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## Reversible Phosphorylation of Photosynthetic PEP Carboxylase: Studies on C4-Leaf PP2A and Recombinant PEPC-Kinase from CAM-Induced *Mesembryanthemum crystallinum*

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# Reversible phosphorylation of photosynthetic PEP carboxylase: Studies on C<sub>4</sub>-leaf PP2A and recombinant PEPC-kinase from CAM-induced *Mesembryanthemum crystallinum*

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**Keywords:** PEP carboxylase (PEPC), protein phosphatase 2A (PP2A), PEPC-kinase (PpcK), regulatory phosphorylation, C<sub>4</sub> and CAM photosynthesis

The activity and allosteric properties of plant PEPC are controlled posttranslationally by specific reversible phosphorylation of a strictly conserved Ser residue near the N-terminus. This up/down-modulation is catalyzed by a transcriptionally regulated, seemingly dedicated Ser/Thr kinase (PpcK) and an opposing Ser/Thr phosphatase (PP2A). We have now partially purified and characterized the native form of this largely “neglected” PP2A from maize leaves using various chromatographic and affinity matrices, and C<sub>4</sub> [<sup>32</sup>P]PEPC as substrate (Dong et al., 2001, *Planta* [in press]). The results indicate that the C<sub>4</sub>-leaf holoenzyme is analogous to yeast and mammalian PP2As in regards to its heterotrimeric structure (~170 kDa), comprised of a ~103-kDa core PP2A-A heterodimer complexed with a ~74-kDa B-type subunit, and its sensitivity to free Me<sup>2+</sup> and various inhibitors, activators and anionic metabolites. Notably, this native PP2A (a) lacks any strict phosphoprotein specificity in that it dephosphorylates C<sub>4</sub> PEPC, mammalian phosphorylase *a*, and casein *in vitro*, and (b) displays, at best, only modest light/dark effects *in vivo* on its apparent *M<sub>r</sub>*, component core subunits, and activity against C<sub>4</sub> PEPC-SerP. In addition, we will also report new findings on a recombinant form of CAM PpcK from *M. crystallinum* (Taybi et al., 2000, *Plant Physiol.*) produced as a highly soluble, active fusion with the ~55-kDa NusA carrier protein in *E. coli*. This NusA–PpcK fusion protein has been purified by sequential IMAC and FPLC, used for detailed analysis of its target-protein specificity and other kinetic properties, and cleaved “on-bead” by thrombin to yield free PpcK for antibody production.