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SHORT COMMUNICATION

HEXOSAMINES IN PURIFIED CHLOROPLASTS FROM SPINACH LEAVES

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Abstract—Hexosamines have been found in purified chloroplasts from spinach leaves.

HEXOSAMINES are generally known to be essential components in glycoproteins, and in animal tissues they have recently been found in subcellular membranous organelles, especially in smooth-surfaced microsomes and purified mitochondria.[1,2] The present communication reports the presence of hexosamines in chloroplast, also a membranous organelle of plant tissues. Thus, the purest chloroplast fraction prepared by repeated fractional centrifugation from the leaves of *Spinacia oleracea* has been found to contain 3·6–4·0 μg/mg protein of hexosamines which are nearly comparable to the content in microsomes and mitochondria from animal cells.[2]

Column- and paper chromatography showed more than 90 per cent of the hexosamines to be glucosamine and the rest to be galactosamine. In order to obtain information on the chemical nature of the hexosamines, the purified chloroplast fraction was suspended in a hypotonic medium (0·002 M potassium phosphate buffer, pH 7·0) and centrifuged at 15,000 *g* for 30 min. The precipitated chloroplast membrane fraction was further extracted thoroughly with acetone and chloroform–methanol (2:1, v/v) successively. A greater part (about 75 per cent) of the hexosamines contained in the whole chloroplast was present in the final insoluble residue. From this finding, it is suggested that the hexosamines are firmly bound to non-lipid macromolecules which constitute the membranous structure in chloroplast.

Proteolytic digestion of the defatted chloroplast membrane was then carried out by suspending it into 0·04% Pronase solution at 37° for 48 hr. After centrifugation, the hexosamines released into the supernatant were estimated, and found to be around 50 per cent of the original content in the membrane. Thus, it seems probable that the hexosamine-containing molecules in the chloroplast are some sorts of glycoproteins.

It should be remembered that the hexosamines in microsomes and mitochondria were suggested also to be bound to non-lipid macromolecules of protein nature.[3] The chemical similarity of structural proteins in these membranous organelles is also known.[4] Doris van Wyk[5] has shown the binding of some neutral sugars to the structural protein in chloroplasts

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from several species of higher plants. Although it remains to be seen whether the hexosamines in spinach chloroplasts are also components of this protein, the results given here strongly suggest that hexosamines, possibly as components of proteins, might be universal constituents of subcellular membranous structures both in the animal and the plant kingdom.

EXPERIMENTAL

Fresh spinach leaves were homogenized with 5 vol. of 0·4 M sucrose–0 06 M potassium phosphate buffer (pH 7 0) in a Waring blendor and centrifuged at 80 *g* for 5 min. The supernatant was further centrifuged at 800 *g* for 12 min. The precipitated chloroplast fraction was suspended in the same buffer and purified by repeating centrifugation at 80 *g* and 800 *g* each twice more. The purest final fraction showed a constant ratio of chlorophyll to protein content (162–166 μg/mg protein).

Hexosamine was determined by the method of Boas[6] after hydrolysis in 2 N HCl at 100° for 16 hr. Identification of hexosamine in the hydrolysate was performed by the column chromatography according to Gardell[7] and also by paper chromatography directly and after ninhydrin degradation.[8] Chlorophyll was determined by the method of Arnon[9] and protein by the method of Lowry *et al.*[10]

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