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## Regulatory Properties of Phosphoenolpyruvate Carboxylase in Crassulacean Acid Metabolism Plants: Diurnal Changes in Phosphorylation State and Regulation of Gene Expression

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**Abstract:** Regulatory properties of phosphoenolpyruvate carboxylase (PEPC, EC 4.1.1.31) in three CAM species, *Kalanchoë pinnata*, *K. daigremontiana* and *Ananas comosus* (pineapple) were examined. PEPC activity in the leaves of the three CAM species exhibited diurnal changes peaking during the first 2-h of darkness in *Kalanchoë* species and at midnight in pineapple, and then decreasing drastically until dawn. The oscillations of PEPC activity were far greater in *Kalanchoë* species than in pineapple. In the presence of 2 mM malate, the activity of PEPC decreased in all three CAM species, but the sensitivity of PEPC to malate was markedly different between pineapple and the *Kalanchoë* species. The malate sensitivity was 2- to 3-times higher in pineapple than in the *Kalanchoë* species during the dark period, but it was almost the same during the light period. PEPC in the three CAM species was phosphorylated only during the dark period. PEPC proteins were highly phosphorylated during the first 2-h of darkness in *Kalanchoë* species and at midnight in pineapple, and then they decreased drastically during the latter part of darkness. CAM-specific isoforms of PEPC in the leaves of the three CAM species contained a highly conserved phosphorylation site of Ser-11 at the N-terminus. These PEPC isoforms displayed diurnal changes in transcript abundance, with the peak of transcripts occurring during the dark period. The day/night changes in PEPC transcript abundance were mirrored by changes in the PEPC protein and corresponding enzyme activity over the diurnal cycle. These findings suggest that the diurnal regulation in PEPC activity is determined by the

amount of PEPC protein as well as the posttranslational control in these CAM species.

**Keywords:** [Crassulacean acid metabolism \(CAM\)](#), [Malate sensitivity](#), [Phosphoenolpyruvate carboxylase \(PEPC\)](#), [Phosphorylation state](#), [Protein abundance](#), [Transcript abundance](#)

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