

S17-027

**Phosphoenolpyruvate carboxylase isoforms from an aquatic monocot:
structure/function relationships**

Rao SK, Reiskind JB, Bowes G

Department of Botany, University of Florida, Gainesville FL 32611-8526, USA, FAX 352-392-3993, email gbowes@botany.ufl.edu

Keywords: C₄ photosynthesis, *Hydrilla*, molecular model, PEPC isoforms

Introduction

Hydrilla verticillata is a submersed monocot with C₃ characteristics, but in low [CO₂] C₄ photosynthesis is induced, producing a unique "minimalist" system that functions without Kranz anatomy (Bowes and Salvucci 1989). Recent studies have focused on the PEPC gene family and its role in the shift from C₃ to C₄ (Rao et al submitted). PEPC catalyzes the initial carboxylation in the C₄ system, as well as anapleurotic reactions in C₃ and C₄ plants. Kinetic and regulatory studies of PEPC from C₄ and C₃ *Hydrilla* shoot extracts (Reiskind et al unpublished) showed similar patterns to the C₄ maize and the C₃ wheat and soybean enzymes, respectively (Jiao and Chollet 1992; Gupta et al 1994).

Among the C₃ and C₄ PEPC sequences in C₄ monocots approximately 80% homology is found, as compared with 95% in *Flaveria* (Bläsing et al 2000). However, this 5% divergence is sufficient to alter significantly the kinetics and regulation of the enzyme. Bläsing et al (2000) have defined specific domains and residues in *Flaveria* PEPCs that relate to C₃ and C₄ function. In *Flaveria*, the Ser-774 residue has been described as C₄ specific, and Lys-347 as linked to Glc-6-P activation in the C₄ enzyme.

Three full-length cDNA sequences were obtained from *Hydrilla*, two from leaf and one from root, and the PEPC isoforms they represented appear to be functionally specific. To investigate this concept we constructed for one *Hydrilla* isoform a homologous model based on the X-ray crystallographic *E. coli* one (Kai et al 1999). Using the *Hydrilla* model and sequence data from the isoforms we have evaluated residues and segments for possible functional determinants. This information will be used to design site/segment directed mutagenesis to further define specific roles.

Materials and methods

Hydrilla verticillata sprigs in the C₃ photosynthetic state were incubated to induce C₄ photosynthesis or maintain the C₃ state (Bowes and Salvucci 1989). Roots were obtained from sprigs planted in sand; tubers were collected from the lake sites. Full- and partial length cDNAs were obtained using RACE-ready cDNA from total RNA from C₃ and C₄ shoots, roots and tubers. A model of *Hvpepc3* was built using SWISS-Model server (www.expasy.ch) (Kai et al 1999), with the Swiss-Pdb viewer for structural analysis.

Results

A nucleotide comparison of the three full-length sequences showed that leaf *Hvpepc3* and *Hvpepc5* from root and tuber were 99% similar, but differed in the 3'-UTR length. The C₄ leaf *Hvpepc4* was 95% similar to *Hvpepc3* and 5. Partial 5' and 3' sequencing of other clones revealed two phenomena. Some clones from root/tuber had a 180 bp deletion at the 5' end and a 22 bp 5'-UTR insertion; the remaining sequence was *Hvpepc5*. A variant clone from C₃ shoots showed a *Hvpepc4*-like 5'-end and a *Hvpepc3*-like 3'-end.

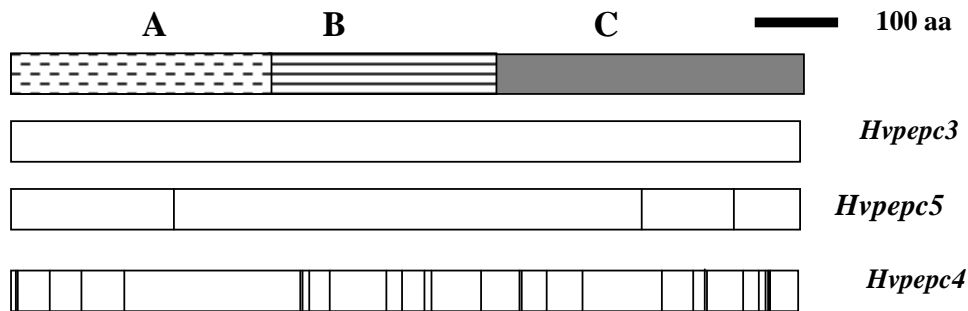


Fig 1. Schematic drawing of the proposed segments identified for chimeric constructs. The primary structure of the *Hydrilla* PEPC deduced sequences are aligned below the construct bar. **A.** N-terminus (1-320 aa); **B.** Mid (321-600 aa); **C.** C-terminus (601-970 aa). Segment A contains the phosphorylation and oxaloacetate-forming site, segment B primarily conserved domains, and segment C the PEP-HCO₃ binding site. The vertical lines indicate the site of divergent residues as compared to *Hvpepc3*.

A multiple alignment of deduced amino acids of the three full-length isoforms is illustrated in Figure 1. When compared with *Hvpepc3*, *Hvpepc5* had three substitutions that include a unique Ser-196 for Cys and Arg-891 for Glu, while *Hvpepc4* had 44 substitutions and two deletions. The *F. trinervia* C₄ PEPC Ser-774 was absent in all the sequences, replaced by the invariant C₃ Ala. The putative C₄ Lys (*Flaveria* 347) was found in *Hvpepc4*, but the typical C₃ Arg occurred in the other isoforms. Other reported C₄ determinants (Dong et al 1998) were absent from the *Hydrilla* sequences.

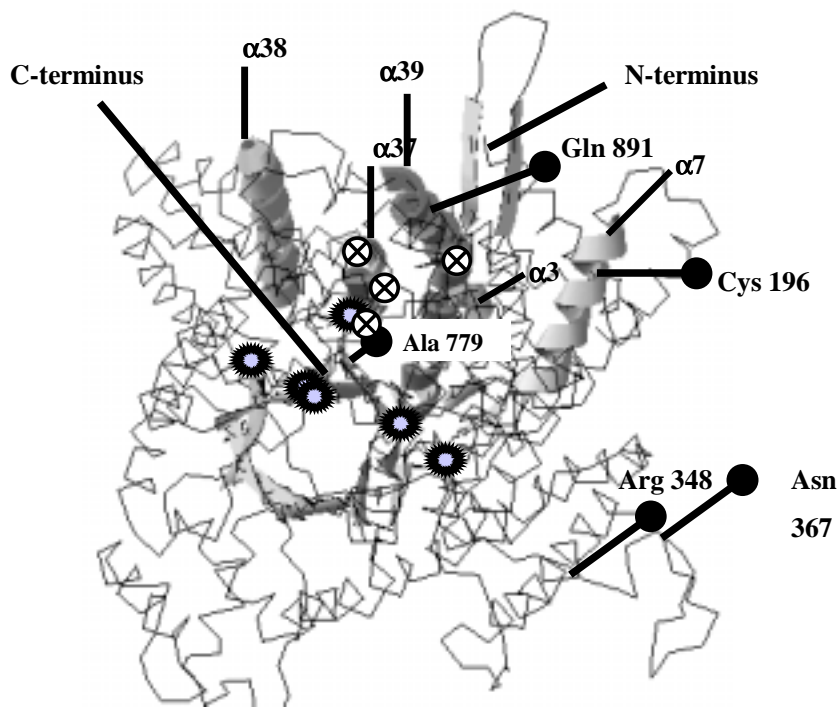


Fig 2. A 3-D view of *Hvpepc3* protein showing some candidate residues for site directed mutagenesis. The model was built using *E. coli* PEPC as the template (Kai et al 1999) that shares 38% homology with the *Hydrilla* PEPC isoforms. Five α -helices and all the β -sheets found in the model are illustrated as ribbons. Stars indicate residues associated with catalytic sites and crossed circles with aspartate binding.

Figure 2 is a 3-D wire frame model of *Hvpepc3* protein showing relative positions of the N- and C-termini, α -helices, and catalytic and regulatory residues. In addition there were several residues common to monocot C₄ PEPCs and *Hvpepc4*, two of which were located in the α -helical regions of segment B. A singular His-367 and several other unique residues at the C terminus, which may function in Asp binding, were also found.

Discussion

All three *Hydrilla* sequences were very similar, as occurs with the *Flaveria* C₃ and C₄ PEPCs. Evidence indicates that *Hvpepc4* is the photosynthetic isoform in *Hydrilla*, despite its lack of a C₄-signature Ser. This isoform was only expressed in, and been isolated from, C₄ leaves; and it least resembled *Hvpepc3* or 5 that were isolated from C₃ and root tissue. It contained a putative C₄ Lys-348, whereas the others had Arg common to the C₃ enzyme (Bläsing et al 2000), and it was expressed early in C₄ induction conditions. Although *Hvpepc3* was also strongly, but differentially, expressed in C₄ leaves, it resembled the root/tuber isoform. Our working hypothesis is that *Hvpepc3* and 5 have anapleurotic roles. C₄ leaves fix carbon in the dark at 12% of the light rate (Reiskind et al 1997), and *Hvpepc3* may function in this process. Matching *Hydrilla* sequences with comparable *Flaveria* C₃ and C₄ regions may help to identify functional variation. A key relates to how the *Hydrilla* photosynthetic isoform can operate as such with the C₄ Ser replaced by Ala at the corresponding position.

For recombinant enzyme studies, based on the sequence and model information, site-directed mutagenesis will be used to replace *Hvpepc4* Lys-348 with Arg and His-367 with Asn, and *Hvpepc5* Ser-196 will be replaced by Cys. The 348 site is associated with Glc-6-P activation, and studies with C₃ and C₄ *Hydrilla* shoot extracts show that kinetic differences exist (Reiskind et al unpublished). Sites involving His and Cys are unique substitutions that also may affect enzyme kinetics. Chimeric constructs of regulatory and catalytic segments should yield general details of function. Thus the following will be constructed: *Hvpepc3* AB *Hvpepc4* C and *Hvpepc3* A *Hvpepc4* B *Hvpepc3* C. The *Hvpepc4* segment A contains the phosphorylation site and studies with C₃ and C₄ shoot extracts indicate differences in phosphorylation status and subsequent regulation (Reiskind et al unpublished). Recombinant proteins of the truncated sequences should give clues as to how the lack of phosphorylation affects kinetics. Segment C contains unique residues that may be involved in Asp binding.

Prior to making alterations, kinetic and regulatory analyses of recombinant proteins of the natural “chimeras” and full-length isoforms are planned. The influence of tetrameric formation will be probed. Western analyses yielded monomers of differing mass, corroborating the deleted-180 bp sequence described above (Rao et al submitted), so it is unclear whether *Hydrilla* PEPC *in vivo* is comprised of four identical monomers. Studies with native protein should reveal true tetrameric structures.

Acknowledgements

Supported by NSF IBN-9604518 and USDA NRICGP 98-35306-6449.

References

- Bläsing OE, Westhoff P, Svensson P (2000) *Journal of Biological Chemistry* **275**, 27917-27923.
- Bowes G, Salvucci ME (1989) *Aquatic Botany* **34**, 232-266.
- Dong L-Y, Masuda T, Kawamura T, Hata S, Isui K (1998) *Plant Cell Physiology* **39**, 865-875.
- Gupta SK, Ku MSB, Lin J-H, Zhang D, Edwards GE (1994) *Photosynthesis Research* **42**,

133-143.

Jiao JA, Chollet R (1992) *Plant Physiology* **98**, 152-156.

Kai Y, Matsumura H, Inoue T, Terada K, Nagara Y, Yoshinaga T, Kihara A, Tsumura K, Isui K (1999) *Proceedings of the National Academy of Science USA* **96**, 823-828.

Reiskind JB, Madsen TV, van Ginkel LC, Bowes G (1997) *Plant Cell Environment* **20**, 211-220.