SOME FACTS AND THOUGHTS ABOUT AMYLOPLAST ENZYME COMPARTMENTATION

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1. Of enzymes so far implicated in starch synthesis, only two -- starch synthetase (ADP-gJucose —• starch + ADP) and phosphorylase (glucose-1-phosphate——• starch + iP) — sediment with normal pea and maize amyloplasts from developing seeds.

2. Substrate affinity (for starch) alone fails to account for amyloplast binding of these enzymes (cf. Tsai, Bchm. Gen. 11:83-95, 1974, for starch synthetase and item 7, below, for phosphorylase).

3. Observation of a significant difference in R/R vs r/r peas with regard to soluble phosphorylase level dates back at least to 1957 (NAS-NRC, Laboratory and Field Studies in Biology: A Sourcebook for Secondary Schools, pp. 681-688, 1957). Matheson and Richardson (Phytochem. 15:887-892, 1976, and 16:1875-1879, 1977), using extraction procedures which are inadequate to release much amyloplast bound phosphorylase (see PNL 10:78), recently quantified this difference in developing, mature, and germinating R/R and r/r peas.

4. Matheson and Richardson (1977) mention that mixtures of phosphorylase II (the larger of two phosphorylase isozymes) extracted from bananas and R/R peas can be resolved electrophoretically, while similar mixtures from bananas and r/r peas cannot. I find that phosphorylase II released from R/R amyloplasts has a slightly slower disc electrophoretic migration rate than soluble phosphorylase II from r/r seeds. Phosphorylase II from R/r seeds is resolved by disc electrophoresis into three bands, confirming Matheson and Richardson's finding that this pea enzyme is dimeric.

5. In the light of 2, 3, and 4, above, I suggest that phosphorylase II has an "amyloplast binding site" which is separate and distinct from its "catalytically active site", and that the former is defective or missing in r phosphorylase II.

6. Among maize, potatoes, and peas, I find that only developing pea amyloplasts can be ruptured osmotically by pelleting from concentrated sucrose solution and resuspending in water. Microscopic examination reveals significant ballooning of the amyloplast membrane even among similarly treated mature pea amyloplasts. Other workers have indicated unusual difficulty in leaching soluble starch from pea amyloplasts (e.g., Potter, et al., J. Am. Chem. Soc. 75:1335-1338, 1953) and in deproteinizing pea starch (Senti and Dimler, Food lech. 63:663-666, 1959).

7. Immature amyloplast fragments from r/r peas bind about 3 times as much R/R as r/r phosphorylase II when exposed under uniform conditions. Pretreatment of the ruptured amyloplasts with papain tends to nullify this difference, indicating that protein(s) plays a role in the specificity of phosphorylase "receptors" in the amyloplast (membrane?). Use of the r gene "probe" adds an interesting dimension to amyloplast-phosphorylase binding studies such as those of Fekete (Arch. Bchein. Bphys. 116:368-374, 1966). I am presently using starch conjugate (-active site?) bound phosphorylase (see Matheson and Richardson 1977) to search for specific amyloplast proteins with phosphorylase affinities. I'm assuming that these "receptors" are probably bound into the amyloplast membrane via hydrophobic regions as discussed by Tanford (Science 200:1012-1018, 1978).

8. The R/R variety 'Alaska' (used by Matheson and Richardson) has a soluble phosphorylase level late in development which is about twice as high as that of at least three other R/R_ varieties I have tested. R/R varietal hybrid seeds from both 'Alaska' x 'Mammoth Melting Sugar' and 'Alaska' x 'Dwarf Grey Sugar' have higher soluble phosphorylase levels when 'Alaska' is used as the female parent than when 'Alaska' is used as the pollen parent. According to Marshall (PNL 2:18-19), 'Alaska' carries the gene di_ (dimpled) which is expressed as a maternal character; di_ may therefore be involved in organization of amyloplast receptors for phosphorylase alluded to in 7, above.

9. For possible future research, I suggest that saturation of amyloplast receptors with phosphorylase may somehow signal a "shutdown" of phosphorylase synthesis. Such a system of regulation could be essential in the developing seed since phosphorylase may act both in starch synthesis (when bound??) and in starch digestion (when unbound??-hence the phenotype of r/r peas and starch??).

10. Suggestions 5 and 9, above, may also hold true for starch synthetase in maize. Tsai (Maize Genetics Newsletter 39:153, 1965) has shown that the wx (waxy) gene decreases amyloplast bound starch synthetase level in a dose dependent' manner. No concomitant increase in soluble enzyme is found, indicating that wx probably disrupts the catalytic site of starch synthetase. The striking dose effect of wx and the lack of increase in soluble enzyme by wx may be due to competitive binding of limited supplies of catalytic (Wx) and non-catalytic (wx) enzyme proteins by amyloplast receptors (presumably, the binding site within the mutant "enzyme" and the system of regulation could be intact) in heterozygotes. Nelson (Maize Genetics Newsletter 50:109-113, 197b) has constructed a fine structure map containing over 30 wx alleles, but very little is known about the actual nature of the wx gene product(s). If antibodies can be raised to maize starch synthetase, I plan a quantitative search for cross reacting material in wx homo- and heterozygotes.

11. I have so far been unable to associate any lesion in the starch synthetase or phosphorylase pathways to starch nor any differences in starch synthetase or phosphorylase solubilities with the rb gene in peas. Nor have I been able to find the solute primarily responsible for higher osmotic potential of rb cytoplasm. Some of my assays need refinement, however.

12. Isolation of new mutants affecting catalytic activity of pea phosphorylases or electrophoretic mobility of both pea and maize phosphorylases and starch synthetases should provide excellent raw material for further study of enzyme compartmentation in the amyloplast. Help in identifying possible candidates is hereby solicited from pea geneticists, especially those who regularly engage in mutagenesis. If, as suggested here, separate sites for organelle binding and catalytic activity exist within the structure of an enzyme (and therefore may be coded within the same locus), they could be expected to mutate separately. Whether or not such neighboring mutants would result in identical phenotype and/or be detected as alleles in classic tests of allelism remains to be seen. Since seed "lethals" may be of value in my studies, I maintain pod and plant sib identification records in my own mutagenic work.