cyanobacterial membrane will be helpful in the characterization of mutants, in the study of membrane assembly, and in our attempts to clone specific genes coding for these membrane proteins.

Acknowledgments

We gratefully acknowledge the expert technical assistance of Ms. Jill Cunningham and Ms. Diana Baker. This research was supported by grant GM21827 from the National Institutes of Health, an NIH Postdoctoral Fellowship (GM07704) to

J.A.G., and, in part, by the University of Missouri Institutional Biomedical Research Grant RR07053 from NIH.

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