CHLOROPHYLL, GLYCEROLIPID AND PROTEIN RATIOS IN SPINACH CHLOROPLAST GRANA AND STROMA LAMELLAE[1]

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SUMMARY

The relative molar amounts of glycerolipids are similar in grana and stroma lamellae, as are the ratios of total glycerolipid to weight of membrane protein. However the chlorophyll content relative to protein of grana lamellae is about 40% higher than that of stroma lamellae from the same preparation. Previous reports of chemical composition or enzyme activity based on chlorophyll alone can be highly misleading. The large functional and conformational differences between these two membranes may be related to these differences in pigment content, but are likely to result primarily from qualitative protein differences. The data are in accord with a membrane model in which nonpolar regions of membrane protein bind lipid in fairly constant amounts.

INTRODUCTION

Detergent separation of the photochemical systems of photosynthesis yields fractions with relatively specific electron transport functions, but of very uncertain origin (1,2). The extent to which detergents specifically solubilize a membrane containing only one photosystem and the extent to which closely situated photosystem 1 and photosystem 2 fractions are actually being separated from a single membrane type is not always evident (3,4). In addition, chemical composition of the detergent fractions may be modified from those in native systems by exchange of chemical substances during the extraction process. In particular, the significance of the nearly identical glycolipid compositions of Triton solubilized photosystem preparations (5) is uncertain. For this reason it is particularly interesting to analyze the composition of fractions enriched in one or the other photochemical system which are prepared by a

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mechanical shearing process in the French Pressure Cell (6). Preparation and some properties of these fractions have already been described (7,8,9). Fractions greatly enriched in photosystem 1 with high chla to chlb ratios[2], high P-700 content, and little cytochrome b_{559} arise from stroma lamellae. Fractions containing both photosystems, all the cytochromes and a lower chla to chlb ratio appear to arise from the grana of the chloroplast. In this paper we show that chlorophyll contents of these two membranes are markedly different, and comparative composition or enzyme activity studies based on chlorophyll content alone are misleading.

MATERIALS AND METHODS

The grana and stroma lamellae fractions were prepared from spinach leaves according to Sane *et al* (7) with the following exceptions. First, the Class II chloroplasts were washed twice in 0.15 M KCl - 0.05 M potassium phosphate buffer pH 7.4 at 1,500 x g for 15 minutes before being passed through an Aminco French Pressure Cell. Second, the high speed centrifugations were carried out by centrifuging the 40,000 x g supernatant for 1 hour at 200,000 x g in a Beckman 60 Ti rotor. The precipitates of whole French Press homogenate, the 10K fraction and the 200K fraction were individually resuspended in 1 mM potassium EDTA, pH 8.0 at 0° for 30 minutes and precipitated 1 hour at 200,000 x g (10). The pellets were again resuspended in a small volume of EDTA solution and applied to linear sucrose density gradients (0-60% w/v). After centrifugation at 100,000 x g for 16 hours in an SW-27 rotor, the green bands, each at the same position in the tube, were carefully removed, diluted with water and centrifuged at 200,000 x g for 1 hour to recover the pellet. These fractions were analyzed for chlorophyll, glycerolipid, and protein content.

Lipids were extracted from aliquots of the suspended particle preparations containing known amounts of chlorophyll and separated and analyzed following the procedure of Allen and Good (11). The insoluble residues remaining after

[2]The abbreviations used are: chla, chlorophyll a; chlb, chlorophyll b; EDTA, ethylenediaminetetraacetate.

extraction were analyzed for protein by the micro-Kjeldahl method (12). It was assumed for the purpose of the calculations, that the proteins present contained 16% nitrogen by weight. Chlorophylls were determined by the method of Arnon (13).

RESULTS AND DISCUSSION

EDTA treatment did not affect the lipid components of the chloroplast, but reduced the protein content by 15 to 30%. The fractions resulting from the EDTA treatment had quite constant lipid to protein ratios and in the case of the 10K fraction a chlorophyll to nitrogen weight ratio of 2.3, a higher value than that reported by Park and Pon (14) for extensively washed chloroplast fragments. The 10K and French Press homogenate fractions were very similar with respect to glycerolipid composition.

The lipid and fatty acid analyses from EDTA purified 10K and 200K fractions are presented in Tables I and II. The proportions of the lipids are quite similar and the only possibly significant difference in fatty acid composition is the several-fold increase in the linoleic acid of phosphatidyl glycerol of the 200K fraction relative to the 10K which was also seen in other runs. As the percentage of this acid is low, the differences could result from minor contamination by foreign membrane fragments containing phosphatidyl glycerol rich in linoleic acid.

Although no large differences exist among the glycerolipids in these two fractions, significant differences do occur in pigment composition. In addition to the difference in chla to chlb ratio, the chlorophyll content of the 10K (grana lamellae) fraction is always 1.4 - 1.5 times greater than that of the 200K (stroma lamellae) fraction expressed either on a protein or a total lipid basis. Thus stroma lamellae are depleted in chlorophyll compared with grana. Although the absolute amount of chlorophyll in the fractions varies somewhat, the ratio of the amounts in the two fractions is maintained. This is shown for three experiments presented in Table III. Besides the differences in chla to chlb ratios and chlorophyll amounts between the two fractions, significant differences in carotenoid kind and amounts also exist. The 200K fraction has

TABLE I

RATIO OF LIPIDS TO PROTEIN IN PURIFIED THYLAKOID FRACTIONS

(μmoles lipid per gram of protein)

Averaged Values

	Thylakoid[a]	10K	200K
Monogalactosyl Diglyceride	280	214	231
Digalactosyl Diglyceride	160	185	172
Phosphatidyl Glycerol	110	66	76
Sulfolipid	78	59	65
Fatty Acid (times 0.5)[b]	0	44	40
Other[c]	0	84	135
Chlorophyll	360	401	278
Total of Above	878	1,053	997

a) Lipid analysis from (19)
Chlorophyll analysis from (14)

b) Fatty acids produced by degradation of glycerolipids

c) Trace lipids from foreign membrame fragments, and lipid degradation products. Phosphatidyl ethanolamine was a major component of this fraction from 200K fractions but not of the 10K material.

less carotenoids and is relatively deficient in xanthophylls compared with the 10K fraction (15). However, the ratio of total chlorophylls to total carotenoids is about 5 for both fractions.

In these experiments the total lipid to protein ratio is found to be fairly constant in each fraction and from experiment to experiment. This observation tends to support a membrane model in which nonpolar regions of the membrane protein are binding lipid in fairly constant amounts (5) as was deduced from analysis of Triton fractions.

Finally, the data of Tables I and II suggest, as had the work of Allen and Good (5) with Triton fractions, that the vast differences in metabolic

TABLE II

Acyl Group Composition of Lipids

(Molar Ratios) [a]

		10K	200K	Thylakoid Fragments [b]
Monogalactosyl Diglyceride	16:0	1	1	
	16:3	21	22	25
	18:1	1	1	
	18:2	1	1	2
	18:3	77	76	72
Digalactosyl Diglyceride	16:0	4	4	3
	16:3	4	5	5
	18:1	1.5	1.5	2
	18:2	1	1	2
	18:3	89	89	87
Phosphatidyl Glycerol	16:0	11	11	11
	16:1	40	38	32
	18:2	1	5	4
	18:3	48	46	47
Sulfolipid	16:0	40	39	39
	16:3	1	1.5	
	18:2	6	7	6
	18:3	52	52	52

a) Fatty acids designated by number of carbons : number of olefinic bonds.

b) Reference 11.

capacity and structural conformation between these two membrane types cannot readily be explained on the basis of differences in glycerolipid composition. The protein is more likely to account for these differences in function and structure. Initial SDS gel electrophoresis observations in Park's laboratory indicate major differences in the protein composition of the grana lamellae and stroma lamellae fractions which will be the subject of a later report.

The enhanced chlorophyll and xanthophyll in the grana may also play a role in structure and function. Two recent galactolipid analyses by Bishop *et al* (16) and Wintermans (17) are relevant to the data reported here. Wintermans finds that stroma lamallae from glutaraldehyde fixed chloroplasts

TABLE III

Chlorophyll Content (in μmoles) of 10K and 200K Fractions Per Gram of Protein

Fraction	Experiment 1	2	3
10K(chla/chlb)	401 (2.5)	351(2.45)	350 (2.4)
200K(chla/chlb)	278 (5.0)	245(5.5)	238 (5.7)
$\frac{\text{10K chlorophyll content}}{\text{200K chlorophyll content}}$	1.44	1.43	1.47

have a galactolipid to chlorophyll ratio 1.5 times higher than grana lamellae. The data of Bishop *et al*, while derived from whole chloroplasts or whole cells rather than purified fractions, suggest the galactolipid to chlorophyll ratio of agranal plastids is 1.5 to 2 times that of grana containing plastids in sorghum and maize. Our data from Table I show the galactolipid to chlorophyll ratio of the 200K fraction is 1.4 times that of the 10K fraction, thus agreeing with the ratios of Wintermans, and Bishop *et al*, but indicating this is due to depletion of chlorophyll in the 200K fraction rather than increase in galactolipid content. In the experiment reported in Table I the galactolipid to chlorophyll ratio of a given fraction is less than that reported by Wintermans though we have also obtained ratios similar to his. From our work it appears there is not a constant ratio between galactolipid and chlorophyll, though the total lipid of the chloroplast lamellae remains fairly constant on a protein basis.

Our results do not tend to support the model of Kreutz (18) which requires a decreased monogalactosyl diglyceride to digalactosyl diglyceride ratio for galactolipid associated with photosystem 1.

It would appear that future work on these two types of photosynthetic membranes can be fruitfully directed along three lines. First, characterization of

the proteins composing the grana and stroma lamellae will contribute to better understanding of the differences in function and ultrastructure of these two membranes. Second, the hypothesis proposed by Sane and Park (8) that stroma lamellae are the first membrane synthesized and are elaborated and folded upon completion of photosystem 2 to form grana needs testing. Third, the interaction of the membrane protein and lipophilic components, and the structural-functional consequences of such interactions needs further investigation.

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