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

L. Dong
University of Nebraska - Lincoln

N. V. Ermolova
University of Nebraska - Lincoln

M. A. Cushman
University of Nevada, Reno, NV

T. Taybi
University of Newcastle, Newcastle-upon- Tyne NE1 7RU, UK

J. C. Cushman
University of Nevada, Reno, NV

See next page for additional authors

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Reversible phosphorylation of photosynthetic PEP carboxylase: Studies on C4-leaf PP2A and recombinant PEPC-kinase from CAM-induced *Mesembryanthemum crystallinum*

L. Dong,[1] N. V. Ermolova,[1] M. A. Cushman,[2] T. Taybi,[3] J. C. Cushman,[2] and R. Chollet [1]

1 Department of Biochemistry, University of Nebraska, Lincoln, NE 68588-0664, USA. Fax: 1-402-472-7842; email: RCHOLLET1@unl.edu

2 Department of Biochemistry, University of Nevada, Reno, NV 89557-0014, USA.

3 Department of Agricultural & Environmental Science, University of Newcastle, Newcastle-upon-Tyne NE1 7RU, UK.

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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_4 [^{32}P]PEPC as substrate (Dong et al., 2001, *Planta* [in press]). The results indicate that the C_4-leaf holoenzyme is analogous to yeast and mammalian PP2As in regards to its heterotrimeric structure (~170 kDa), comprised of a ~103-kDa core PP2Ac-A heterodimer complexed with a ~74-kDa B-type subunit, and its sensitivity to free Me^{2+} and various inhibitors, activators and anionic metabolites. Notably, this native PP2A (a) lacks any strict phosphoprotein specificity in that it dephosphorylates C_4 PEPC, mammalian phosphorylase *a*, and casein *in vitro*, and (b) displays, at best, only modest light/dark effects *in vivo* on its apparent M_r, component core subunits, and activity against C_4 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.