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By HARTMUT K. LICHTENTHALER** and RODERIC B. PARK

THE photosynthetic capacity of higher plant cells is localized within the chloroplast[1,2]. When viewed in thin section by electron microscopy, the chloroplast is seen to consist of a lamellar phase (grana and stroma lamellae) embedded in a matrix (the stroma). These two phases are surrounded by a membrane. The lamellar structures can be separated from the stroma matrix[3]. The separated lamellae contain the chlorophyll and about 50% of the protein nitrogen in the chloroplast[4]. They perform the light reactions and associated electron transport reactions of photosynthesis which lead to O_2 production, PPNR reduction and photosynthetic phosphorylation. The separated stroma material on the other hand contains the enzyme involved in the CO_2 fixation reactions of photosynthesis[3]. The lamellae appear to be made from subunits (quantasomes). Intact lamellae are not necessary to perform the light and electron transport reactions. Aggregates of 5 or 6 quantasomes are fully active in quantum conversion and electron transport as assayed by Hill reaction[4].

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Quantasome is the name given to the lamellar subunits which were initially observed by Frey-Wyssling and Steinman[5]. Further studies on the quantasome density and chemical composition have suggested that the quantasome may be a morphological expression of the physiological photosynthetic unit. Since quantasomes may represent the smallest functional photosynthetic unit able to carry out quantum conversion and electron transport, it is desirable to obtain as complete a chemical and physical picture of these particles as possible. The available experimental data on quantasomes can be summarized as follows. Quantasomes appear in the electron microscope as oblate spheres 100 x 200 Å. These particles are readily observed in shadowed preparations. They do not stain and are not readily observed in sectioned material. However, they are closely associated with the unit membrane structure of a chloroplast lamella. The quantasome aggregates are composed of 50% lipid and 50% protein[6]. Associated with the protein are the transition elements, Fe, Cu and Mn. The lipid portion includes the photosynthetic pigments, several quinones and tocopherols[7]. The pigments constitute ca. 23% of the lipid material. The remaining lipid is accounted for by various other lipids reported associated with photosynthetic tissue. These reports are widespread in the literature and often presented without recognizing the important role of these lipids in the structure of chloroplast lamellae. During the past 30 years a number of papers have reported the total amount of lipids, protein and pigments in leaves and chloroplasts of various higher plants. Most chloroplast analyses in fact have been performed on spinach, which is widely used in photosynthetic studies. In the

present paper we summarize the information on spinach chloroplast lipids and protein in the lamellae, on the basis of a minimum molecular weight of 960,000 per mole of manganese.

The early results on lipid and protein constituents of spinach chloroplasts suffer from uncertainties concerning chloroplast purity. However, a brief review of these analyses is useful as a background for our present knowledge of spinach chloroplast composition. In an investigation of cell organelles in spinach leaves, Menke separated the leaf tissue into 4 fractions[8]. The cytoplasm made up 13.5% and chloroplasts 21.1% of the leaf tissue. In this work, the cytoplasmic material contained 91% protein and only 0.5% lipids, on a dry weight basis. The chloroplasts contained 53% protein and 31% lipids. Menke's observations indicated that most of the leaf lipids were located in the chloroplasts. The chloroplast and cytoplasmic fractions in these experiments were not obtained by centrifugation, but were precipitated from leaf homogenates by ammonium sulfate fractionation.

In a second paper Menke isolated spinach chloroplasts by centrifugation and found that their composition was 48% protein and 37% lipids[9]. He pointed out that the "chloroplast substance" isolated in his earlier experiment was in fact about 15% cytoplasm. Menke specified that the ether soluble chloroplast lipids were a mixture of fatty acids, glycerides, phosphatides and pigments.

In 1942 Menke and Jacob separated the spinach chloroplast lipids into an acetone soluble and acetone insoluble fraction[10]. The acetone soluble fraction in which 80% of the total chloroplast lipids were present contained pigments, triglycerides, and sterols.

The acetone insoluble fraction consisted of phosphatides and waxes. This experiment, in fact, was again carried out on precipitated "chloroplast substance" and not on pure chloroplasts. Since Menke found that the cytoplasm contained only 1% of the leaf lipid, its presence in the chloroplast substances was not regarded as a serious contamination to the chloroplast lipids.

Zill and Harmon, however, found in spinach that "as much as one third of the whole leaf lipids were not present in chloroplasts"[11]. Thus the amounts of chloroplast lipids given by Menke and Jacob are uncertain, since it cannot be decided to what extent their chloroplasts were contaminated by cytoplasm. Moreover, it is not sure whether minor constituents such as sterols reported by those authors in their chloroplast substance also represent cytoplasmic contamination. Zill and Harmon also detected sterols in isolated chloroplasts, thus lending support to Menke's earlier observation concerning sterols. Since the sterol determination by Zill and Harmon was not quantitative, the sterol concentration found by Menke and Jacob (approximately 1.5% of the total chloroplast lipids) should be redetermined.

In 1942, Bot investigated the chemical composition of spinach "grana"[12]. The grana were isolated by repeated centrifugation of a ground spinach leaf suspension. They contained 42-54% protein, 4-6% chlorophyll, 26-32% lipoids (ether-alcohol soluble products) and a residue of 16-25%. This composition varied with the season and with the age of the tissue. Bot's results are similar to those of Menke. But since Bot gives no centrifugation data for the isolation of the grana, we doubt the purity of the grana fraction. This view is supported by the large unidentified residue obtained in the analysis.

Park and Pon found that fragments of spinach chloroplast lamellae washed free from stroma protein had a lipid to protein weight ratio of approximately 1[6]. Bot obtained a lipid to protein weight ratio of about 0.6[12]. Bot's protein value may be high, due to the inclusion of stroma protein or possibly cytoplasmic protein. On the other hand, incomplete extraction of the lipids would also yield a low ratio and may also have accounted for the high residue value of 16-25%.

Weber reported that lipids make up 33-36% of the chloroplast dry weight[13]. The residue after lipid extraction consisted primarily of protein, 10-20% of which was soluble in salt solution, the remainder (38-46%) being termed structural protein. Thus the lamellar lipid to protein weight ratio in this work was between 0.7 and 1.

Park and Pon prepared highly purified chloroplast lamellae which were active in Hill reaction and consistantly found a lipid to protein weight ratio of about 1[4,6]. Thus during the past 20 years analyses of the energy conversion apparatus of photosynthesis have yielded increasing lipid to protein weight ratios, primarily because early preparations contained non-lamellar protein.

Wintermans has determined the amount of chloroplast phospholipids as 53 moles of phospholipids per 105 moles of chlorophyll[14]. The distribution of compounds among the 53 moles was: 6 glycerophosphoryl inositol (GPI), 24 glycerophosphoryl glycerol (GPG), 3 glycerophosphoryl ethanolamine (GPE), 19 glycerophosphoryl choline (GPC), and 1 glycerophosphate (GP). He has also determined that the digalactosyldiglyceride and monogalactosyldiglyceride concentration in chloroplasts is 66 and 158 moles per 105 moles of

chlorophyll, respectively[15]. Benson et al. reported the presence of a sulfolipid 1-O-oleoyl-3(β-galactopyranosyl-6-sulfate)-1-glycerol in chloroplasts[16]. Wintermans has reported its abundance as 22 moles of sulfolipid per 105 moles of chlorophyll[15]. Zill and Harmon separated the lipids of spinach chloroplasts and whole spinach leaves up into several fractions by chromatography on silicic acid[11]. They found that glycolipids, phospholipids and sulfolipids are the major lipid classes in chloroplasts. Non-polar lipids such as waxes, hydrocarbons, cerylalcohol, which make up a significant fraction of the lipids of whole leaves, were not present. They reported that pigments, phospholipids, glycolipids, digalactosylglycolipids and diglyceride make up 86% of the total spinach chloroplast lipids. The rest consisted of waxes and hydrocarbons (2%), probably a contamination from the non chloroplast portions of the leaf, and other unidentified lipids.

The fatty acids for whole spinach leaves were given by Speer, Wise and Hart with linoleic acid 34.7%, oleic acid 26.3%, and linolenic acid 12.7%, as the main components[17]. According to this report, 53% of the fatty acids occurred in the free form and 47% as glycerides. The total fatty acid content of spinach chloroplasts was determined more recently by Wolf et al., and Debuch, as linolenic acid 68.9 and 47.8%, an unidentified C_{16} acid with 3 double bonds 10.8 and 19.5%, palmitic acid 11.2 and 15.5%, linoleic acid 4.6 and 5.0%, respectively, and trace amounts of others[18, 19]. These values were obtained by gas chromatography and are certainly more accurate than those obtained by distillation of the saponified lipid extract from whole leaves. However, neither

Wolf et al. nor Debuch made distinction between free and esterified fatty acids and their relative abundance compared to other lipids was not indicated. Thus the amount of free fatty acids in spinach chloroplasts on a weight basis or in relation to other chloroplast lipids is still uncertain.

The concentrations of quinones in spinach chloroplasts were determined more recently[20,21]. The distribution of quinones and carotenoids in relation to the chlorophylls for chloroplasts and quantasomes was determined in this laboratory[7].

From all the data now available it is possible to calculate the relative concentrations of lipid components in spinach chloroplast lamellae. Since manganese is present in low concentration in chloroplast lamellae and is required for oxygen evolution in photosynthesis[22], Park and Pon calculated a minimum molecular weight of 9.6×10^5 for a photosynthetic unit, based on 1 manganese atom[6]. The calculation of the quantasome mass, the morphological 200 x 100 Å subunit of chloroplast lamellae, from density and volume measurements yielded a molecular weight between 1 and 2 times the minimum molecular weight given above. Park and Pon reported that lipids make up 52% of the chloroplast lamellae, while the remaining 48% is protein. Thus lipid and protein contribute to the minimum molecular weight 495,000 and 465,000 respectively.

In Table I, the lipid and protein portion of the lamellae are presented. The lipid portion is broken down to show the relative amounts of various lipids based on the analyses reviewed above. It is an interesting fact that the quinone tocopherol fraction contributes more to the lipid weight than the total carotenoids.

Table 1. Representative distribution of substances in spinach chloroplast lamellae on basis of minimum molecular weight of 960,000 per mole of manganese.

Lipid (Composition in Moles/Mole Mn)			
115 chlorophylls[6,7]		103,200	
80 chl. a	71,500		
35 chl. b	31,700		
24 carotenoids[7]		13,700	
7 β-carotene	3,800		
11 lutein	6,300		
3 violaxanthin	1,800		
3 neoxanthin	1,800		
23 quinone compounds[7]		15,900	
8 plastoquinone A	6,000		
4 plastoquinone B	4,500		
2 plastoquinone C[21]	1,500		
4.5 α-tocopherol	1,900		
2 α-tocopherylquinone	1,000		
2 vitamin K_1	1,000		
58 phospholipids[14] (phosphatidylglycerols)		45,400	
7 GPI			
26 GPG			
3 GPE			
21 GPC			
1 GP			
72 digalactosylglyceride[15]		67,000	
173 monogalactosyldiglyceride[15]		134,000	
24 sulfolipid[15]		20,500	
? sterols[10]		7,500	
Unidentified lipids		87,800	
			495,000
Protein			
4,690 N atoms as protein		464,000	
1 Mn		55	
6 Fe		336	
3 Cu		159	
			465,000
LIPID + PROTEIN			960,000

The known components make up roughly 82% of the total lipids in chloroplast lamellae. The unidentified portion (ca. 18%) probably consists mostly of minor constituents which are present in chloroplasts. These constituents are either not reported quantitatively or the values available are inaccurate. To these unidentified constituents belong free fatty acids, free phytol, protochlorophyll, phaeophytin, xanthophyll esters, antheroxanthin, phytoene, phytofluene, tocopherols and tocopherylquinones other than α-tocopherol and α-tocopherylquinone, as well as other not yet identified lipids. The lipid composition shown in Table 1 undoubtedly undergoes considerable modification from species to species and within a plant species depending on the physiological conditions. However, such a catalog of lamellar composition is useful for it provides some of the information from which it may be possible to construct models of the photosynthetic quantum conversion apparatus.

Two kinds of information are still needed for construction of an accurate model for photosynthetic quantum conversion and electron transport. First, we must characterize the various proteins making up one half of the lamellar structure. Second, we must find the spatial arrangement between the lamellar proteins and the various lipids. Studies utilizing fluorescence, spectrophotometry and electron miscroscopy, and dichrpism measurements, are beginning to provide information as to the nature of this arrangement. For example, such studies have shown that chlorophyll exists in lamellae in several physical forms[23,24]. One of these forms of chlorophyll (700 mμ) is oriented with respect to the quantasome axis[25]. Sauer and Calvin have suggested this oriented chlorophyll may be the site for actual conversion of an exciton (electromagnetic energy) into charge separation (chemical energy).

However, the structural relationships on a molecular or supramolecular level between the many other lamellar lipids and as yet undefined lamellar proteins are unknown. The catalogue in Table 1 presents both the basic chemical composition of a photosynthetic unit and in electron microscopic terms the basic chemical composition of the photosynthetically specialized unit membrane. It hopefully will become even more useful with future additions and corrections. When this catalogue is combined with other physical measurements it will provide some of the boundary conditions necessary for construction of an accurate molecular model for the quantum conversion apparatus in photosynthesis.

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