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# Freeze-fractured Thylakoids of Some Marine Red Algae

By

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With 6 Figures

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Summary

TSEKOS I. & REISS H.-D. 1988. Freeze-fractured thylakoids of some marine red algae. – Phyton (Austria) 28 (2): 201–214, with 6 figures. – English with German summary.

The chloroplast membranes of four marine Ceramiales *(Ceramium rubrum, Callithamnion corymbosum, Callithamnion caudatum, Polysiphonia deusta)* are studied in replicas of rapidly frozen and fractured cells. Freeze-fractured thylakoid membranes of these red algae exhibit only two types of fracture faces (EF and PF), because the lamellae in red algal chloroplasts are not stacked. The particle densities of the PF and EF range from 3000 to 4200 and 900 to 1400 particles/$\mu m^2$ respectively and appear similar to those of the EFu and PFu faces of unstacked thylakoid membranes of higher plants and green algae. The particle size distribution for *C. corymbosum* and *P. deusta* is uniform in the two types of faces with an average diameter of about 10.3–10.7 nm; additionally in *C. rubrum* and *C. caudatum* a second minor size class of particles between 14–15 nm may occur on the PF and EF. The EF and PF particles of the thylakoids are randomly distributed in all four species. The phycobilisomes of *C. rubrum* (flattened discs of 30–35 nm in diameter) are also randomly distributed. *Ceramium* cells possess about 501±40 phycobilisomes/$\mu m^2$ of thylakoid surface, while the number of EF particles is about 1394±232/$\mu m^2$. Therefore, the ratio of phycobilisomes to EF particles ($\mu m^{-2}$) is about 1:3.

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## Zusammenfassung

TSEKOS I. & REISS H.-D, 1988. Gefrierbruch an Thylakoiden einiger mariner Rotalgen. – Phyton (Austria) 28 (2): 201–214, mit 6 Abblidungen. – Englisch mit deutscher Zusammenfassung.

Die Chloroplastenmembranen von vier marinen Rotalgen der Ordnung *Ceramiales (Ceramium rubrum, Callithamnion corymbosum, Callithamnion caudatum, Polysiphonia deusta)* wurden mit der Gefrierbruchtechnik untersucht. Die Thylakoidmembranen zeigen nur zwei Bruchflächen (EF und PF), da bei Rotalgen keine Thylakoidstapel vorkommen. Auf der EF und PF variiert die Partikeldichte zwischen 3.000 und 4.200 bzw. 900 und 1.400 pro $\mu m^2$ und ist vergleichbar mit der EFu und PFu von nicht gestapelten Stroma-Thylakoidmembranen in Grünalgen und höheren Pflanzen. Bei *C. corymbosum* und *P. deusta* ist die Partikelgröße mit einem Durchmesser von 10,3–10,7 nm auf beiden Bruchflächen einheitlich; bei *C. rubrum* und *C. caudatum* findet man zusätzlich Partikel zwischen 14 und 15 nm auf PF und EF. In allen vier Arten sind die Partikel zufallsmäßig verteilt. Die Phycobilisomen von *C. rubrum* (flache Scheibchen mit einem Durchmesser von 30–35 nm) sind ebenfalls zufallsmäßig verteilt. Die *Ceramium*-Zellen besitzen 501±40 Phycobilisomen/$\mu m^2$ Thylakoidoberfläche, während die Anzahl der EF-Partikel 1394±232/$\mu m^2$ beträgt. Daraus ergibt sich ein Verhältnis von etwa 1:3 zwischen Phycobilisomen und EF-Partikel.


## Introduction

Within the algal divisions, plastids have been shown to have various, distinctive membrane arrangements within them (HOOBER 1984 for review).

Due to the absence of stacked lamellae in red algal chloroplasts, thylakoids present only two types of fracture faces (PF and EF), one with many particles (PF) and one with few (EF) (STAEHELIN & al., 1978). These appear similar to the unstacked membranes of chloroplasts of higher plants and green algae in relation to the density of PF and EF particles (STAEHELIN & al. 1978). In red and blue-green algae the light-harvesting pigments, the phycobilins, are aggregated into large particles (the phycobilisomes) and attached to the stroma (external) surface of thylakoids (GANTT 1980).

Studies of blue-green, cryptophyte and red algal chloroplasts (STAEHELIN & al. 1978, WOLLMAN 1979, DWARTE & VESK 1983, GIDDINGS & al. 1983, KURSAR & ALBERTE 1983, SPEAR-BERNSTEIN & MILLER 1985, STAEHELIN 1986) have suggested that, by analogy with higher plant chloroplasts, the EF-particles represent photosystem II (PS II). The phycobilins (pigment-protein complexes) are functionally equivalent to the chlorophyll b containing light harvesting complexes of higher plants and green algae. As the main light gathering antennae (STAEHELIN & al. 1978, STAEHELIN 1986) the light energy trapped by phycobilisomes is channeled primarily into PS II reaction centers (LEFORT-TRAN & al. 1973, BOGORAD 1975, LEY & BUTLER 1977).

The functional association existing between phycobilisomes and EF particles of red algal thylakoids may be expressed by a spatial relation. In *Griffithsia pacifica* (WAALAND & al. 1974, STAEHELIN & al. 1978) and

*Spermothamnion turneri* (GANTT & CONTI 1966, STAEHELIN & al. 1978) both the intramembrane EF particles of the thylakoids and phycobilisomes are randomly distributed. In contrast, in *Porphyridium cruentum, P. aerugineum* (NEUSHUL 1970, 1971), *Cyanidium caldarium* (LEFORT-TRAN & al. 1973, WOLLMAN 1979) and probably also in *Bangia fusco-purpurea* (BISALPUTRA & BAILEY 1973) both the EF particles and the phycobilisomes appear organized into rows. Finally, in *Antithamnion glanduliferum* (LICHTLÉ & THOMAS 1976) while the phycobilisomes seem to be arranged into rows, the EF particles are randomly distributed. When the rows are set parallel to each other as in the case of *Porphyridium cruentum* (GANTT & CONTI 1966, NEUSHUL 1970, 1971) the spacing of the two types of rows is essentially the same, 50 nm. This has led to the suggestion that the EF particles and the phycobilisomes are associated with each other (GANTT 1980). The ratios of phycobilisomes to EF particles have been found to range between 0,2–2,0 (NEUSHUL 1970, WAALAND & al. 1974, LICHTLÉ & THOMAS 1976, STAEHELIN & al. 1978, KURSAR & ALBERTE 1983, Table II of GANTT 1980).

The chloroplasts of red algae appear to occupy an intermediate position between those of blue-green algae, and those of green algae and higher plants (STAEHELIN & al. 1978). For this reason and the fact that the structure of red algal chloroplasts has not been sufficiently studied, it is of considerable interest to investigate the supramolecular architecture of photosynthetic membranes of more red algal species. In addition, it should be stressed that freeze-etching has been among the most informative structural tools for the study of biological membranes (MILLER 1978, STAEHELIN 1986).

## Materials and methods

Carposporophytes of *Ceramium rubrum* (HUDS.) C. AG. and tetrasporophytes of *Callithamnion corymbosum* (SMITH) LYNGB., *Callithamnion caudatum* J. AG. and *Polysiphonia deusta* (ROTH) J. AG. were collected at Epanomi and Nea Mechaniona (Thermaikos Gulf, Greece) and were shipped immediately to the Institute of Cytology, University of Heidelberg, where they were maintained in a seawater aquarium. The thalli were cut into small pieces, and immediately after sectioning were frozen in nitrogen slush. The specimens were fractured with the double-replica device in a Balzers BAF 400-T apparatus (REISS & al. 1984).

The *Ceramium* material for ultrathin sections was fixed in the field for 2 hrs at 22°C, in 4% glutaraldehyde in 0.1 M sodium cacodylate buffer at pH 7.0, to which had been added NaCl (30 mg/ml) and $CaCl_2$ (20 $\mu$g/ml) (MC DONALD 1972). The initial 30 mg/ml sodium chloride solution was diluted by 25% with each successive step (KUGRENS 1974).

After rinsing in 0.1 M cacodylate buffer without NaCl the samples were post-fixed for 2 hours in 1% $OsO_4$ in 0.1 M cacodylate buffer at pH 7.0. The

material was dehydrated in an ethanol series, with an overnight staining in 2% uranyl acetate in 10% ethanol and then gradually transferred to propylen oxide (TSEKOS 1981). Ultrathin sections were also stained with uranyl acetate and lead citrate (REYNOLDS 1963). Both the replicas and ultrathin sections were examined either on a Philips EM 400 or on a Zeiss EM 9 S-2 electron microscope.

Abbreviations in the text and in the figures: EF = extraplasmatic fracture faces, PF = plasmatic fracture faces, PSI and PSII = photosystem I and II respectively.

## Results and Discussion

Ultrathin sections of *Ceramium rubum* were prepared for comparative purposes (Fig. 1). Numerous discoid plastids were oserved in all cell types examined. The mature chloroplasts of cortical cells contain one peripheral thylakoid which encloses single unstacked thylakoids. The inner thylakoids terminate close to the peripheral thylakoid. Thylakoids, approximately 17.5 nm thick, are arranged parallel to each other lying 50–60 nm apart in the stroma. Interconnections between adjacent thylakoids were frequently observed. Interconnections have also been observed in *Batrachospermum moniliforme* (BROWN & WEIER 1968). The phycobilisomes are located on the stroma surface of the thylakoids. The phycobilisomes of *Ceramium rubrum* have the appearance of flattened discs (30-35 nm in diameter) and are randomly distributed (Fig. 1).

The two distinct fracture faces of the thylakoid membranes of *Ceramium rubrum, Callithamnion corymbosum, Callithamnion caudatum* and *Polysiphonia deusta* were studied in oblique and tangential fractures. The EF and PF particles of the thylakoids in *Ceramium rubrum, Callithamnion corymbosum, C. caudatum* and *Polysiphonia deusta* are randomly distributed (Figs. 2, 3, 4 and 5). The PF faces exhibit numerous, tightly packed particles (Figs. 2, 3, 4, 5, and Table 1), while the EF faces, with a smooth background, possess relatively few particles (Figs. 2, 3, 4, 5 and Table 1).

The results show that the thylakoids of *Ceramium rubrum, Callithamnion corymbosum, C. caudatum* and *Polysiphonia deusta* are structured asymmetrically, which agree with the results obtained for blue-green, other red, cryptophyte, brown and green algae, and higher plants (LICHTLÉ & THOMAS 1976 and literature cited therein, STAEHELIN & al. 1978, STAEHELIN 1981, BERKALOFF & al. 1983, SPEAR-BERNSTEIN & MILLER 1985). The density of PF and EF particles of the investigated red algae (Table 1) appears similar to other red algae (LICHTLÉ & THOMAS 1976, STAEHELIN & al. 1978, STAEHELIN 1986) and to the unstacked membranes of chloroplasts of higher plants, green and brown algae (STAEHELIN & al. 1978, STAEHELIN 1981, BERKALOFF & al. 1983).

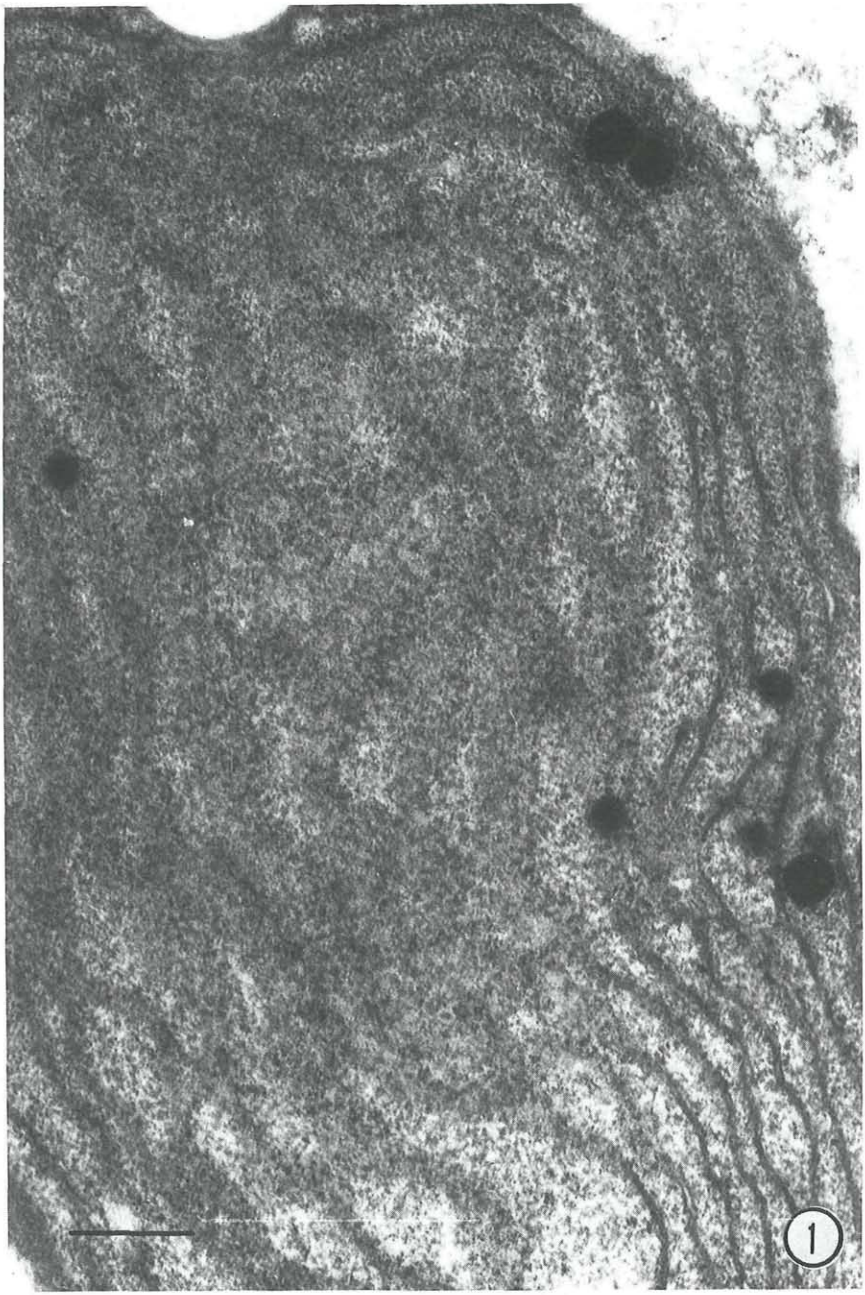



Fig. 1. Phycobilisomes can be observed to cover the tangential thin sectioned chloroplast lamellae of the red alga *Ceramium rubrum*. x 60,000. Index bar (even as in Figs. 2–5) = 0.3 $\mu$m.

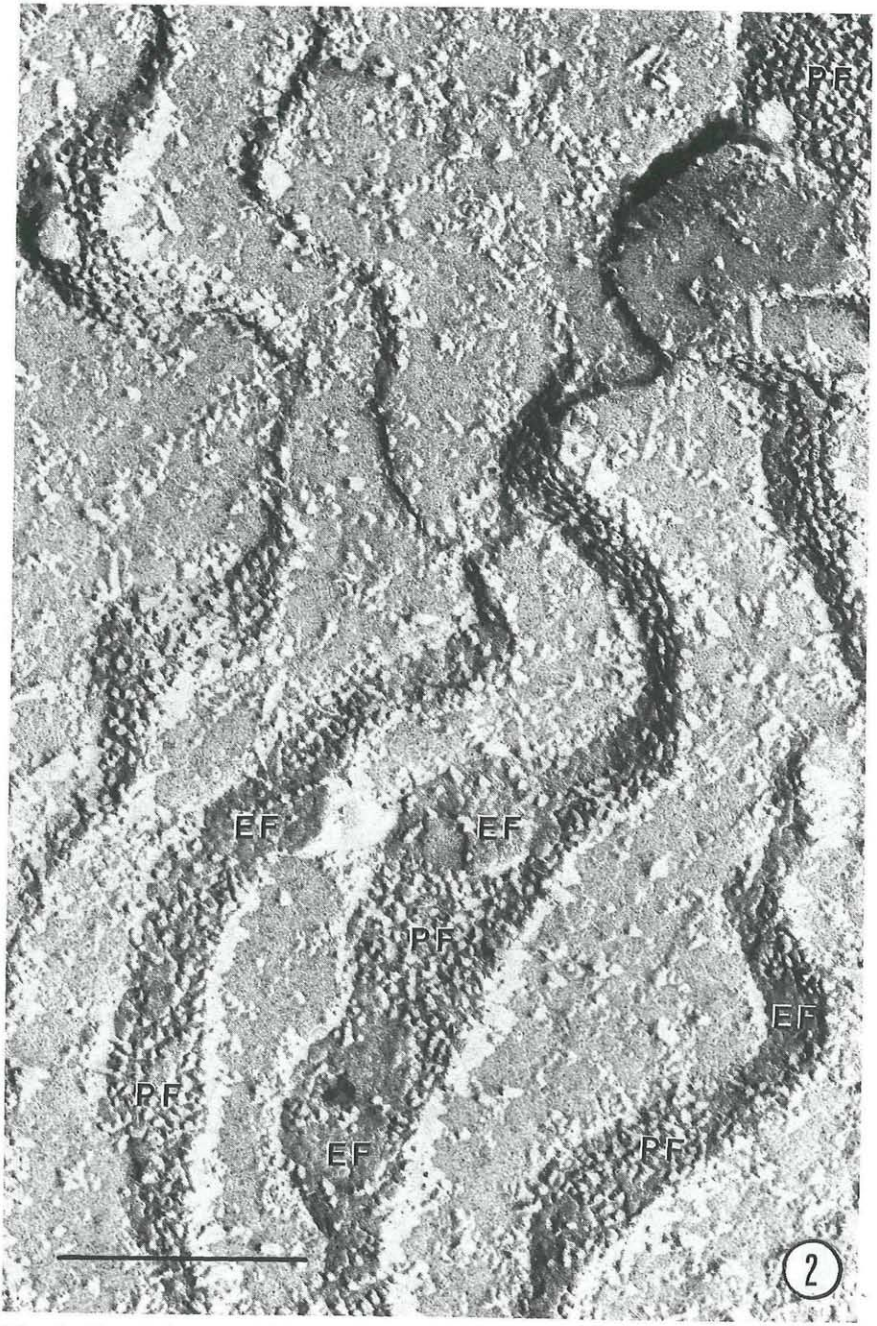
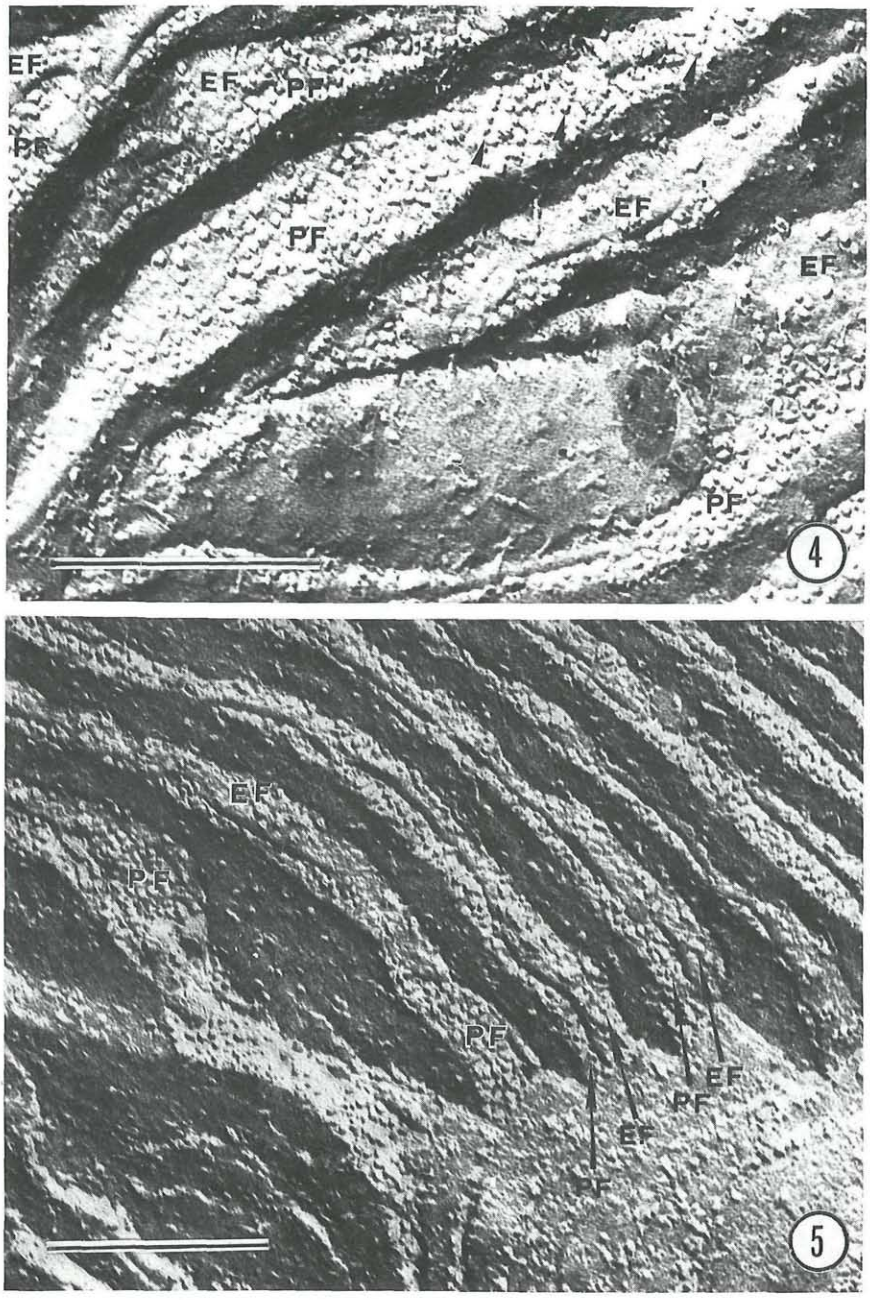



Fig. 2. Freeze-fractured thylakoid membranes of *Ceramium rubrum*. Randomly distributed particles are seen on both EF and PF faces. (EF = extraplasmatic fracture face, PF = plasmatic fracture face.) x 110,000.


Fig. 3. Freeze-fractured thylakoid membranes of *Callithamnion corymbosum*. Randomly distributed particles are seen on both EF and PF. (EF and PF see Fig. 2) x 100,000.




Figs. 4, 5. Freeze-fractured thylakoid membranes of *Callithamnion caudatum* (Fig. 4) and *Polysiphonia deusta* (Fig. 5). Randomly distributed particles are seen on both EF and PF in the two species; in *Callithamnion caudatum* tend to be organized into rows (arrows). (EF and PF see Fig. 2.) Fig. 4, x 125,000; Fig. 5, x 100,000.

Table 1

Size and density of the particles on the thylakoid membranes

| Species | Average diameter of all particles (nm) | | Density of particles ($\mu m^{-2}$) | |
|---|---|---|---|---|
| | PF–face | EF–face | PF-face | EF–face |
| *Ceramium rubrum* | 12.3 | 11.9 | 4195±341 | 1394±232 |
| *Callithamnion corymbosum* | 10.4 | 10.5 | 4066±177 | 1404±338 |
| *Callithamnion caudatum* | 11.6 | 11.5 | 3091± 63 | 965±150 |
| *Polysiphonia deusta* | 10.3 | 10.7 | 4040±227 | 1078± 31 |

The situation found in *Ceramium rubrum* where both the EF particles (Fig. 2) and the phycobilisomes (Fig. 1) are randomly distributed, is in accord with that in the red algae *Griffithsia pacifica* (WAALAND & al. 1974, STAEHELIN & al. 1978) and *Spermothamnion turneri* (STAEHELIN & al. 1978). However, in another study regularly arrayed phycobilisomes were observed in *Spermothamnion* (GANTT & CONTI 1966). GANTT (1980) has suggested that culture conditions, particulary illumination or specimen preparation, may influence the regularity or randomness of these thylakoid structures. STAEHELIN (1986) notes that under low light conditions the phycobilisomes display a tendency to become organized into rows.

In *Porphyridium cruentum* (NEUSHUL 1970), *Antithamnion glanduliferum* (LICHTLÉ & THOMAS 1976) and *Griffithsia pacifica* (WAALAND & al. 1974, STAEHELIN & al. 1978), a simple relationship between the number of phycobilisomes and that of the EF particles per surface unit of the thylakoid has been noticed (cf. also GANTT 1980, Table II). *Ceramium rubrum* cells grown under natural conditions possess 501±40 phycobilisomes/$\mu m^2$ of thylakoid surface, while the number of EF particles is 1394±232/$\mu m^2$. The ratio of phycobilisomes to EF particles per $\mu m^2$ is about 1:3, which is in agreement with the ratio obtained for *Griffithsia* cells grown under low light conditions (50 ft.-c.) (WAALAND & al. 1974, STAEHELIN & al. 1978, GANTT 1980) and for *Antithamnion glanduliferum* cells (LICHTLÉ & THOMAS 1976). On the other hand, based on biochemical measurements a 1:4 phycobilisomes to PS II ratio has been calculated für the red alga *Neogardhiella* and close to a 1:2 ratio for the cyanobacterium *Anacystis* (KURSAR & ALBERTE 1983). Since both the measurements of phycobilisome to EF particle and phycobilisome to PS II reaction center ratios yield similar values, STAEHELIN (1986) suggests that it seems highly likely that each EF particle is equivalent to a PS II complex.

It has been shown in red algae that light energy absorbed by phycobiliprotein pigments is tranferred primarily to the chlorophyll a involved in PS II (DUYSENS & al. 1961, LUDLOW & PARK 1969, NISHIMURA 1968, LEY & BUTLER 1977). If the 10 nm EF particles correspond to PS II complexes in phycobilisome-carrying thylakoids (cf. also STAEHELIN & al. 1978, WOLLMAN 1979, GIDDINGS & al. 1983, STAEHELIN 1986, BRICKER & al. 1986), then *Ceramium rubum* chloroplasts would have on the average 1 phycobilisome delivering excitation energy to 3 PS II units. EF particles and phycobilisomes are propably also closely associated from a spatial standpoint (cf. also GANTT 1980, STAEHELIN 1986). However, not every EF particle seems to be intimately associated with a phycobilisome (STAEHELIN 1986).

The particles on both fracture faces (PF and EF) of red algal thylakoids seem to belong to one major size class with a modal distribution of abouth 10–11 nm (cf. NEUSHUL 1970, 1971, LEFORT-TRAN & al. 1973, BISALPUTRA & BAILEY 1973, LICHTLÉ & THOMAS 1976, STAEHELIN & al. 1978, WOLLMAN 1979, GANT 1980). From our histograms in Fig. 6 and Table 1 we can conclude that a second minor size class of particles between 14–15 nm may occur at least on the PF and EF of *Ceramium rubrum* and *Callithamnion caudatum*. Similar supramolecular organization is observed in the thylakoids of another red alga, *Porphyra*, where the freeze-fracture particles fall into two size classes, 10 to 11 nm (major size class) and 14 to 15 nm (TSEKOS & REISS, unpublished results).

The smaller size (10.5–11.9 nm) of the EF particles of the investigated red algae thylakoid membranes (Figs. 2, 3, 4, 5, 6 and Table 1) is different from those of higher plants and green algae (STAEHELIN & al. 1978, STAEHELIN 1981) and most likely reflects the absence of the chlorophyll a/b light harvesting pigment-protein. In higher plants and green algae this protein aggregates with PS II core units thus producing larger EF particles (about 16 nm) in the thylakoids (ARMOND & al. 1977). This view is further strengthened by the fact that the particles present in the thylakoids of chloroplasts grown in intermittent lights have a diameter of abouth 8 nm (STAEHELIN & al. 1981, STAEHELIN 1986) and these are believed to lack the light harvesting pigment-protein (ARMOND & al. 1977). Large 16 nm EF particles are also absent in brown algae (BERKALOFF & al. 1983) and in *Gonyaulax polyedra* (SWEENEY 1981), which do not contain the chlorophyll a/b light harvesting pigment-proteins. In *Gonyaulax* the principal light-harvesting protein is the small water-soluble peridin-chlorophyll-protein (SWEENEY 1981). BERKALOFF & al. (1983) propose that the light-harvesting proteins in brown algae are rather small and/or associated with all the particles. The cryptophyte algae (cryptomonads) do not contain chlorophyll a/b light harvesting complex and therefore it is not surprising that their EFs particles (15 nm in diameter) have a different size from those of higher plants (DWARTE & VESK 1983).

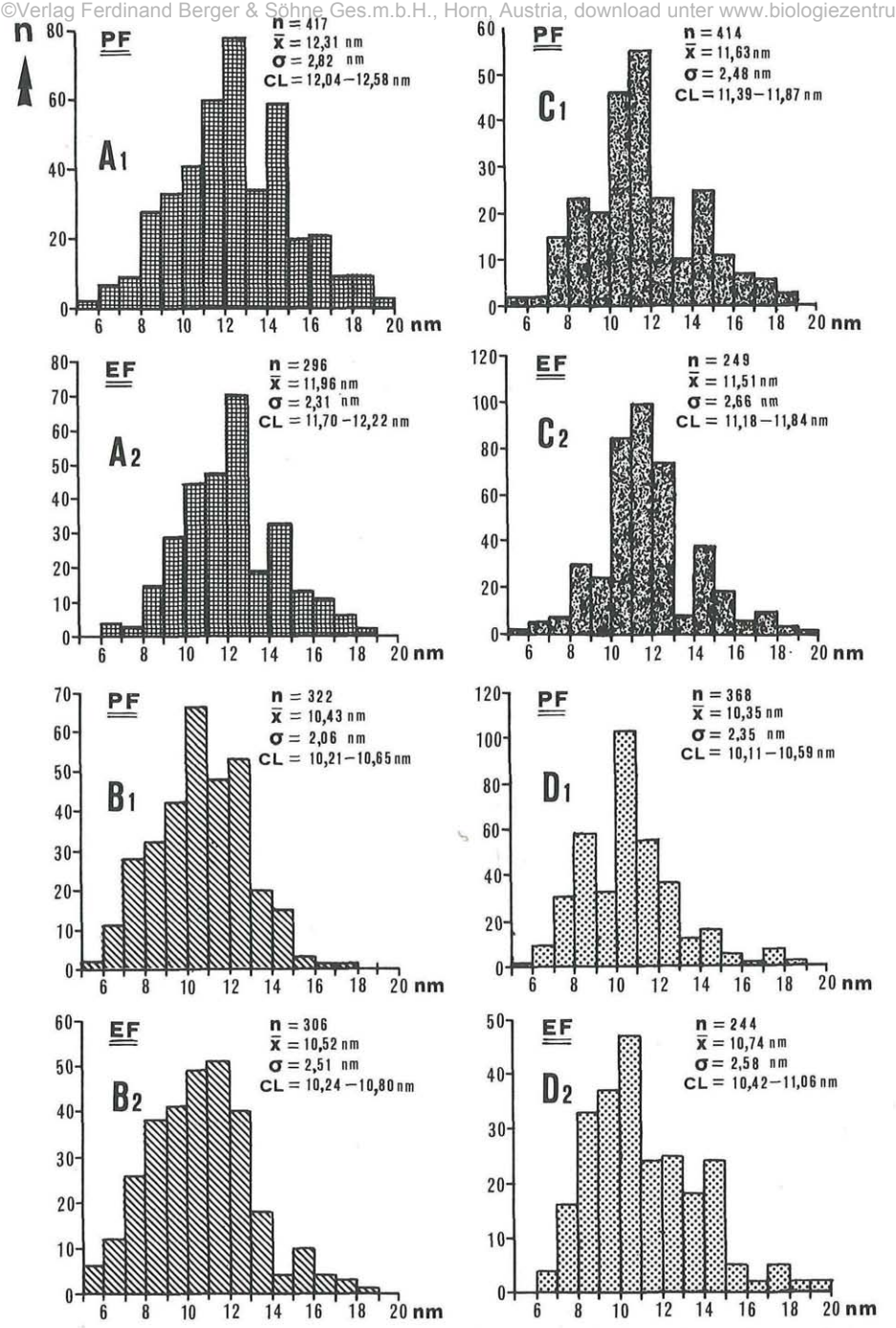



Fig 6. Histograms of the particle sizes found on the PF and EF faces of *Ceramium rubrum* ($A_1$, $A_2$), *Callithamnion corymbosum* ($B_1$, $B_2$), *Callithamnion caudatum* ($C_1$, $C_2$) and *Polysiphonia deusta* ($D_1$, $D_2$). The confidence limits (CL) are 95%.

The PF particles of red algal thylakoid membranes probably represent photosystem I (ARNTZEN & al. 1969, STAEHELIN 1986). The functional significance of the 14–15 nm PF and EF particles of *Ceramium rubrum* and *Callithamnion caudatum* is still unclear at the present time, however, one has to entertain the possibility that PS I and PS II complexes of variable size may exist in thylakoid membranes of red algae.

In accordance with STAEHELIN & al. (1978) for *Spermothamniom turneri*, the spread of the PF face particles sizes in *Ceramium rubrum* and *Callithamnion caudatum* is somewhat greater than for the EF face particles (Fig. 6), suggesting that several categories of particles with similar sizes could be present on the PF face.

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