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Metal Ion Interactions with Phosphoenolpyruvate Carboxylase from *Crassula argentea* and *Zea mays*[1]

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ABSTRACT

Metal ion interactions with phosphoenolpyruvate carboxylase from the CAM plant *Crassula argentea* and the C_4 plant *Zea mays* were kinetically analyzed. Fe^{2+} and Cd^{2+} were found to be active metal cofactors along with the previously known active metals Mg^{2+}, Mn^{2+}, and Co^{2+}. In studies with the *Crassula* enzyme, Mg^{2+} yielded the highest V_{max} value but also generated the highest values of $K_{m(metal)}$ and $K_{m(PEP)}$. For these five active metals lower $K_{m(metal)}$ values tended to be associated with lower $K_{m(PEP)}$ values. PEP saturation curves showed more kinetic cooperativity than the corresponding metal saturation curves. The activating metal ions all have ionic radii in the range of 0.86 to 1.09 Å. Ca^{2+}, Sr^{2+}, Ba^{2+}, and Ni^{2+} inhibited competitively with respect to Mg^{2+}, whereas Be^{2+}, Cu^{2+}, Zn^{2+}, and Pd^{2+} showed mixed-type inhibition. V_{max} trends with the five active metals were similar for the *C. argentea* and *Z. mays* enzymes except that Cd^{2+} was less effective with the maize enzyme. $K_{m(metal)}$ values were 10- to 60-fold higher in the enzyme from *Z. mays*.

In CAM (18) and C_4 (6) plants PEP[3] carboxylase (EC 4.1.1.31) serves as the primary carboxylating enzyme in photosynthesis. The enzyme requires a divalent metal ion cofactor and in assays of the purified enzyme Mg^{2+} and Mn^{2+} are commonly used. Co^{2+} also supports the activity of the enzyme but various other metals act as inhibitors (4, 10, 12, 14). The ability of a given metal ion to activate the enzyme may depend on the source of the enzyme. For example a lack of activation by Mn^{2+} has been reported for the PEP carboxylase from immature pods of chickpea (16), although this finding has not been corroborated. In this study we report metal ion effects on PEP carboxylase from two sources, the CAM plant *Crassula argentea* and the C_4 plant *Zea mays*. A number of kinetic properties of the enzyme from these two species have been previously described (13, 21).

Mildvan and co-workers (8, 9) have presented evidence that the metal ion serves as a bridge for the binding of PEP to the enzyme and that the metal specifically coordinates the phosphoryl moiety of the substrate. In addition to the active site, the enzyme may have a second type of metal binding site that affects the stability of the enzyme (3).

In order to identify features of the metal ions, such as size, which may govern their interactions with the enzyme, we have tested the kinetic effects of a variety of divalent metal ions on the PEP carboxylase of *C. argentea*. Two previously unknown metal ion activators of this enzyme, Fe^{2+} and Cd^{2+}, were found in the course of this study. Kinetic constants of activation or inhibition were determined for each metal ion tested. The effect of metal ion type on the kinetics of PEP utilization was also analyzed. Finally, kinetic properties relating to metal ion utilization were examined with PEP carboxylase from *Z. mays* in order to compare the kinetic characteristic of the C_4 and CAM enzymes.

MATERIALS AND METHODS

Chemicals. PEP (trisodium salt), NADH, EDTA, 2-mercaptoethanol, Tris, polyvinyl pyrrolidone (average mol wt 40,000), Triton X-100, PEG (average mol wt 6,000), and diphenyl thiocarbazone were purchased from Sigma Chemical Co. Hepes, DTT, and malate dehydrogenase were purchased from Boehringer Mannheim Biochemicals. $MgCl_2$ was obtained from Mallinckrodt Chemical Works. $CaCl_2$, $CuCl_2$, and $CdCl_2$ were purchased from Baker Chemical Co. $CoCl_2$ was obtained from Allied Chemical. $MnCl_2$ and $SrCl_2$ were purchased from Matheson Coleman and Bell. $BeSO_4$, $FeSO_4$, $BaCl_2$, $ZnCl_2$, $NiCl_2$, and ascorbic acid were purchased from Aldrich Chemical Co. DEAE-cellulose (microgranular DE 52) was purchased from Whatman Chemical Co. All chemicals were used without further purification except $MgCl_2$, $MnCl_2$, and $CaCl_2$ which were purified using the method described by Morrison (11) to eliminate trace levels of contamination by heavy-metal ions. Fe^{2+} solutions were freshly prepared each day by dissolving the $FeSO_4$ in a solution containing a 5-fold excess of ascorbic acid titrated to pH 4.0 and purged of O_2 by flushing with nitrogen gas for 3 min.

Plant Materials. *Crassula argentea* was grown in a field plot where it was watered once a week and fertilized once a month. Mature leaves were harvested in the morning. (about 8:00 AM).

Enzyme Purification. Leaves of *C. argentea* were washed and sliced into thin sections before being homogenized in 40 g batches at 4°C for 15 s in a Brinkmann homogenizer containing 100 ml of extraction buffer (100 mM Hepes, pH 8.5; containing 5 mM cysteine, 1 mM EDTA, 1% Triton X-100, 2% polyvinyl pyrrolidone). The homogenizer was equipped with a PTA 20TS generator and was used on a power setting of 7. The homogenate was filtered through cheese cloth and the pH was adjusted to 7.5. The crude homogenate was then centrifuged at 10,000*g* for 30 min. The supernatant was then fractionated by sequential addition of PEG in 4% (w/v) increments. At each step solid PEG was added slowly with stirring and the resulting precipitate was collected by centrifugation at 13,000*g* for 30 min. PEG carboxylase was precipitated by 12% PEG and the pellet was dissolved in a minimal volume of resuspension medium (50 mM Hepes, pH 7.5; with 1 mM EDTA and 1 mM DTT).

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[3] Abbreviations: PEP, phosphoenolpyruvate; MDH, malate dehydrogenase; OAA, oxalacetate.

The redissolved 12% PEG pellets were pooled and applied to a DEAE-cellulose column (1.65 × 43 cm) equilibrated with 50 mM Tris-Cl (pH 7.5) containing 1 mM DTT. The column was washed with this buffer and the enzyme was then eluted with a gradient of 50 to 200 mM NaCl in the same buffer. The active fractions were pooled and concentrated by precipitation with 16% PEG. The pellet was dissolved in a minimal amount of 65 mM Hepes (pH 7.5) containing 1 mM PEP, 50 mM NaCl, and 1 mM DTT. The redissolved enzyme was then loaded on a gel filtration column (Sepharose 6B, 1.65 × 43 cm) and eluted with the same buffer. The active fractions were concentrated by PEG precipitation, redissolved in 50 mM Hepes (pH 7.5) with 1 mM DTT and stored in liquid N_2 until used. The enzyme purified in this way had a specific activity of 4.0 to 5.8 μmol min^{-1} (mg protein)$^{-1}$. The enzyme was about 83% pure on the basis of SDS gels, a value which is consistent with previously published estimates of purity of the *C. argentea* enzyme when purified to a comparable specific activity (20).

PEP carboxylase from *Z. mays* was purchased from Sigma Chemical Co. and used without further purification. The maize enzyme had a specific activity of 1.7 μmol min^{-1} (mg protein)$^{-1}$. While this value suggests that the enzyme was less than 10% pure, contamination by NADH oxidase activity was not significant (6 nmol min^{-1} [mg protein]$^{-1}$).

Enzyme Assays. PEP carboxylase activity was assayed spectrophotometrically by following the disappearance of NADH at 340 nm in a coupled assay using malate dehydrogenase. Unless otherwise indicated the assays were initiated by the addition of enzyme to 1.0 ml of an assay mixture which contained 4.0 mM PEP, 4.0 mM $NaHCO_3$, 0.1 mM NADH, 30 μg malate dehydrogenase, 50 mM Hepes (pH 7.5), and metal ion at 20°C. Control assays lacking metal ion showed background levels of NADH oxidation that were less than 2% of the rate obtained in the presence of a saturating level of Mg^{2+}. In each case the observed background rate was subtracted from the rates obtained in the presence of metal ion. Assays with Fe^{2+} were conducted in sealed cuvettes under N_2. Assay mixtures were purged of O_2 by bubbling with N_2 gas for 3 min prior to sealing the cuvettes with a serum stopper. Bicarbonate, MDH, and PEP carboxylase were then added and the assay was started by the addition of Fe^{2+}. A blank containing the complete reaction mixture except for PEP carboxylase was also assayed at each concentration of Fe^{2+} to correct for the slight absorbance increase (corresponding to less than 3% of the rate with saturating levels of Fe^{2+}) arising from background levels of Fe^{2+} oxidation. Assays with Cd^{2+} were initiated by addition of the metal ion to otherwise complete assay mixtures in order to avoid unnecessary exposure of MDH to Cd^{2+} prior to the start of the assay.

Data Analysis. The observed velocities in PEP or metal ion saturation series were fitted to Eq. 1,

$$v = \frac{V_{max} \times S^n}{K_m{}^n + S^n} \quad (1)$$

using the Logistic fitting program on the Prophet computer system. In this equation v is the observed velocity, n is the Hill coefficient, and V_{max} and K_m have their usual meanings. Each saturation series contained data from 12 to 16 individual assays. Inhibition constants were determined from replots of the $K_{m(app)}/V_{max(app)}$ and $1/V_{max(app)}$ *versus* inhibitor concentration (2). For each metal ion inhibitor, four to six separate Mg^{2+} saturation curves were performed at different fixed concentrations of inhibitor. For Ca^{2+} and Ni^{2+}, which gave nonlinear replots, apparent K_i values were determined at each fixed concentration of inhibitor using Eq. 2.

$$K_{i(app)} = \frac{I}{\frac{K_{m(app)}}{K_m} - 1} \quad (2)$$

The actual K_i values were obtained by plotting $1/K_i$(app) *versus* I according to Eq. 3 (15).

$$1/K_{i(app)} = (1/K_i^2)I + 2/K_i \quad (3)$$

RESULTS

Metal Ion Activators. Various metal ions with stable divalent oxidation states were tested for their ability to active PEP carboxylase from *C. argentea*. Of the 13 metal ions tested, only Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, and Cd^{2+} activated the enzyme. Of these, Fe^{2+} and Cd^{2+} were not previously known to activate PEP carboxylase. The kinetic properties of the enzyme with these activating metal ions are shown in Table I. V_{max} values with the different activators varied by as much as a factor of five, with Mg^{2+} generating the highest value. However Mg^{2+} also gave the highest value of $K_{m(metal)}$. The observed variation in $K_{(metal)}$ was large, with a 23-fold difference in the values for Mg^{2+} and Co^{2+}. Saturation curves for the active metals were essentially hyperbolic, with Hill coefficients close to one.

PEP saturation curves were performed with each of the active metal ions and the results of these studies are summarized in Table II. $K_{m(PEP)}$ varied by less than 6-fold, with Mg^{2+} yielding the highest value. The PEP saturation curves tended to show more kinetic cooperativity, as indicated by higher n values, than was observed in the metal saturation series.

Metal Ion Inhibitors. All of the remaining ions tested inhibited the enzyme to some extent. Ca^{2+}, Sr^{2+}, Ba^{2+}, and Ni^{2+} were competitive inhibitors with respect to Mg^{2+}, with Sr^{2+} and Ba^{2+} generating linear replots of $K_{m(app)}/V_{max(app)}$ *versus* inhibitor concentration for K_i estimations. However, these replots were parabolic for both Ca^{2+} (Fig. 1A) and Ni^{2+} (not shown). The nonlinearity of the replots suggested more than one inhibitor binding site and precluded the determination of the K_i by the usual method (15). Consequently, the K_i was obtained by plotting the apparent K_i, determined at each inhibitor concentration using

Table 1. *Metal Ion Activation Parameters*

All assays performed as described under "Materials and Methods" using 2.8 μg of PEP carboxylase from *C. argentea*. The range of concentrations used for each metal ion were: Mg^{2+}, 0 to 5 mM; Cd^{2+}, 0 to 0.2 mM; Mn^{2+}, 0 to 0.75 mM; Fe^{2+}, 0 to 0.2 mM; Co^{2+}, 0 to 0.2 mM.

Metal Ion	V_{max}	$V/K_{(metal)} \times 10^3$	$K_{(metal)}$	n
	$\mu M\ min^{-1}$	min^{-1}	mM	
Mg^{2+}	10	48	0.21	0.76
Cd^{2+}	8.3	360	0.023	1.2
Mn^{2+}	6.3	190	0.034	1.3
Fe^{2+}	4.7	247	0.019	1.0
Co^{2+}	1.9	210	0.009	1.1

Table II. *Metal Ion Dependence of PEP Kinetic Parameters*

All assays were performed as described under "Materials and Methods" with 2.8 μg of PEP carboxylase from *C. argentea* and the following concentrations of the activators: 4.0 mM Mg^{2+}, 0.1 mM Cd^{2+}, 0.3 mM Mn^{2+}, 0.27 mM Fe^{2+}, and 0.1 mM Co^{2+}.

Metal Ion	V_{max}	$V/K_{(PEP)} \times 10^3$	$K_{(PEP)}$	n
	$\mu M\ min^{-1}$	min^{-1}	mM	
Mg^{2+}	10	24	0.42	1.8
Cd^{2+}	8.3	75	0.11	1.7
Mn^{2+}	6.0	43	0.14	1.8
Fe^{2+}	4.3	61	0.071	1.2
Co^{2+}	2.0	22	0.091	1.2

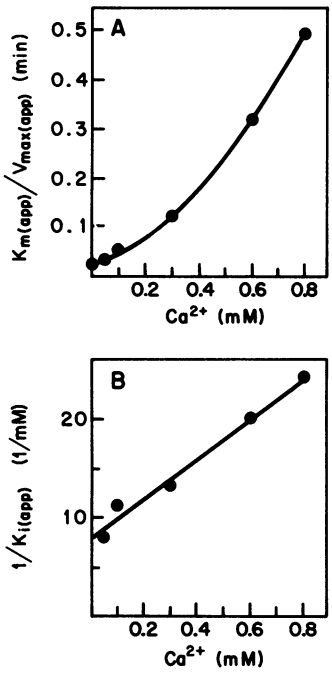

FIG. 1. A, $K_{m(app)}$ $V_{max(app)}$ for Mg^{2+} as a function of the Ca^{2+} concentration; B, dependence of the apparent inhibition constant, $K_{i(app)}$, on the concentration of Ca^{2+}. The correlation coefficient for the fitted line is 0.99.

Table III. *Metal Ion Inhibition Constants*

All assays performed as described under "Materials and Methods" using 2.8 μg of PEP carboxylase from *C. argentea.*

Metal Ion	K_i[a]	K_i'
	mM	
Be^{2+}	0.12	0.39
Ca^{2+}	0.25	
Sr^{2+}	3.8	
Ba^{2+}	21	
Ni^{2+}	0.020	
Cu^{2+}	0.0025	0.13
Zn^{2+}	0.0083	0.09
Pd^{2+}	0.33	5.0

[a] K_i and K_i' are the dissociation constants for the enzyme-inhibitor complex and the enzyme-Mg-inhibitor complex, respectively.

Eq. 2, *versus* the inhibitor concentration according to Eq. 3 (Fig. 1B). This plot gives a straight line for parabolic replot systems where two molecules of inhibitor bind to the enzyme. The K_i values for Ca^{2+} (0.25 mM) and Ni^{2+} (0.02 mM) were determined from the intercept, which is equal to $2/K_i$.

Cu^{2+}, Be^{2+}, Zn^{2+}, and Pd^{2+} were mixed inhibitors of the enzyme, affecting both V_{max} and K_m. K_i and K_i' values were obtained from $K_{m(app)}/V_{max(app)}$ and $1/V_{max(app)}$ replots, respectively, and are summarized in Table III.

Comparison of the CAM and C_4 Enzyme. Because of apparent differences in the kinetic behavior of the *C. argentea* enzyme and the enzyme from C_4 plant *Z. mays* (14), we analyzed the metal activation kinetics of the maize enzyme with the same five active metals (Table IV). V_{max} trends with these metals (Mg^{2+} > Mn^{2+} > Fe^{2+} > Co^{2+} > Cd^{2+}) were similar to those found with the *C. argentea* enzyme (Mg^{2+} > Cd^{2+} > Mn^{2+} > Fe^{2+} > Co^{2+}) except that Cd^{2+} was significantly less effective with the maize enzyme. $K_{m(metal)}$ values ranged from 10- to 60-fold higher with the maize enzyme than with the enzyme from *C. argentea.* For both sources of the enzyme Mg^{2+} gave by far the highest $K_{m(metal)}$ value, In assays with Mg^{2+}, the $K_{m(PEP)}$ of the maize enzyme was 4.5 mM, which is also an order of magnitude higher than that obtained with the *C. argentea* enzyme.

Table IV. *Metal Ion Activation of Maize PEP Carboxylase*

All assays were performed as described in "Materials and Methods" except using 25 mM PEP and 28 μg PEP carboxylase from *Z. mays.* The range of concentrations used for each metal ion were: Mg^{2+}, 0 to 25 mM; Mn^{2+}, 0 to 3 mM (rates declined above this limit); Fe^{2+}, 0 to 6 mM mM; Co^{2+}, 0 to 5 mM; Cd^{2+}, 0 to 1.5 mM.

Metal Ion	V_{max}	$V/K_{(metal)}$ $\times 10^3$	$K_{m(metal)}$	n
	$\mu M\ min^{-1}$	min^{-1}	*mM*	
Mg^{2+}	48	11	4.3	1.0
Mn^{2+}	39	52	0.75	1.1
Fe^{2+}	21	39	0.54	1.4
Co^{2+}	18	33	0.55	1.2
Cd^{2+}	10	43	0.23	1.6

DISCUSSION

Of the 13 divalent metal ions tested, only Mg^{2+}, Mn^{2+}, Fe^{2+}, Co^{2+}, and Cd^{2+} activated the PEP carboxylase from *C. argentea.* Cd^{2+} and Fe^{2+} have not, to our knowledge, previously been reported to activate PEP carboxylase. In fact, Stiborova and Leblova (17) reported that Cd^{2+} inactivates the PEP carboxylase from maize. However, the inactivation observed in their study can be explained by the fact that the enzyme and Cd^{2+} were preincubated at least 5 min before the assay. Such preincubation in the absence of substrate may allow Cd^{2+} to react with an essential suflhydryl group, as these authors suggest (17). In our Cd^{2+}-initiated assays, where the enzyme was not exposed to Cd^{2+} in the absence of substrates, we found significant activity. Cd^{2+} activated both the CAM and the C_4 enzymes, as did Fe^{2+} and the three previously known activators of this enzyme. While Cd^{2+} is not a normal physiological cation, it may prove useful for *in vitro* studies of this enzyme as it offers the prospect of identifying active site residues involved in metal ion binding through ^{113}Cd-NMR.

Use of any of the active transition metal ions in place of Mg^{2+} causes substantial changes in the kinetic properties of the enzyme. A 5-fold range in V_{max} values was observed with these five active metals, a similar range of values was seen in $K_{m(PEP)}$ and $K_{m(metal)}$ varied by 23-fold. Although a physiological role for Cd^{2+} or Co^{2+} is unlikely, the biological occurrence of Mn^{2+} and Fe^{2+} makes these ions at least potential alternatives to Mg^{2+} within the cell. For the related enzyme PEP carboxykinase, Bentle and Lardy (1) have in fact proposed that Fe^{2+} may play a role as a physiological activator in the rat liver cytosol. Comparisons of $V/K_{m(metal)}$, the apparent first order rate constant for the reaction at low metal concentrations, indicate that the PEP carboxylases from *C. argentea* and *Z. mays* have a moderately high intrinsic preference for both Mn^{2+} and Fe^{2+} relative to Mg^{2+}. For either source of the enzyme, Mg^{2+} gives the lowest values of $V/K_{m(metal)}$. Nonetheless, a role for Fe^{2+} or Mn^{2+} in the physiological functioning of PEP carboxylase in plant tissues remain in doubt in the absence of precise data on the relative concentrations of Mg^{2+}, Mn^{2+}, and Fe^{2+} in the cytosol. For iron there is also the problem of oxidation of the ferrous ion in the oxygen rich cytosol of photosynthetic cells, the prospect of which argues against a physiological role for this metal.

Hill coefficients for the metal and PEP saturation curves indicated modest kinetic cooperativity for PEP binding. This sigmoidicity may contribute to the physiological regulation of the enzyme. Lower Hill coefficients were found in the corresponding metal saturation curves.

K_m values for metal ion with the enzyme from *C. argentea* increased in the order $Co^{2+} < Fe^{2+} < Cd^{2+} < Mn^{2+} < Mg^{2+}$ (Table I). For these active metals lower values of $K_{m(metal)}$ tended to be associated with lower values of $K_{m(PEP)}$ (with a correlation coefficient of 0.99 for the trend). This is consistent with the proposal of Mildvan and co-workers (8, 9) that PEP binds to the enzyme in part through a metal ion bridge, since tighter binding through this bridge would be expected to give lower K_m values for both metal ion and PEP. O'Leary *et al.* (14) have shown that it is the uncomplexed, fully ionized form of PEP which binds to the enzyme.

The activating metal ions all have a preference for octahedral coordination geometry and in this form have ionic radii in the range 0.86 to 1.09 Å (5). The failure of the other ions tested to activate the enzyme can be interpreted in terms of an inappropriate size or coordination geometry. For example, the alkaline earth inhibitors, while capable of octahedral coordination, nonetheless have ionic radii which are outside the observed range of the activators, with Be^{2+} (0.59 Å) being too small and Ca^{2+} (1.14 Å), Sr^{2+} (1.32 Å) and Ba^{2+} (1.49 Å) being too large. Size may also play a partial role in determining the strength of the enzyme-metal interaction. Evidence for this can be found with the group II metals where K_i (or K_m) increases with atomic number (and hence size) of the metal ion, *i.e.* $Be^{2+} < Mg^{2+} < Ca^{2+} < Sr^{2+} < Ba^{2+}$. Although ionic radius appears to be an important element in determining the nature and strength of the metal-enzyme interaction, many kinetic and thermodynamic features of metal ions depend on their size. Hence, it is possible that a size related property of the metal, such as the rate of ligand exchange, is the key factor. For some metals the coordination geometry may also be a factor. For example, Be^{2+}, Cu^{2+}, Ni^{2+}, Zn^{2+}, and Pd^{2+} often exist in complexes with a coordination number of 4.

Ni^{2+} and Ca^{2+} were the only inhibitors to yield parabolic inhibition kinetics. Further analysis of the data suggested that these ions can each bind at two sites on the enzyme. However, an alternative explanation is that the parabolic inhibition might result from metal ion interference with the coupled assay system. Metal ions can inhibit the malate dehydrogenase reaction by nonezymically decarboxylating OAA or by converting the keto form of OAA to the enol form, which is not a substrate for malate dehydrogenase (19). The extent of decarboxylation and tautomerization is directly related for various metals (7). While these nonenzymic processes may contribute to the inhibition caused by Ni^{2+} or other metals, such artifacts are unlikely with Ca^{2+}, which has little tendency to decarboxylate OAA (7). Also, the proposal that there are two distinct types of binding sites for Ca^{2+} on the enzyme is consistent with the previous findings of Gavalas *et al.* (3). They found that with PEP carboxylase from C_4 plants, Ca^{2+} not only is a competitive inhibitor with respect to Mg^{2+}, but also stabilizes the enzyme in assays at low concentrations of PEP in a way that cannot be replaced by Mg^{2+}. This finding suggests the possibility of a separate binding site for Ca^{2+} which is distinct from the active site. With its low K_i for Ca^{2+} the enzyme has roughly the same affinity for this inhibitor as it has for Mg^{2+}, and thus Ca^{2+} inhibition of the enzyme is a potential physiological issue in CAM plants. Gavalas and Manetas (4) have suggested that Ca^{2+} may be a physiologically significant inhibitor of PEP carboxylase in C_4 plants.

While in general there is no strict quantitative relationship between the ionic radius of an inhibitor and its inhibition constant, there is nonetheless an interesting size-dependent relationship that holds for inhibitors from various groups of the periodic table. The reciprocals of their K_i values are stability constants and they follow the order $Ba^{2+} < Sr^{2+} < Ca^{2+} < Ni^{2+} < Cu^{2+} > Zn^{2+}$. This follows exactly the order of their appearance in the Irving-Williams series of stability. This well known series describes the stability of complexes formed with divalent metal ions, and in part derives from size effects (5).

V_{max} trends with the five active metals were similar in the CAM and C_4 enzymes, except that Cd^{2+} was relatively much less active in the latter. This difference in relative activity with Cd^{2+} may stem from minor differences in composition or topography of the active site that effect the ability these two enzymes to utilize Cd^{2+}, the largest of the active metals. The active site of PEP carboxylase from chickpea, a legume, may be quite different from either the *Z. mays* or *C. argentea* enzymes in regard to metal ion binding. Singal and Singh (16) report that the chickpea enzyme is specifically activated by Mg^{2+}, and is not activated by Mn^{2+}.

Significant differences were seen between the *Z. mays* and *C. argentea* enzymes with respect to apparent affinities for metal ion and PEP. For a given metal ion, the $K_{m(metal)}$ was 10- to 60-fold higher in the enzyme from the C_4 plant *Z. mays*. Also the $K_{m(PEP)}$ for the *Z. mays* enzyme in the presence of Mg^{2+} was more than 10-fold higher than the $K_{m(PEP)}$ obtained with the enzyme from the CAM plant *C. argentea*. It has been previously noted that $K_{m(PEP)}$ values for the enzyme from C_4 plants are generally much larger than those of the enzyme from C_3 plants (13). The results of the present study are consistent with the view that PEP carboxylase from C_4 plants has a low apparent affinity for both PEP and metal ions.

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