Biochimica et Biophysica Acta, 766 (1984) 62–69
Elsevier

BBA 41523

CYTOCHROME DISTRIBUTION ACROSS CHLOROPLAST THYLAKOID MEMBRANES

CONTROLLED PROTEOLYSIS OF INSIDE-OUT AND RIGHT-SIDE-OUT VESICLES

ROYSTON W. MANSFIELD * and DEREK S. BENDALL

Department of Biochemistry, University of Cambridge, Tennis Court Road, Cambridge, CB2 1QW (U.K.)

Key words: Cytochrome; Transverse distribution; Thylakoid membrane; Proteolysis; (Pea chloroplast)

The transverse distribution of chloroplast cytochromes *b*-559 (high and low potentials), *b*-563 and *f* in pea thylakoid membranes was studied by the effects of trypsin and pronase on inside-out and right-side-out thylakoid vesicles. The high potential (HP) form of cytochrome *b*-559 was degraded to a low potential (LP) form most rapidly in right-side-out vesicles. In either type of vesicle there was no overall loss of the cytochrome from the membrane. This suggests that the haem group is buried in the membrane but that the cytochrome environment is most labile at the outer surface. Cytochrome *b*-563 was unaffected by trypsin and only slightly degraded by pronase in inverted vesicles. However, pronase caused the loss of an M_r 1000, non-haem fraction from the cytochrome *f* polypeptide in inside-out vesices only. The total cytochrome *f* content (measured spectrophotometrically and by staining polyacrylamide gels for haem associated peroxidase activity) decayed only slightly in either type of vesicle. These observations suggest that cytochrome *f* is, in part, exposed to the intrathylakoid lumen, whilst its haem group is retained in a more hydrophobic region.

Introduction

The cytochromes of chloroplast thylakoid membranes occur in such stoichiometries with other functional units of the electron transport chain to suggest that they too have distinct functional roles. Evidence supporting possible roles has been extensively reviewed [1–3] and it is clear that the physical proximity of cytochromes to other, non-uniformly distributed components is an important factor in controlling their activity. There is some debate about the lateral organisation of cytochromes, although it is well established that cytochrome *b*-559 HP ($E_{m,7}$ + 370 mV) is found in the granal stacks in the vicinity of photosystem two (PS II) [4]. Cytochrome *b*-559 also exists in two lower potential forms that are indistinguishable by the hydroquinone vs. ferricyanide method used here. One of these (with $E_{m,7}$ + 110 mV) is a modified form of the high potential cytochrome [5] and as such is found in the grana. The other appears to be a discrete entity ($E_{m,7}$ + 20 mV) with a similar α-band absorbtion maximum and is found in loose attachment to the cytochrome *b*-563-*f* complex [6]. The latter is found in stroma-exposed thylakoids and, when mechanical disruption is used, also in grana enriched fractions [7,8] although perhaps not in the closely appressed partitions [9]. Detergent fractionation studies tend to support the exclusion of this complex from partition membranes [10,11]. Two recent reports have, by theoretical considerations [12] and by study of agranal maize bundle sheath thylakoids [13], put forward the postulate that the *b*-563-*f* complex encroaches from the stromal lamellae into the fret regions of the grana but no further.

An understanding of cytochrome distribution in

* To whom correspondence should be addressed at CSIRO, Division of Plant Industry, Black Mountain, P.O. Box 1600, Canberra ACT 2601, Australia

Abbreviations: E_m, midpoint potential at specified pH; HP, high potential; LP, low potential; P-680, primary chlorophyll electron donor of Photosystem II; P-700, primary chlorophyll electron donor of Photosystem I; PS, photosystem.

the second, transbilayer, dimension of thylakoids is also essential, particularly as it is the vectorial arrangement of electron transport components [14] that is widely held as being necessary for energy transduction, the fundamental role of chloroplasts. Information on transverse distribution of the cytochromes has been derived from experiments in which proteinases, antibodies, labelled modifying agents and redox reagents interact with the outer surface of the thylakoids or with disrupted membranes. From such studies it has become apparent that cytochrome *b*-559 HP may be found adjacent to the outer membrane surface [15,16] but not whether it physically spans the membrane to interact with oxidising side of PS II. Because of a rapid interaction with exogenous reductants it has been suggested that the native low potential form of the cytochrome is located near the external surface [1]. Similarly, cytochrome *f* has been shown to be variably accessible (depending on light conditions) to the negatively charged oxidant, ferricyanide at the outer aqueous interface [15]. It also appears to have antigenic determinants on the inner surface (Refs. 17–19, although see Ref. 20) and is able to interact with exogenous plastocyanin when that surface is exposed [7]. Cytochrome *b*-563 appears to be well 'buried' within the lipid matrix, as demonstrated by limited accessibility of electron donors [7,12] and also by analysis of the hydrophobicity pattern of its amino acid sequence [22]. However, it has been suggested that antibodies may interact with the cytochrome, primarily from the inner surface [19].

In this report, the effect of trypsin (which catalyses the hydrolysis of bonds involving the carboxyl groups of arginine and lysine [23]) or pronase (a mixture of enzymes giving an overall wider specificity including endo-, amino- and carboxypeptidase activities [24]) on the cytochrome content of inside-out and right-side-out thylakoid vesicles has been investigated. As noted in Ref. 7 and 8 these vesicles were found to contain the full complement of cytochromes, as such they are more valuable for symmetry analysis than the purified partition-derived vesicles described in Ref. 9.

Materials and Methods

Membrane preparation. Inside-out and right-side-out vesicles were prepared from chloroplasts isolated from 10–12 day old pea seedlings (Superb variety) using the procedures described in Refs. 25 and 26. Yeda-press-disrupted membranes were added to an aqueous two-phase system containing 5.9% polyethylene glycol and 5.9% dextran. After equilibration to 2°C, mixing and centrifugation, the upper and lower phases were repartitioned with fresh lower and upper phases, respectively. This was repeated a third time yielding inside-out vesicles (as detected by reversed light-induced proton pumping activity) in the third bottom phase (B3 fraction) and right-side-out vesicles in the third top phase (T3 fraction). Vesicles were recovered from the polymers by 5 × dilution in a washing medium containing 0.05 M sucrose, 0.01 M NaCl and 0.05 M sodium phosphate (pH 7.4) followed by centrifugation (30 min at 35000 × *g*).

Proteinase treatment. Samples were resuspended by hand homogenisation into a medium containing 0.01 M NaCl and 0.01 M Tris-HCl (pH 7.6) to give a final chlorophyll concentration of about 200 μg/ml as determined by the method in Ref. 27. Samples were then equilibrated to 25°C in a shaking-water bath and trypsin (bovine pancreas, type XI; 10800 BAEE units/mg solid at 25°C from Sigma Chemical Company) or pronase (ex. *Streptomyces griseus*, 120 PUK * mg solid at 40°C, from Calbiochem-Behring Corp.) were added to give chlorophyll/proteinase ratios as detailed in figure legends. At specified times, samples were taken and the proteolytic reaction stopped by 50 × dilution in the above ice-cold washing medium containing 1 mM phenyl methyl sulphonylfluoride and trypsin inhibitor (soybean, type 1-s, from Sigma Chemical Company) at 20-times the initial proteinase concentration. The membranes were immediately recovered by centrifugation (45 min at 35000 × *g*) and were resuspended by hand homogenisation into an appropriate medium for subsequent analysis. Control samples underwent the same procedures without the initial addition of proteinase.

Spectrophotometric analysis. Pellets were resuspended in a medium containing 0.33 M sorbitol, 1 mM $MgCl_2$, 1 mM $MnCl_2$, 2 mM EDTA and 0.05 M sodium phosphate (pH 6.5) to given a final

* PUK, proteolytic units (1 unit liberates the equivalent of 25 g tyrosine per min)

chlorophyll concentration of 150 μg/ml and were then analysed according to the procedures given in Ref. 28 on a Johnson foundation split-beam spectrophotometer. All specta closely resemble those shown in Ref. 28. The contribution of cytochrome *f* absorbance to the cytochrome *b*-559 HP signal was calculated by assuming a cytochrome *f* concentration of 1 nmol haem/mg chlorophyll and using the correction factor of 0.29 as in Ref. 29. The concentrations of cytochromes *b*-559 LP (in total) and *b*-563 were distinguished by their different rates of reduction upon addition of 0.5 mg/ml sodium dithionite. The absorption maximum 30 s after addition was at 559 nm, this peak shifted towards 563 nm during the ensuing 10 mins. The peak heights at the end point were firmly established by addition of a small amount of solid dithionite 10 min after the first addition.

Polyacrylamide gel electrophoresis. Pellets were hand homogenised into a medium containing 20% glycerol, 4% SDS, 5% β-mercaptoethanol, 0.002% bromophenol blue and 0.125M Tris (pH 6.8) to give 1 mg chlorophyll/ml. This mixture was allowed to stand on ice for 15 min, 50 μl samples were then electrophoretically separated overnight on 10–17% polyacrylamide gradient gels as in Ref. 29. Gels were then stained for 6 h with 0.04% Coomassie blue in a 25% isopropanol, 10% acetic acid solution, then destained in 25% isopropanol and 10% acetic acid. Haem-containing polypeptides were detected by peroxidase activity in the presence of 3,3′5,5′-tetramethyl benzidine as in Refs. 30, 31. Gel scanning was carried out using an RFT Transidyne 2955 scanning densitometer.

Results

The concentration of cytochrome *b*-559 HP in control samples (2.82 ± 0.18 nmol/mg chlorophyll in inside-out vesicles and 2.32 ± 0.24 nmols/mg chlorophyll in right-side-out vesicles) was similar to that found in Ref. 7 (2.77 and 2.11 nmol/mg chlorophyll, respectively, but somewhat lower than that found in Ref. 8. Fig. 1 shows the loss of cytochrome *b*-559 HP, with a parallel increase in cytochrome *b*-559 LP, upon trypsination of inside-out vesicles. A similar parallelism occurred with right-side-out vesicles and intact thylakoids, and also upon pronase treatment of the two types of

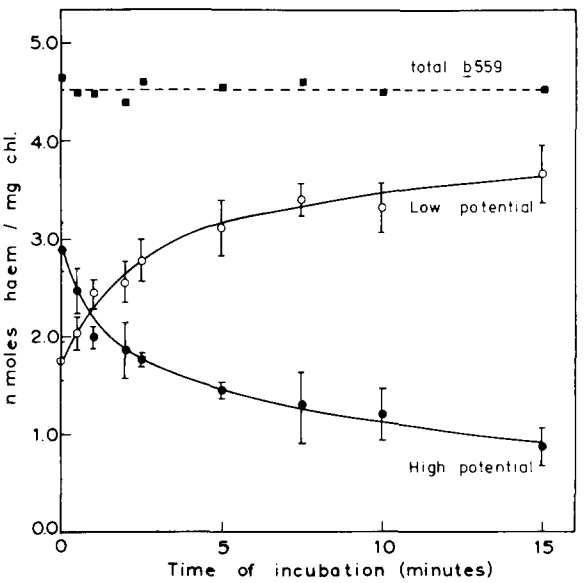

Fig. 1. The conversion of cytochrome *b*-559 HP (●) to the low potential form (○) upon trypsination of inside-out vesicles. The sum of the low and high potential forms is also shown (■). The chlorophyll/trypsin ratio was 50:1 (w/w). Error bars represent the standard deviation of between 5–10 measurements on different preparations.

vesicle. The total cytochrome *b*-559 content (high and low potentials) remained constant, at 4.5 ± 0.13 nmol/mg chlorophyll, throughout the incubation period shown, and did not change with further treatment of up to 2 h (not shown). In control samples incubated at the same temperature but in the absence of proteinase, there was no loss of high potential cytochrome during the period shown. These results indicate that a direct interconversion of the high to a low potential form of cytochrome *b*-559 took place upon proteolysis, but that there was no absolute loss of cytochrome from either side of the membrane.

Fig. 2 shows the rate of decay of cytochrome *b*-559 HP in various samples after trypsin (Fig. 2a) or pronase (Fig. 2b) treatment. The cytochrome was degraded by trypsin in a first order manner in both righ-side-out vesicles and intact thylakoids. The slower loss from the latter membranes may be due to restricted access of the proteinase to susceptible sites in partially stacked regions, present even under the low salt conditions used during treatment. In contrast, the decay slope of the inverted

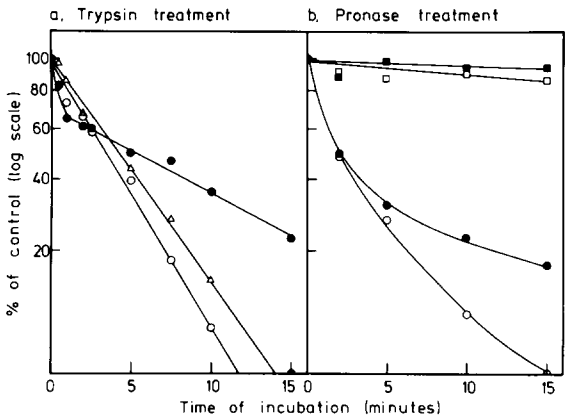

Fig. 2. The effect of proteinases on cytochrome *b*-599 HP in membranes of different sidedness. Inside-out vesicles (●), right-side-out vesicles (○) and intact thylakoids (△) were treated at a chlorophyll/protease ratio of 50:1 (w/w). The sum of cytochrome *b*-559 HP and LP in inside-out (■) and right-side-out (□) vesicles after treatment with pronase is also given. The points are expressed as percentages of control samples not exposed to the proteinase and are the means of 5–10 different preparations.

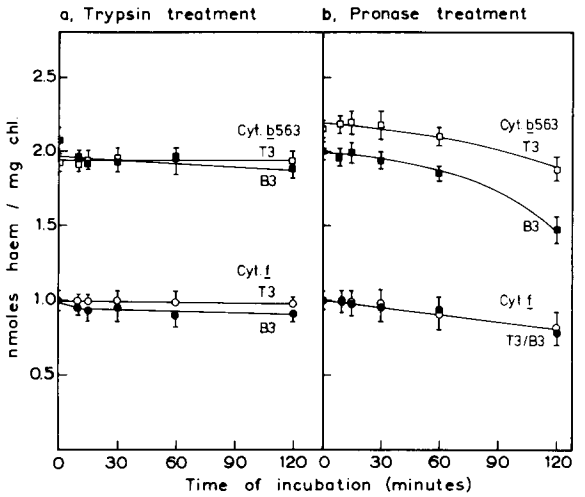

Fig. 3. The effect of proteinase treatment on cytochrome *b*-563 (■, □) and cytochrome *f* (●, ○) in inside-out (■, ●) and right-side-out (□, ○) vesicles. Proteinase concentrations were as in Fig. 2. The error bars represent the standard deviation of measurmements on 5 different preparations for each proteinase.

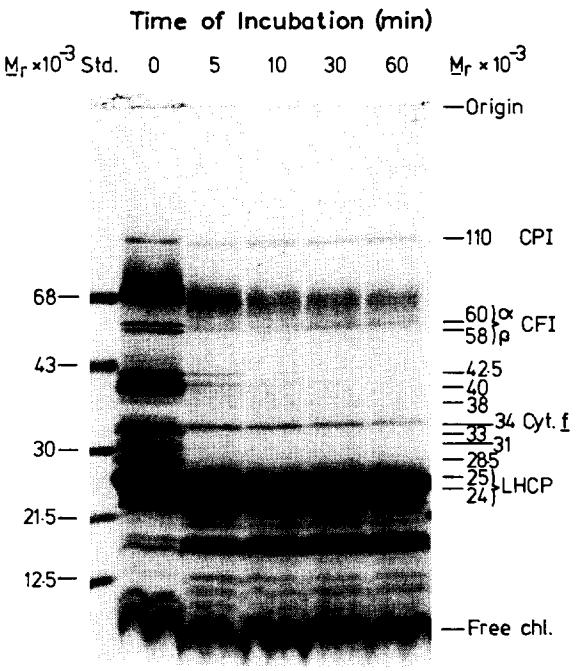

Fig. 4. The effect of trypsin on the cytochrome *f* polypeptide in inside-out vesicles. The chlorophyll/trypsin ratio was 6:1 (w/w). The cytochrome *f* polypeptide was identified by a prior treatment of the gel with tetramethylbenzidine and hydrogen peroxide to detect haem-associated peroxidase activity. The gel was then stained with Coomassie blue to show the total protein content. M_r standards (Std.) were bovine serum albumin (68000), ovalbumin (43000), carbonic anhydrase (30000), trypsin inhibitor (21500) and cytochrome *c* (12500).

vesicles shows biphasic kinetics indicating that two first order reactions took place simultaneously. The initial fast phase accounts for 30% of the sample and is possibly the result of contamination of the B3 fraction by right-side-out vesicles [25]. The differences in slopes between the various samples over this period were not greater than the experimental error. The remaining 70% of the sample (by extrapolation of the slow phase to zero time) gave a considerably lower rate of decay (60% slower) indicating that the cytochrome is less susceptible to proteolysis in inverted vesicles. Similar differences, although not as marked, were seen for the effect of pronase (Fig. 2b). Here, neither slope was linear, an effect that is probably related to the multi-enzymic nature of the proteinase. Pronase had a minimal effect on the total *b*-559 (HP and LP) content as can also be seen in Fig. 2b. Again further treatment up to 2 h had little effect.

Cytochromes *f* and *b*-563 were resistant to tryptic digestion in both right-side-out and inside-out vesicles (Fig. 3a). At higher trypsin concentrations (6 mg chlorophyll : 1 mg trypsin) some decrease in the content of both cytochromes occurred, particularly in the inverted membranes but this may have been the result of differential loss of

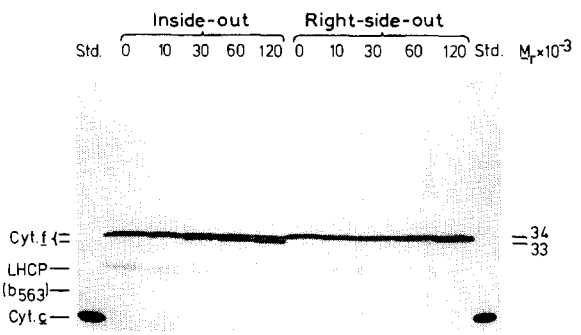

Fig. 5. The effect of pronase on the 'haem-staining' pattern of inside-out and right-side-out vesicles. The chlorophyll/pronase ratio was 50:1 (w/w). The relative molecular weight of bands showing haem-associated peroxidase activity was established by subsequent staining with Coomassie blue and comparison with known standard (as in Fig. 4).

membrane integrity. Incubation with pronase led to a 20% decrease in the spectrophotometrically measured cytochrome *f* content over the assay period in either type of vesicle (Fig. 3b). Cytochrome *b*-563, however, was slightly more sensitive in inside-out vesicles, losing 27% of the original content over the incubation period as opposed to a 13% loss from right-side-out membranes. The progressively downward slope of the B3 curve indicates that a shielding group may first be degraded, later allowing greater access of the proteinase to a susceptible site. It is shown, in Fig. 4, that cytochrome *f* is remarkably resistant to tryptic digestion in comparison with polypeptides of similar or higher molecular weight. It neither changed mobility (in contrast to the report in Ref. 32) nor significantly decreased in staining intensity. The same resistance was found with intact thylakoids and right-side-out vesicles from peas and also equivalent membranes from spinach. In contrast, pronase had a marked effect on the cytochrome *f* polypeptide as visualized by staining gels for haem-associated peroxidase activity (Fig. 5). Under the conditions used here, cytochrome *f* from both peas and spinach had M_r 34000. It is apparent that an M_r 1000 fraction was removed from the cytochrome in inside-out vesicles only. This effect became noticeable after 30 min treatment (Fig. 5, track 4) whereas a change of M_r 500 in the right-side-out sample was noted only after 2 h. The effect in the inside-out vesicle sample is unlikely, therefore, to be due to contamination by

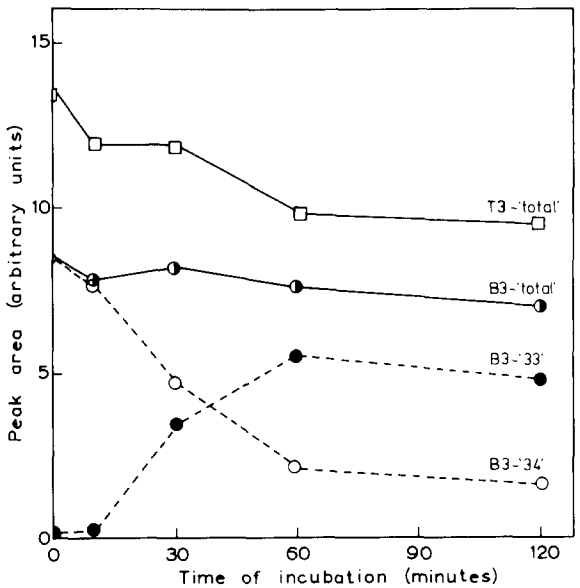

Fig. 6. The change in 'haem-stain' intensity following pronase treatment of inside-out (B3) and right-side-out (T3) vesicles. The area under densitometer peaks at M_r 34000 (○, '34'), M_r 33000 (●, '33') and the combined total of these two (○) is shown for inside-out vesicles. Right-side-out vesicles showed no stain at M_r 33000, only the change in the total staining intensity is shown for these samples (□). The gel was scanned 10 min after addition of hydrogen peroxide to the staining mixture and the first track scanned gave the same peak area at the end of the measurements. Chlorophyll/pronase ratio was 50:1 (w/w).

normally orientated vesicles. A similar gel was analysed by densitometry and the integrated area of total haem-stain, as well as that under the individual M_r 34000 and 33000 peaks, was plotted (Fig. 6). In inside-out vesicles the loss of stain intensity at M_r 34000 was partly matched by an increase at M_r 33000. Over the 2 h incubation period there was an absolute loss of 20% of haem-staining from both types of vesicle. This gross loss was exactly matched by a loss of the cytochrome *f* spectral signal in either population (Fig. 3b) and possibly reflects a slight but progressive breakdown of the membrane. It is clear from Fig. 7, which shows the integrated peak areas at M_r 34000 and 33000 of a Coomassie blue stained gel with similar samples to those in Fig. 6, that the increase in haem-stain at M_r 33000 is the result of an actual increase in protein at that position. The inside-out vesicles progressively lost protein at M_r 34000 (cytochrome *f*) whereas the same band in

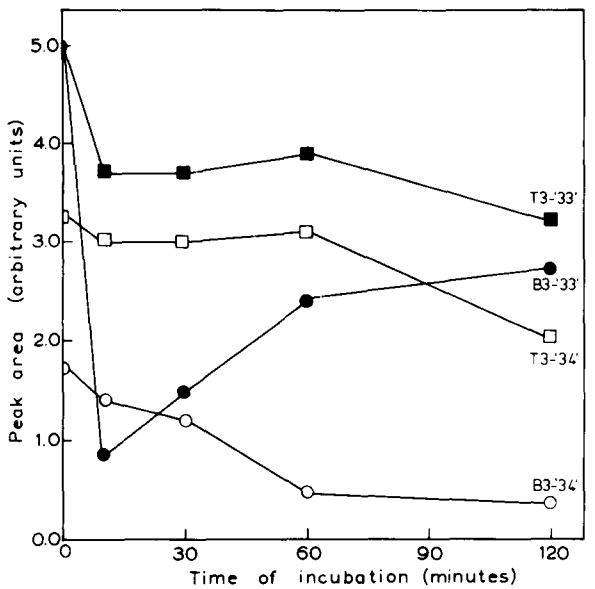

Fig. 7. The change in Coomassie blue stain intensity at M_r 34000 (○, □; '34') and M_r 33000 (●, ■; '33') in inside-out (○, ●; B3) and right-side-out (□, ■; T3) vesicles following pronase treatment. The chlorophyll/pronase ration was 50 : 1 (w/w).

right-side-out vesicles changed only slightly. Initially, the M_r 33000 polypeptide was rapidly degraded in inside-out thylakoids, much less so in normaly orientated membranes (it is likely that this protein is the same as that affected by trypsin in Ref. 33). After 10 min incubation, the staining intensity of this band in the inside-out sample began to increase whilst that in the right-side-out sample continued to slowly decrease. It is evident than, that the increase in peorxidase activity at M_r 33000 in inverted membrane samples was due to an actual increase in haem-containing protein at that position. The results presented in Fig. 7 make it unlikely that the change in haem-stain position (Fig. 6) is due to loss of haem-binding ability of the M_r 34000 polypeptide upon proteolysis, coupled with non-specific binding of the displaced haem (as demonstrated in Ref. 31) to the M_r 33000 polypeptide. From these results we conclude that a M_r 1000 non-haem containing fraction of the cytochrome *f* polypeptide is exposed to the intrathylakoid lumen and is susceptible to hydrolysis by pronase when that surface is exposed.

Discussion

It has become well established that there is heterogeneity in the lateral distribution of photosystems I and II within thylakoid membranes. Similarly, it is apparent that cytochrome *b*-559 HP is enriched in granal regions [7,8]. It was shown (Fig. 2) that this cytochrome is converted to lower potential forms by proteolytic attack at the outer surface more readily than at the inner surface of membranes derived from the grana. The change of potential may be due to proteinase interaction directly with the cytochrome or with components in its environments. The first order nature of the plots of tryptic digestion might suggest that a single peptide bond is responsible for maintaining the potential of the cytochrome. However, this does not appear to be the case since hydrolysis by pronase gives plots that are far from first order. Tryptic hydrolysis of arginyl bonds can occur at 25-times the rate of lysyl-bond hydrolysis [34]; this may explain why, apparently, only a single process was detected (Fig. 2a). The lower rate of degradation upon proteolytic attack at the inner surface might be due to an absence of susceptible peptide bonds on parts of the protein that may, nevertheless, be exposed. This seems unlikely since a proteinase with broad specificity (pronase) was found to have esssentially the same effect as the lysine/arginine specific trypsin. A possible explanation is that there is greater steric hinderance by other proteins at the inner surface. Such proteins might include the M_r 3300 polypeptide known to be removed by trypsin or Tris-treatment [33] or the M_r 23000 and 16000 polypeptides removed by high-salt washes [35]. Removal of the latter two polypeptides by a salt-wash might render cytochrome *b*-559 HP more susceptible to proteolysis at the inner surface. However, it has been suggested that such a pre-treatment is sufficient, in itself, to lower the potential of the cytochrome [36]. Clearly, the potential of this cytochrome is strongly influenced by its proteinaceous as well as its lipid [37] environment.

The results presented in this paper would support a model in which the cytochrome *b*-559 HP polypeptide fully spans the membrane. It may then be shielded from the inner aqueous phase by one or more of the M_r 33000, 23000 or 16000

proteins yet remain unprotected at the outer surface by the M_r 32000 'herbicide-binding' polypeptide. Physical interaction with both sides of the membrane would be advantageous if the cytochrome were involved in cyclic electron flow around PS II. Loss of oxygen evolution activity (with phenyl-parabenzoquinone or ferricyanide as electron acceptor) upon trypsination of the two types of vesicle was compared with the loss of high potential *b*-559 (result not shown). The rate at which activity declined coincided with the loss of high potential cytochrome in inside-out vesicles. However, in right-side-out vesicles (as previously demonstrated with intact thylakoids [38]) the loss of cytochrome *b*-559 HP occurred more rapidly (about 3-times as fast) than loss of activity with either electron acceptor. It therefore seems unlikely that cytochrome *b*-559 HP is obligatorily involved in oxygen evolution, although a modified form, with intermediate potential and not detectable in the hydroquinone vs. ferricyanide assay, might still support activity, albeit with reduced efficiency. The cytochrome may also have an essential protective role in transporting electrons from the reducing side of PS II, or from external donors such as ascorbate, to highly oxidising P-680 which may be generated in units with impaired water-splitting abilities.

Cytochromes *b*-563 and *f* in inside-out membranes showed specific reactions to pronse, therefore we support the conclusion reached in Refs. 7 and 8 that granal stacks contain the cytochromes. The loss of mass by the cytochrome *f* polypeptide upon pronase digestion did not occur in a single M_r 1000 step but as a more progressive change (Fig. 5). This suggests that no one discrete group was removed and probably reflects the broad specicity of this particular proteinase. Previous workers have found that cytochrome *f* loses M_r 1000 upon trypsination of maize thylakoids [32]. Presumably this was an effect on the stroma exposed surface. Using pea and spinach thylakoids we have found trypsin to be ineffective at altering the mobility of cytochrome *f* under conditions when many other membrane-bound polypeptides show distinct proteolytic degradation (this has been confirmed elsewhere with another pea variety, Feltham First; D.L. Willey and J.C. Gray, personal communication). There is extensive evidence that cytochrome *f* is, in part, located near the inner membrane surface (reviewed in Ref. 2), although some antibody studies [20] and use of hydrophilic modifying agents [39,40] suggest that there may not be a complete barrier between the cytochrome and the external phase. Recent amino acid sequence data [41,42] suggest that an extensive hydrophilic region exists at the N-terminus of the protein, and it is suggested in Ref. 41 that this section projects into the intrathylakoid lumen. The specific susceptibility of cytochrome *f* to pronase attack at the inner surface noted here should allow critical examination of the exposed fraction. It may be that the labile section of the polypeptide is involved in binding internally located plastocyanin [7]. Investigations of plastoquinol-plastocyanin oxidoreductase activity and plastocyanin mediated cytochrome *f*-P-700 interaction (noting the reduced P-700 content of these membranes) in inverted and normally orientated vesicles subjected to controlled pronase digestion are now in progress.

Cytochrome *b*-563 is known to exist in a highly hydrophobic environment and this is reflected in the identification of 8–9 hydrophobic (membrane spanning) regions in its amino acid sequence [22]. Numerous sections of the aforementioned sequence are assumed to loop into the hydrophilic spaces either side of the membrane, therefore one would expect to see proteolytic digestion of the polypeptide with little or no effect on its haem-binding, as was observed for cytocrome *f*. We find that staining gels for haem-associated peroxidase activity does not give reproducible results with cytochrome *b*-563 and intend to approach this problem by immunochemical means. When assayed spectrophotometrically it was found that the cytochrome was slightly more susceptible to pronase attack at the inner surface, a result that concurs with the finding of antigenic determinants at that surface [19]. In contrast, we have noted, as did the authors in Ref. 7, that the rate of reduction of the cytochrome by dithionite is as slow with inverted vesicles as with normal membranes (result not shown). This indicates that a similar barrier exists on either side of the membrane; the transverse distribution of cytochrome *b*-563, therefore, remains somewhat enigmatic.

Acknowledgements

RWM would like to thank the S.E.R.C. for a post-doctoral fellowship.

References

1 Bendall, D.S. (1982) Biochim. Biophys. Acta 683, 119–151
2 Hauska, G. Hurt, E., Gabellini, N. and Lockau, W. (1983) Biochim. Biophys. Acta 726, 97–133
3 Cramer, W.A. and Whitmarsh, J. (1977) Annu. Rev. Plant Phys. 28, 133–172
4 Boardman, N.K. and Anderson, J.M. (1967) Biochim. Biophys. Acta 143 187–203
5 Rich, P.R. and Bendall, D.S. (1980) Biochim. Biophys. Acta 591, 153–161
6 Hurt, E. and Hauska, G. (1981) Eur. J. Biochem. 117, 591–599
7 Cox, R.P. and Andersson, B. (1981) Biochem. Biophys. Res. Comm. 103, 1336–1342
8 Anderson, J.M. (1982) FEBS Lett. 138, 62–66
9 Henry, L.E.A. and Moller, B.L. (1981) Carlsberg Res. Commun. 46, 227–242
10 Berthold D.A., Babcock, G.T. and Yocum, C.F. (1981) FEBS Lett. 134, 231–234
11 Lam, E., Baltimore, B., Ortiz, W., Chollar, S., Melis, A. and Malkin, R. (1983) Biochim. Biophys. Acta 724, 201–211
12 Barber, J. (1983) Plant, Cell and Environ, 6, 311–322
13 Ghirardi, M.L. and Melis, A. (1983) Arch. Biochem. Biophys. 224, 19–28
14 Mitchell, P. (1965) J. Theor. Biol. 62, 327–367
15 Horton, P. and Cramer, W.A. (1974) Biochim. Biophys. Acta 368, 348–360
16 Cramer, W.A., Whitmarsh, J. and Widger, W. (1981) Proc. 5th Int. Photosynthesis Congress (Akoyunoglou, G., ed.), Vol. II, pp. 509–522, Balaban International Science Services, Philadelphia, PA
17 Hauska, G. (1972) Angew. Chem. 84, 123–124
18 Racker, E., Hauska, G.A., Lien, S., Berzborn, R.J. and Nelson, N. (1972) Proc. 2nd Int. Photosynthesis Congress (Forti, G., Avron, M. and Melandri, A., eds.), pp. 1097–1113, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
19 Morschel, E. and Staehelin, L.A. (1983) J. Cell Biol. 97, 301–310
20 Schmid, G.H., Radunz, A. and Menke, W. (1977) Z. Naturforsch. 32C, 271–280
21 Cox, R.P. (1979) Biochem. J. 184, 39–44
22 Widger, W.R., Cramer, W.A. Herrmann, R. and Trebst. A. (1984) Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 505–508, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
23 Kiel, B. (1971) The Enzymes, Vol. III (Boyer, J., ed.) pp. 250–275, Academic Press, New York
24 Seber, J.F., Toomey, T.P., Powell, J.T., Brew, K. and Awad, W.M. (1976) J. Biol. Chem. 251, 204–208
25 Andersson, B. and Åkerlund, H.-E. (1978) Biochim. Biophys. Acta 503, 462–472
26 Mansfield, R.W., Nakatani, H.Y., Barber, J., Mauro, S. and Lannoye, R. (1982) FEBS Lett. 137, 133–136
27 Arnon, D.I. (1959) Science 149, 1460–1470
28 Bendall, D.S., Davenport, H.E. and Hill, R. (1971) Methods Enzymol. 23, 327–344
29 Laemlli, U.K. (1970) Nature 227, 680–685
30 Thomas, P.E., Ryan, D. and Levin, W. (1976) Anal. Biochem, 75, 168–176
31 Hoyer-Hansen, G. (1980) Carlsberg Res. Commun. 45, 167–176
32 Bricker, T. and and Sherman, L.A. (1982) FEBS Lett. 149, 197–202
33 Åkerlund, H.-E. and Jansson, C. (1981) FEBS Lett. 124, 229–232
34 Wang, S.-S. and Capenter, F.H. (1967) Biochemistry 6, 215–224
35 Åkerlund, H.-E., Jansson, C. and Andersson, B. (1982) Biochim. Biophys. Acta 681, 1–10
36 Larsson, C., Jansson, C., Ljungberg, U., Åkerlund, H.-E. and Andersson, B. (1984) Advances in Photosynthesis Research (Sybesma, C., ed.), Vol. I, pp. 363–366, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
37 Matsuda, H. and Butler, W.L. (1983) Biochim. Biophys. Acta 724, 123–127
38 Cox, R.P. and Bendall, D.S. (1974) Biochim. Biophys. Acta 347, 49–59
39 Smith, D.D., Selman, B.R., Voegli, K.K., Johnson, G. and Dilley, R.A. (1977) Biochim. Biopys. Acta 459, 468–482
40 Alam, J., Bhatnagar, D., Dilley, R.A. and Krogmann, D.W. (1981) Plant Physiol. (Suppl.) 67, p87
41 Willey, D.L. and Gray, J.C. (1984) in Advances in Photosynthesis Research (Sybesma, C., ed.), Vol IV., pp 567–570, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague
42 Alam, J and Krogmann, D.W. (1984) Advances in Photosynthesis Research (Sybesma C., ed.), Vol I, pp 521–524, Martinus Nijhoff/Dr. W. Junk Publishers, The Hague