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Non-random Distribution of Calvin Cycle Enzymes in the Pea Leaf Chloroplast

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Immunocytolocalization experiments indicate that carbonic anhydrase, phosphoribulokinase, and P-glycerate kinase are near neighbors of Rubisco [1], that P-glycerate kinase and glyceraldehyde-3-P dehydrogenase subunit A are located close to one another [2], that the enzymes glyceraldehyde-3-P dehydrogenase, triose-P isomerase and aldolase are located close to one another, that aldolase is located close to sedoheptulose bisphosphatase [3], that glyceraldehyde-3-P dehydrogenase subunit B is co-localized with transketolase (Fig. 1), that aldolase and phosphoriboisomerase are located close to transketolase, and that phosphoriboisomerase is located close to phosphoribulokinase, in the pea leaf chloroplast stroma [this abstract]. Direct transfer of intermediates between these enzymes during CO_2 fixation is then a possibility. Spatial organization of these enzymes may be important for efficient CO_2 fixation in photosynthetic organisms.

In contrast, there is no indication that fructose bisphosphatase is co-localized with aldolase [3], and neither fructose bisphosphatase, sedoheptulose bisphosphatase [this abstract], or the A subunit of glyceraldehyde-3-P dehydrogenase (Fig. 2) is co-localized with transketolase. Substrate channeling between these enzymes seems unlikely.

With the exception of fructose bisphosphatase and sedoheptulose bisphosphatase, each enzyme of the Calvin cycle is apparently co-localized with the enzyme that forms its substrate and with the enzyme that utilizes its product (Fig. 3). Even if not every one of these co-localized enzymes are directly adjacent, their proximity to one another will obviate the need for high levels of phosphorylated intermediates and is almost certainly necessary for efficient photosynthetic CO_2 fixation.

Thin sections were prepared from pea leaf tissue fixed in 1% acrolein, 0.1% glutaraldehyde and embedded in LR White resin. The grids were floated on solution containing the primary antibodies overnight. Exposure to the gold labeled secondary antibodies was for 4 hours the following morning. We used the method of J.B. Anderson et al. [2] for analysis of nearest neighbor distances on the micrographs from the double labeling experiments. For a population of two different non-interacting species the expression $n/N = 1 - \exp(-\pi r^2 \rho)$ gives the fraction n/N corresponding to position in an ordered list of samples with increasing nearest-neighbor distance r, where n is the number of the measurement in rank order, N is the total number of measurements, r is the distance between nearest neighbors, and ρ is the species density. A plot of $-\ln(1-n/N)$ versus r^2 produces a straight line, if the two species are distributed randomly. Where there is positive interaction the initial data points will be displaced towards the $-\ln(1-n/N)$ axis and the curve will balloon out toward that axis. We measured the distance from the center of each large gold particle to the center of the nearest small gold particle using Scion Image (Scion Corporation, Frederick, MD) and plotted $-\ln(1-n/N)$ against r^2.

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[2] J.B. Anderson et al. J. Structural Biol. 143 (2003) 95.
[3] L.E. Anderson et al. Photosyn. Res. 83 (2005) 317.

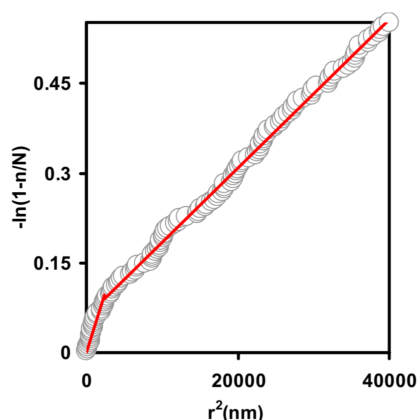

Fig. 1. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking transketolase and gold particles marking glyceraldehyde-3-P dehydrogenase subunit B. The biphasic curve indicates co-localization.

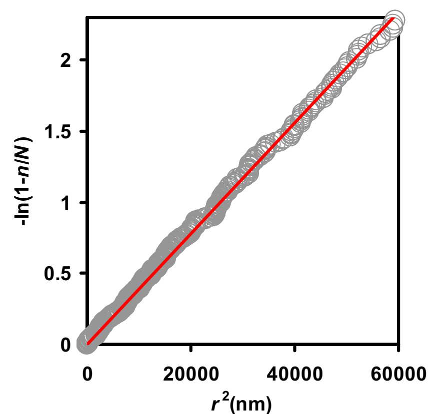

Fig. 1. Plot of the negative log of 1 – fraction in the ordered list of measurements against the square of the distance between nearest neighbor gold particles marking transketolase and gold particles marking glyceraldehyde-3-P dehydrogenase subunit A. The straight line indicates that the enzymes are distributed randomly with respect to one another.

	CA	RUB	PGK	A	B	TPI	ALD	FBP	SBP	TK	PRI	PRK
CA		+										
RUB	+		+	+	+	-					+	+
PGK		+		+	-							
A		+	+		+	+	+			-		+
B		+	-	+		+	+			+		+
TPI		-		+	+		+			-		
ALD				+	+	+		-	+	+		
FBP							-			-		
SBP							+			-		
TK				-	+	-	+	-	-		+	
PRI		+								+		+
PRK		+		+	+						+	

Fig. 3. Co-localization of Calvin cycle enzymes within the chloroplast. '+' denotes co-localization, '-' no evidence for co-localization. Red symbols above diagonal, enzymes that act in sequence. CA, carbonic anhydrase; RUB, Rubisco; A, glyceraldehyde-3-P dehydrogenase A subunit; B, B subunit, PGK, P-glycerate kinase; TPI, triose-P isomerase; ALD, aldolase; FBP, fructose bisphosphatase;.SBP, sedoheptulose bisphosphatase; TK, transketolase; PRI, phosphoriboisomerase; PRK, P-ribulokinase.

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