

Distribution of Protein-bound Hexosamine in Chloroplasts

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ABSTRACT

Intact chloroplasts of spinach (*Spinacia oleracea* L.), sunflower (*Helianthus annuus* L.), and maize (*Zea mays* L.) mesophyll cells contained 0.33, 0.50, and 0.14% of bound hexosamine on a protein basis, respectively. Undifferentiated maize chloroplasts contained 0.19%. Values for chloroplast lamellae were, respectively, 0.16, 0.18, 0.12, and 0.06% and for envelope membranes they were 1.6, 2.5, 3.8, and 2.7%. Thus most of the hexosamine of chloroplasts is located in the envelope membrane.

Hexosamine and Protein Assays. Protein was determined with Folin reagent (9) with BSA as a standard. Protein-bound hexosamine was measured by the Cessi modification of the Elson-Morgan reaction as described previously (6, 17). The protein-bound hexosamine was separated from hexosamine associated with lipids and low mol wt polar compounds by extraction with 80% acetone to remove the latter. After acid hydrolysis in 1 N HCl at 100 C for 18 hr. the liberated hexosamine was converted to 2-methylpyrrole and steam distilled into *p*-dimethylbenzaldehyde. The resulting chromogen was determined colorimetrically at 548 nm with glucosamine as a standard.

RESULTS AND DISCUSSION

Hexosamine in bound form is a common constituent of green leaves (17). In spinach leaves part of this hexosamine is bound to chloroplast proteins (4). These hexosamines are often associated with glycoproteins, which occur in cell walls and membranes, or as soluble proteins and enzymes (2, 12). Since the major soluble stromal protein of chloroplasts, ribulose biphosphate carboxylase, is not a glycoprotein (18; unpublished data of D. Racusen), it follows that chloroplastic hexosamine must be localized in the envelope membrane, the lamellae, or in some stromal protein present in low concentration. We show that most of the hexosamine in chloroplasts of spinach, maize, and sunflower leaves is located in the envelope membrane and a much lesser amount is found in the lamellae.

In the only previous investigation of hexosamine in chloroplasts (4), its location within the chloroplast was uncertain. The present value of 0.33% for intact spinach chloroplasts agrees with that determined by Izumi (4). Table I shows that most of the protein-bound hexosamine of spinach, sunflower, and maize chloroplasts (1.4-5 $\mu\text{g}/\text{mg}$ protein) is present in the envelope membranes (16-38 $\mu\text{g}/\text{mg}$ protein) as opposed to the lamellae (0.6-1.8 $\mu\text{g}/\text{mg}$ protein). This and other differences between the two kinds of membranes in Chl content, the lipid composition, and the types of ATPase undoubtedly reflect their functional differences; *i.e.*, the involvement of the envelope membrane in CO₂ assimilation and metabolite transport, and of lamellar membranes in photosynthetic electron transport (1, 10, 13-16).

MATERIALS AND METHODS

Plant Material and Growing Conditions. Maize (*Zea mays* L., hybrid 595 S, Agway Inc.), spinach (*Spinacia oleracea* L., var Viroflay, Asgrow Seed Co.), and sunflower (*Helianthus annuus* L., Hybrid 896 [cmsHA 89 \times RHA 266], a gift of G. Fick, U.S. Department of Agriculture at North Dakota State University) were grown in vermiculite in a greenhouse. Nutrient solution was added twice weekly.

The relatively low hexosamine content of the young maize lamellae (Table I) would appear to correlate with the immaturity of these membranes, since undifferentiated maize chloroplasts have poorly developed lamellae (11). The mesophyll maize chloroplasts, with fully developed lamellae, have lamellar hexosamine contents twice as great (Table I).

Chloroplasts. Intact mesophyll chloroplasts were isolated from 10-g batches of freshly harvested, rinsed leaves (4-6 weeks of age) from spinach (5), maize (20), and sunflower (3). Young maize leaves 4 to 6 days old were used to prepare undifferentiated intact chloroplasts (11).

An approximation of the envelope membrane protein and protein-bound hexosamine content given as a percentage of the total protein and hexosamine present in the chloroplast is shown in Table II. These estimates are based on the data in Table I and the assumptions that the stromal protein contains no hexosamine and it constitutes 50% of the total chloroplast protein. These values (Table II) indicate that most of the protein-bound hexosamine of the chloroplast is located in the envelope membrane.

Chloroplast Envelope Membranes and Lamellae. Complete envelope membranes and lamellae were isolated from the above chloroplasts on a three-phase discontinuous sucrose gradient, as described previously (16, 16a). These gradient fractions, after addition of isopropyl alcohol to 10% (v/w) to prevent microbial attack, were dialyzed against running tap H₂O and finally distilled H₂O.

These results are only estimates, because of the possible contamination or loss of material during the isolation of the subcellular fractions. Another possible source of error results from the use of six analyses (three for hexosamine and three for protein); thus the errors may be additive. Another uncertainty results from the above assumptions regarding the stromal protein content.

As noted earlier, the major protein of the stroma is not a glycoprotein, but still there may be small amounts of other stromal protein that are rich in hexosamine. The other assumption that one-half of the mesophyll chloroplast protein is in the stroma is a reasonable approximation for spinach and maize (7, 8). This is probably true for sunflower chloroplasts also, but less certain for undifferentiated maize chloroplasts with their poorly developed lamellae and perhaps a correspondingly higher stromal content (11). However, the major conclusion shown in Table II is not changed even if the assumptions err. Calculations assuming 50% stromal protein compared to those assuming no stromal protein do not alter the conclusion that the envelope membrane contain most of the hexosamine.

Direct estimates from sucrose gradients of the percentage of envelope membrane protein on a total chloroplast protein basis range from 2 to 4% for the species under discussion (16a). These are lower than the calculated values (Table II), but may suffer from uncertainties in total recovery of envelope membrane protein. Assuming lower values estimated from protein recovery are correct, significant amounts of hexosamine are still bound to the envelope membrane protein.

The high levels of protein-bound hexosamine and glycolipids present in the chloroplast envelope membrane (Table I; refs. 1, 10, 13) in comparison to the low levels of phospholipids indicate a predominantly glycoprotein structure. This is in contrast to animal mitochondria, where the envelope membrane appears to

be primarily lipoprotein, with the bulk of the lipids being phospholipids (19). What effect these differences in composition of the membrane have upon structure and permeability is presently unknown.

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Table I. Hexosamine Content of Chloroplast Proteins

	Envelope Membrane	Lamellar	Intact Chloroplast
		% Protein	
Spinach	1.6	0.16	0.33
Maize (young)	2.7	0.056	0.19
Maize (old)	3.8	0.12	0.14
Sunflower	2.5	0.18	0.50

Table II. Estimates of Envelope Membrane Protein and Hexosamine on Basis of Total Chloroplast Protein and Hexosamine

	Chloroplast Protein in Envelope Membranes	Chloroplast Hexosamine in Envelope Membranes
	% of total	
Spinach	17	82
Maize (young)	6.2	89
Maize (old)	2.2	59
Sunflower	18	90