

Table 1. *Distribution of Glycolate and DHAP Between the Chloroplast Pellet and Supernatant Fraction After Assay Under Varying Levels of Pi and NaHCO₃*

After 90 s of incubation in the dark, the chloroplasts were illuminated for 6 min prior to centrifugation to obtain chloroplasts and the extrachloroplastic fraction. Photosynthetic O₂ evolution is expressed as maximum rates obtained during the assay period and the total amount of O₂ evolved during the 6-min assay. In separate experiments, following centrifugation the distribution of exogenous sucrose (20 mM) and NADP-glyceraldehyde-3-P dehydrogenase (NADP-G3PDH) was determined to correct the cross contamination of the chloroplast and cytoplasmic fractions (see under "Materials and Methods"). From two replications, the percentage of the sucrose retained in the chloroplast fraction was only 1.15%, whereas the percentage NADP-G3PDH in the supernatant fraction was 30.8%. The corrected values for distribution of glycolate and DHAP were calculated assuming sucrose is in the extrachloroplastic fraction and NADP-G3PDH is a chloroplastic enzyme.

Where: *C* = percentage of metabolite in the chloroplast,
E = percentage of metabolite in the extrachloroplastic fraction,
P = percentage of metabolite in the pellet, and
S = percentage of metabolite in the supernatant, then, $C + E = P + S$

$$C = \frac{P - (\text{fraction of sucrose in the pellet}) (E)}{(\text{fraction of intact chloroplasts})} = \frac{P - (0.0115) (E)}{0.692}$$

$$E = \frac{S - (\text{fraction of broken chloroplasts}) (C)}{(\text{fraction of sucrose in the supernatant})} = \frac{S - (0.308) (C)}{(0.9884)}$$

Determination	Condition		Maximum Rate of O ₂ Evolution	O ₂ Evolved	Metabolite Formed	Measured Values of Metabolite or Enzyme Distribution		Corrected Value for Distribution of Metabolites	
	Pi	NaHCO ₃				Precipitate (P)	Supernatant (S)	(C)	(E)
	mm	μmol/mg Chl·h				μmol/mg Chl·6 min	%	%	
Glycolate	0	0.3	40.0	3.08	1.69	8.5	91.5	10.8	89.2
Glycolate	0	0.3	39.0	3.11	1.48	11.1	88.9	14.6	85.4
Glycolate	0.025	0.3	30.4	2.34	1.09	2.3	97.7	1.6	98.4
Glycolate	0.025	0.3	28.7	2.06	0.87	5.1	94.9	5.7	94.3
DHAP	0.1	10	99.0	7.10	0.56	2.6	97.4	2.2	97.8
DHAP	0.1	10	89.2	6.43	0.51	2.3	97.7	1.6	98.4