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Intracellular localisation of phosphoglycollate phosphatase and glyoxalate reductase

The mechanism of formation of glycollic acid in photosynthesis is as yet uncertain. It is most probably derived from a sugar phosphate of the photosynthetic carbon reduction cycle[1,2] although some workers have suggested that it is derived *de novo* from an as yet unknown carboxylation reaction[3,4].

A possible precursor of glycollate is phosphoglycollic acid and a specific phosphatase (phosphoglycollate phosphohydrolase, EC 3.1.3.18) has been described by RICHARDSON AND TOLBERT[5]. Whilst this enzyme has been reported to be predominantly cytoplasmic, its site of action has not been rigorously established.

A glyoxalate reductase (glycollate:$NADP^+$ oxidoreductase) which catalysed the reduction of glyoxalate to glycollate by NADPH has been described and partially purified by ZELITCH AND GOTTO[6]. Apart from the observation by ZELITCH AND BARBER[7] that NAD glyoxalate reductase activity was present in dilute suspensions of chloroplasts, its site of action has not been investigated in higher plants.

The distribution within the cell of phosphoglycollate phosphatase and glyoxalate reductase has been determined and the results discussed in relation to glycollate synthesis and metabolism.

Chloroplasts were isolated from freeze-dried spinach leaves on a non-aqueous density gradient as described by BIRD, PORTER AND STOCKING[8]. A suitable discontinuous density gradient which gives high yields of chloroplasts with minimal cytoplasmic contamination consisted of 15 ml of density 1.36 g/cm^3 containing 50 mg of homogenised and filtered leaf material followed by an equal volume of density 1.34 g/cm^3 and 2 ml density 1.32 g/cm^3. The solvents used in making up the different densities were carbon tetrachloride and hexane. The yield of chloroplasts on a dry weight basis was between 4–6 % of the initial freeze-dried material. More than 55 % of the total cell protein remained with the chloroplasts. No mitochondrial activity (for which fumarase and succinate dehydrogenase were considered as characteristic) was detected in these preparations. Under the light microscope, cell debris could not be seen in these preparations. The chloroplasts appear to have the stroma matrix intact[9,10]. Chlorophyll was measured by the method of ARNON[11] and protein by the method of LOWRY *et al.*[12].

The distribution of enzyme between the chloroplast and the cytoplasm was calculated by the method of HEBER[13]. From the specific activities of chloroplasts, intact tissue and chloroplast depleted tissue, it is possible to obtain a value for the specific activity of pure cytoplasm. The percentage of the total enzyme activity of the homogenate present in the chloroplast fraction could then be calculated. The intracellular distribution of phosphoglycollic acid phosphatase and glyoxalate reductase is shown in Tables I and II. Between 89–97 % of the total cell phosphoglycollate phosphatase and 86–98 % of the whole cell glyoxalate reductase were found to be localised in the chloroplasts.

Neither enzyme could be detected in chloroplasts from a glycerol gradient (prepared as described by LEECH[14] and JAMES AND DAS[15]). Assuming that the loss of activity was not due to solvent inactivation it was concluded that both enzymes were localised in the soluble stroma of the chloroplasts rather than in the lamellae.

The enzyme localisation is consistent with the view that glycollate is synthesised in a chloroplast sited reaction from both phosphoglycollate and glyoxalate. The chloroplast is also most probably the site of phosphoglycollate synthesis. KEARNEY AND TOLBERT[16] found phosphoglycollate as well as glycollate in the supernatant fluid

TABLE I

ACTIVITY AND DISTRIBUTION OF PHOSPHOGLYCOLLATE PHOSPHATASE

Reaction mixtures contained in a final volume of 0.31 ml the following components in μmoles: Mg^{2+}, 0.3; Tris buffer (pH 7.5), 30; enzyme suspension and water. The reaction was started by the addition of 2 μmoles of phosphoglycollate and the amount of inorganic phosphate released was measured colorimetrically by the method of HURST[19].

	Expt. No.	
	1	*2*
Chloroplast protein in total protein (%)	73.5	56.0
Specific activity (μmoles P_i/min per mg protein)		
in chloroplasts	3.2*	4.46
	3.53**	5.3
in intact tissue	2.64	2.63
in chloroplast depleted tissue	2.00	1.73
in cytoplasm	1.08*	0.3
	0.202**	0.057
Yield of isolated chloroplasts		
(% of total chlorophyll content of tissue)	32.5	45.0
Total enzyme present in chloroplasts (%)	89.0*	94.5
	96.8**	98.7

* Results uncorrected for cytoplasmic contamination.

** Corrected values assuming that not more than 10% of the total cytoplasmic activity is associated with the chloroplasts.

TABLE II

ACTIVITY AND DISTRIBUTION OF NADPH GLYOXALATE REDUCTASE

Enzyme activity was measured according to ZELITCH AND GOTTO[6].

	Expt. No.	
	1	*2*
Chloroplast protein in total protein (%)	71.6	72.0
Specific activity (mμmoles NADPH/min per mg protein)		
in chloroplasts	21.53*	6.0
	24.5**	6.86
in intact tissue	16.3	4.5
in chloroplast depleted tissue	14.4	3.63
in cytoplasm	3.11*	0.64
	6.2**	0.204
Yield of isolated chloroplasts		
(% of total chlorophyll content of tissue)	49.7	35.0
Total enzyme present in chloroplasts (%)	94.0*	96.0
	86.0**	98.2

* Results uncorrected for cytoplasmic contamination.

** Corrected values assuming that not more than 10% of the total cytoplasmic activity is associated with the chloroplasts.

after separation of chloroplasts. It is probable that phosphoglycollate and glycollate are both derived from a sugar diphosphate of the photosynthetic carbon reduction cycle.

The function of glyoxalate reductase in chloroplasts is as yet unknown. It may be involved in the synthesis of amino acids within the chloroplast. Both SMITH, BASSHAM AND KIRK[17] and STEWARD, BIDWELL AND YEMM[18] have found that although glycine was a frequent constituent of plant leaf protein, the free acid was synthesised very slowly. It was suggested that the glycine residues in chloroplast protein were derived from the cytoplasm—perhaps from a 2-carbon compound such as glycollate. KEARNEY AND TOLBERT[16] have demonstrated glycine and glycollate formation in chloroplasts from glyoxalate.

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