

Variations in Kinetic Properties of Ribulose-1,5-bisphosphate Carboxylases among Plants

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ABSTRACT

Studies of ribulose-1,5-bisphosphate (RuBP) carboxylase from taxonomically diverse plants show that the enzyme from C_3 and crassulacean acid metabolism pathway species exhibits lower $K_m(\text{CO}_2)$ values (12–25 micromolar) than does that from C_4 species (28–34 micromolar). RuBP carboxylase from aquatic angiosperms, an aquatic bryophyte, fresh water and marine algae has yielded consistently high $K_m(\text{CO}_2)$ values (30–70 micromolar), similar in range to that of the enzyme from C_4 terrestrial plants. This variation in $K_m(\text{CO}_2)$ is discussed in relation to the correlation between the existence of CO_2 -concentrating mechanisms for photosynthesis and the affinity of the enzyme for CO_2 . The $K_m(\text{RuBP})$ of the enzyme from various sources ranges from 10 to 136 micromolar; mean \pm SD = 36 ± 20 micromolar. This variation in $K_m(\text{RuBP})$ does not correlate with different photosynthetic pathways, but shows taxonomic patterns. Among the dicotyledons, the enzyme from crassinucellate species exhibits lower $K_m(\text{RuBP})$ (18 ± 4 micromolar) than does that from tenuinucellate species (25 ± 7 micromolar). Among the Poaceae, RuBP carboxylase from Triticeae, chloridoids, andropogonoids, *Microlaena*, and *Tetrarrhena* has yielded lower $K_m(\text{RuBP})$ values (29 ± 11 micromolar) than has that from other members of the grass family (46 ± 10 micromolar).

Kinetic studies on RuBP³ (EC 4.1.1.39) from grasses have revealed variation in $K_m(\text{CO}_2)$ values associated primarily with the differences in photosynthetic pathway, and correlated to some extent with taxonomic groupings (19). It seemed desirable to explore the extent to which the situation in grasses reflects that in the plant kingdom as a whole, and to extend the observations to include $K_m(\text{RuBP})$. The present article reports on the $K_m(\text{CO}_2)$ and $K_m(\text{RuBP})$ of RuBP carboxylases from taxonomically and ecologically diverse samples of plants, including cryptograms, seed plants, and both terrestrial and aquatic forms, with a view to discover the extent of variation in K_m values, and whether these reflect differences in photosynthetic pathway, taxonomic relationships, or ecology.

MATERIALS AND METHODS

Plant Material. Plants were grown from seeds or collected from the field, and their identities were checked with reference to appropriate floristic works.

Enzyme Preparation and Assay. All extraction and purification procedures were carried out at 0 to 4 C. Leaves (about 2 g) were

ground in a mortar with 100 mM Bicine buffer (pH 8.0) containing 25 mM MgCl_2 and 1 mM DTT (about 4–10 ml). The homogenate was centrifuged at 25,000g for 15 min, and 0.5 ml of the supernatant was eluted through a 0.8×15 cm column of Sephadex G-25, equilibrated in the same buffer. The void volume which contained RuBP carboxylase was collected and saved. The partially purified enzyme extract (1.0 ml) was preactivated in 5 mM NaHCO_3 , and then assayed by measuring the fixation of [^{14}C]-bicarbonate. The reaction mixture containing 100 mM Bicine and 25 mM MgCl_2 (pH 8.0) was prepared CO_2 -free, and flushed with N_2 prior to using it. Assays (total volume of 400 μl) were performed in 1-ml stoppered vials (Pierce Reacti-vials No. 13221) which had been flushed with N_2 . For $K_m(\text{CO}_2)$ determination, the HCO_3^- concentration ranged from 0.4 to 16.5 mM, with RuBP fixed at 0.5 mM; and for $K_m(\text{RuBP})$ determination, the RuBP concentration ranged from 7.5 to 500 μM , with HCO_3^- fixed at 10.6 mM. Reaction was started by injection of fully activated enzyme (5 μl for $K_m[\text{CO}_2]$ assays; 2–40 μl for $K_m[\text{RuBP}]$ assays), and stopped after 1 min at 25 C by injection of 0.2 ml 2 N HCOOH . The reaction mixture was then quantitatively transferred to a glass scintillation vial and evaporated to dryness on a hot plate. After the vial had cooled, 1.0 ml distilled H_2O was added, followed by a 9.0-ml mixture of 5 g PPO in 1 liter toluene plus 500 ml Triton X-100. Each vial was then counted for 5 min in a Searle Delta 300 liquid scintillation counter. The bicarbonate introduced into the assay solution with the enzyme aliquot was taken into consideration when calculating HCO_3^- concentration and specific radioactivity. The CO_2 concentration was calculated from the pH and HCO_3^- concentration using the Henderson-Hasselbach equation and pK' value of 6.37 at 25 C (16). The K_m values were statistically calculated using Wilkinson's method (18).

Some plants (listed below) did not show any enzyme activity when extracted with the above buffer system, but produced active enzyme only when extracted with 200 mM Bicine buffer (pH 8.0), containing 25 mM MgCl_2 , 1 mM DTT, and 1% (w/v) PVP-10. Spinach material was also extracted with this buffer to check for possible variations in K_m values, attributable to the new buffer. No significant differences in K_m values were detected.

Plant materials extracted with 200 mM Bicine, 25 mM MgCl_2 , 1 mM DTT, and 1% (w/v) PVP-10 (pH 8.0): *Casuarina*, *Clematis*, *Fragaria*, *Fissidens*, *Ilex*, *Ginkgo*, *Kalanchoë* spp., *Macrozamia*, *Magnolia*, *Pellaea*, *Pinus*, *Populus*, *Pteridium*, *Rumex*, and *Selaginella*.

RESULTS AND DISCUSSION

$K_m(\text{CO}_2)$ of RuBP Carboxylase from Terrestrial Plants. The $K_m(\text{CO}_2)$ values of RuBP carboxylase from 52 species (46 genera) of taxonomically diverse plants are given in Tables I and II. The results obtained for terrestrial plants (Table I) are strikingly similar to those obtained previously for grasses (19), in that all the C_3 species have lower $K_m(\text{CO}_2)$, ranging from 12 to 25 μM CO_2 (mean

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³ Abbreviations: RuBP, ribulose-1,5 bisphosphate.

Table I. $K_m(\text{CO}_2)$ and $K_m(\text{RuBP})$ Values of RuBP Carboxylase from Terrestrial Plants

C_4 species are indicated by bold face, CAM species by asterisks, and alpine plant materials by (alpine). *Ceratopteris* is here regarded as "terrestrial" because its fronds are normally aerial, and submerged plants remain sterile.

Species	$K_m(\text{CO}_2)$	$K_m(\text{RuBP})$
μM		
Bryophyta		
<i>Funaria</i> sp.	23 \pm 1	23 \pm 1
Pteridophyta		
<i>Ceratopteris thalictroides</i>	16 \pm 3	49 \pm 7
<i>Pellaea falcata</i>	19 \pm 1	31 \pm 4
<i>Psilotum nudum</i>	23 \pm 2	42 \pm 7
<i>Pteridium esculentum</i>	20 \pm 1	35 \pm 4
<i>Selaginella kraussiana</i>	18 \pm 3	15 \pm 1
Gymnospermae		
<i>Ginkgo biloba</i>	23 \pm 1	36 \pm 2
<i>Macrozamia communis</i>	14 \pm 1	30 \pm 2
<i>Pinus montezumae</i>	24 \pm 1	20 \pm 3
Monocotyledonae		
<i>Allium cepa</i>	17 \pm 1	19 \pm 2
<i>Cyperus eragrostis</i>	15 \pm 1	28 \pm 3
<i>Poa hiemata</i> (alpine)	20 \pm 2	ND ^a
<i>Cyperus rutilans</i> ^b	34 \pm 14	ND
Dicotyledonae		
"Crassinucelli":		
<i>Atriplex patula</i>	19 \pm 1	17 \pm 2
<i>Casuarina cunninghamiana</i>	14 \pm 1	19 \pm 2
<i>Clematis</i> sp.	12 \pm 2	10 \pm 2
<i>Fragaria vesca</i>	18 \pm 2	22 \pm 2
<i>Gossypium hirsutum</i>	19 \pm 3	17 \pm 2
<i>Ilex aquifolium</i>	25 \pm 3	18 \pm 1
<i>Lupinus angustiflorus</i>	22 \pm 4	16 \pm 2
<i>Magnolia grandiflora</i>	12 \pm 1	22 \pm 4
<i>Papaver nudicaule</i>	22 \pm 2	16 \pm 2
<i>Populus nigra italica</i>	19 \pm 3	18 \pm 5
<i>Rumex acetosella</i>	17 \pm 1	14 \pm 1
<i>Rumex acetosella</i> (alpine)	17 \pm 2	12 \pm 1
<i>Spinacia oleracea</i>	17 \pm 2	17 \pm 3
<i>Kalanchoë daigremontiana</i> *	15 \pm 1	17 \pm 1
<i>Kalanchoë pinnata</i> *	16 \pm 1	18 \pm 1
<i>Zygocactus truncatus</i> *	17 \pm 2	14 \pm 1
<i>Amaranthus edulis</i>	28 \pm 1	20 \pm 2
<i>Atriplex suberecta</i>	31 \pm 2	17 \pm 2
<i>Gomphrena globosa</i>	31 \pm 2	27 \pm 6
"Tenuinucelli":		
<i>Aciphylla glacialis</i> (alpine)	15 \pm 1	16 \pm 2
<i>Buddleia davidii</i>	19 \pm 1	34 \pm 1
<i>Lactuca sativa</i>	22 \pm 2	27 \pm 3
<i>Mentha aquatica</i>	22 \pm 1	22 \pm 5
<i>Microseris lanceolata</i> (alpine)	22 \pm 2	34 \pm 2
<i>Petroselinum crispum</i>	21 \pm 3	20 \pm 1
<i>Solanum tuberosum</i>	17 \pm 1	18 \pm 4
<i>Verbascum thapsus</i>	17 \pm 2	27 \pm 2

^a ND, not determined.

^b C_4 species are indicated by bold face.

\pm SD = 19 \pm 3 μM), than do the C_4 species which vary from 28 to 34 μM (mean \pm SD = 31 \pm 2 μM); the difference between the mean values being statistically significant at 1% probability level. Even in terms of this wide-ranging sample, the C_3/C_4 distinction is manifest regardless of whether one considers closely or distantly related species. For example, *Cyperus eragrostis* and *Atriplex patula*, C_3 species from the monocotyledons and dicotyledons, respectively, have yielded lower $K_m(\text{CO}_2)$ (15 and 19 μM CO_2) than

Table II. $K_m(\text{CO}_2)$ and $K_m(\text{RuBP})$ Values of RuBP Carboxylase from Aquatic Plants

Submerged leaves are used, unless indicated.

Species	$K_m(\text{CO}_2)$	$K_m(\text{RuBP})$
μM		
Chlorophyta		
<i>Chara</i> sp. (fresh water)	42 \pm 4	30 \pm 2
<i>Codium fragile</i> (marine)	43 \pm 1	19 \pm 2
<i>Nitella</i> sp. (fresh water)	44 \pm 1	29 \pm 1
<i>Ulva</i> sp. (marine)	70 \pm 4	17 \pm 1
Bryophyta		
<i>Fissidens rigidulus</i>	40 \pm 3	33 \pm 5
Monocotyledonae		
<i>Egeria densa</i>	30 \pm 2	19 \pm 1
<i>Elodea canadensis</i>	39 \pm 5	14 \pm 3
<i>Potamogeton crispus</i>	43 \pm 6	25 \pm 5
<i>Potamogeton ochreatus</i>	49 \pm 2	29 \pm 6
<i>Potamogeton tricarlinatus</i> (FL) ^a	43 \pm 8	26 \pm 7
Dicotyledonae		
<i>Hygrophila polysperma</i>	36 \pm 6	27 \pm 3
<i>Myriophyllum propinquum</i> (EL) ^a	37 \pm 8	ND ^a
<i>Myriophyllum propinquum</i>	36 \pm 7	ND
<i>Myriophyllum verrucosum</i>	48 \pm 6	19 \pm 2

^a (FL), emergent leaf; (EL), floating leaf; ND, not determined.

have their C_4 counterparts, *Cyperus rutilans* and *Atriplex suberecta* (34 and 31 μM CO_2). Carboxylases from pteridophytes and gymnosperms, which are taxonomically very distant from the monocotyledons and dicotyledons, also exhibit $K_m(\text{CO}_2)$ values ranging from 16 to 24 μM CO_2 and are comparable with those from other C_3 plants, as is that from a terrestrial bryophyte. Increasing the taxonomic range of the sample beyond the Poaceae has not increased the total range of known variation in the $K_m(\text{CO}_2)$ of plant carboxylase i.e. the ranges of C_3 and C_4 K_m values presented in Table I are closely similar to those previously given for grasses; and our earlier conclusion (19) that variation in $K_m(\text{CO}_2)$ of RuBP carboxylase is closely linked with the difference in photosynthetic pathway can be extended to terrestrial plants in general.

As before, there is no over-riding correlation with taxonomic groupings, and this observation is now seen to be applicable across the plant kingdom as a whole. Enzymes from taxonomically diverse genera representing a terrestrial bryophyte, pteridophytes, gymnosperms, monocotyledons, and dicotyledons exhibit similar ranges in $K_m(\text{CO}_2)$ provided comparisons are confined to plants of the same photosynthetic pathway. Neither is there any obvious correlation between the $K_m(\text{CO}_2)$ of RuBP carboxylase and the natural habitats of the plants listed in Table I. Thus, species from moist environments (e.g. *Cyperus* and *Ceratopteris*) have given $K_m(\text{CO}_2)$ levels similar to those of species with the same photosynthetic pathway from drier environments; and plants (e.g. *Aciphylla*, *Microseris*, *Rumex*, and *Poa*) collected from alpine regions (1500 m) do not differ in their $K_m(\text{CO}_2)$ values from relatives found at lower altitudes. RuBP carboxylases extracted from *Gossypium* (C_3) and *Zea* (C_4) which were grown from seeds in CO_2 -enriched atmosphere (640 μbar CO_2) have given $K_m(\text{CO}_2)$ values similar to those grown in normal CO_2 atmosphere (330 μbar CO_2).

Plants with the CAM pathway (*Kalanchoë* and *Zygocactus*) are here seen to have RuBP carboxylases with $K_m(\text{CO}_2)$ values similar to those of C_3 plants (15 to 17 μM CO_2), confirming the earlier findings of Badger *et al.* (2).

$K_m(\text{CO}_2)$ of RuBP Carboxylase from Submerged Aquatic Plants. RuBP carboxylase from 13 species (ten genera) of submerged aquatic plants, representative of bryophytes, algae, and monocotyledonous and dicotyledonous angiosperms, have yielded $K_m(\text{CO}_2)$ values ranging from 30 to 70 μM CO_2 (mean \pm SD = 43 \pm 9 μM ; Table II); i.e. values consistently higher than those of the

C₃ terrestrial plants (mean \pm SD = $19 \pm 3 \mu\text{M}$; Table I), but similar to those of C₄ grasses (19). The range in $K_m(\text{CO}_2)$ levels of the carboxylases from the aquatic monocotyledons, *Egeria*, *Elodea*, and *Potamogeton* (30–49 μM CO₂; see also *Hydrilla* [17]), is similar to that shown by the aquatic dicotyledons, *Hygrophila* and *Myriophyllum* spp. (36–48 μM CO₂). Carboxylases from green algae and from an aquatic moss (*Fissidens*) also exhibit high $K_m(\text{CO}_2)$ levels in line with those of the aquatic angiosperms. Fresh water Chlorophyta (*Chara* and *Nitella*) and an unrelated marine siphonaceous species (*Codium*) have yielded closely similar $K_m(\text{CO}_2)$ (42–44 μM CO₂), but another marine species (*Ulva*) exhibits the extremely high $K_m(\text{CO}_2)$ of 70 μM CO₂.

When emergent and floating leaves of aquatic angiosperms differ morphologically from the submerged leaves, the question arises as to whether RuBP carboxylase operating in different "habitats" in the same plant is constant in its kinetics. In fact, observations on enzyme extracted from the emergent and submerged leaves of *Myriophyllum propinquum*, the floating leaves of *Potamogeton tricarlinatus*, and the submerged leaves of *Potamogeton crispus*, *Potamogeton ochreatus*, and *Myriophyllum verrucosum* show similar (high) $K_m(\text{CO}_2)$ values, suggesting that only one form is operating in these heterophyllous plants.

$K_m(\text{RuBP})$ of RuBP Carboxylase. The $K_m(\text{RuBP})$ values for RuBP carboxylase from 109 species (95 genera) of terrestrial and aquatic plants range from 10 to 136 μM RuBP, with a mean \pm SD of $36 \pm 20 \mu\text{M}$ RuBP (Tables I, II, and III), but have failed to show any clear-cut patterns comparable with those for $K_m(\text{CO}_2)$. There is no correlation between $K_m(\text{RuBP})$ and $K_m(\text{CO}_2)$ (correlation coefficient, $r = -0.06$), and $K_m(\text{RuBP})$ is not correlated with differences in photosynthetic pathway. Nor is variation in $K_m(\text{RuBP})$ attributable to differences in natural habitat: plants from sand dunes (*Festuca littoralis*, *Spinifex*, and *Zoysia*, Table III), from moist habitats (*Ceratopteris*, *Cyperus*, *Oryza*, and *Phragmites*, Tables I and III) and from alpine regions (*Aciphylla*, *Microseris*, and *Rumex*, Table I) have given similar (large) ranges in $K_m(\text{RuBP})$. Even the RuBP carboxylases of assorted submerged aquatics (*Chara*, *Codium*, *Nitella*, *Ulva*, *Egeria*, *Elodea*, *Hygrophila*, *Myriophyllum*, and *Potamogeton*, Table II), although showing a good deal of variation, all fall well within the range exhibited by terrestrial versions of the enzyme. One notes, however, that the $K_m(\text{RuBP})$ values of the two fresh water algae sampled are higher than those of the two marine species.

Although there is a great deal of overlap in the $K_m(\text{RuBP})$ of carboxylases from taxonomically diverse sources, some systematic pattern is detectable. The systematic arrangement of dicotyledonous families remains a contentious subject for taxonomist (14). However, they are conveniently separable *via* correlations involving numerous morphological, anatomical, and other criteria, into the major groups, *i.e.* Crassinucelli and Tenuinucelli (20) which are detectable in most schemes from the nineteenth century to the present day (*cf.* 4, 6). Dicotyledonous material has shown $K_m(\text{RuBP})$ values from 10 to 34 μM RuBP (Table I). The sample from this large plant group is relatively small, but it is noticeable that Crassinucelli generally occupy the lower part of the flowering plant range, falling below 22 μM RuBP (mean \pm SD = $18 \pm 4 \mu\text{M}$); while the Tenuinucelli, except for *Aciphylla*, *Petroselinum*, and *Solanum*, range from 22 to 34 μM RuBP (mean \pm SD for all tenuinucellate species = $25 \pm 7 \mu\text{M}$), the difference between the two means being statistically significant at 5% probability level. Even the aquatic dicotyledons (Table II) fall into taxonomic line in this context, in that a crassinucellate species (*Myriophyllum*) has yielded a lower $K_m(\text{RuBP})$ value (19 μM RuBP) than has a tenuinucellate one (*Hygrophila*, 27 μM RuBP).

RuBP carboxylases from monocotyledons seem to exhibit a wider range of $K_m(\text{RuBP})$ values than do those from dicotyledons, although the extremes are mainly confined to the Poaceae (Table III), which have been more extensively sampled. Some members

of the Poaceae (Triticeae, chloridoids, andropogonoids, and "oddments") have $K_m(\text{RuBP})$ values in the range 15 to 42 μM RuBP (mean \pm SD = $29 \pm 11 \mu\text{M}$), *i.e.* comparable with the other monocotyledons; the rest of the grasses, however, exhibit $K_m(\text{RuBP})$ ranging from 25 to 64 μM RuBP (mean \pm SD = $46 \pm 10 \mu\text{M}$), and *Bromus* spp., *Stipa mollis*, *Isachne globosa*, *Panicum milioides*, and *Panicum stapfianum* have yielded $K_m(\text{RuBP})$ levels in excess of 75 μM RuBP, the highest values found in this wide-ranging sample. Pteridophytes, except for *Selaginella*, have shown higher $K_m(\text{RuBP})$ values than have algae, bryophytes, dicotyledons, and monocotyledons (except for Poaceae), but overlap with those of gymnosperms (Tables I, II, and III).

In interpreting the above $K_m(\text{RuBP})$ values we were concerned with the possibility that some error in the assay procedure could produce random fluctuations in these values. Plants which gave $K_m(\text{RuBP})$ values greater than 50 μM RuBP were determined several times with similar results and species giving lower $K_m(\text{RuBP})$ values were included for assay at the same time, demonstrating that the differences between the species were consistently reproducible.

K_m Values, Function and Evolution of RuBP Carboxylase. Variation in the kinetic properties of RuBP carboxylase from phylogenetically diverse sources may represent evolutionary changes in function of the enzyme, necessary for it to operate in environments of changed substrate levels. However, the lack of functional correlation (*i.e.* in terms of photosynthetic types and ecology) for the variation in $K_m(\text{RuBP})$ values is consistent with the possibility that steady-state levels of RuBP in photosynthetic organisms have not been significantly changed during evolution of the photosynthetic system. It may also reflect the fact that $K_m(\text{RuBP})$ is relatively unimportant in relation to the enzyme concentration found *in vivo*, as suggested by Farquhar's model for the kinetics of RuBP carboxylase (8). At present, there is no obvious teleological or physiological explanation for the enzyme from some species (*Bromus* spp., *S. mollis*, *I. globosa*, *P. milioides*, and *P. stapfianum*) showing considerably lower affinities for RuBP. However, the slight taxonomic pattern in $K_m(\text{RuBP})$ values is suggestive of phylogenetic divergence in the structure of the enzyme.

The situation regarding $K_m(\text{CO}_2)$ is quite different. Organisms presumed to exemplify primitive photosynthetic systems, namely blue-green algae and unicellular algae, seem to possess CO₂-concentrating mechanisms and have RuBP carboxylase with low CO₂ affinity (1, 3, 13). Even the more complex green algae, as exemplified by *Chara*, *Nitella*, *Codium*, and *Ulva*, exhibit lower CO₂ affinity RuBP carboxylase (Table II). C₃ "higher plants," apparently lacking the ability to concentrate CO₂, may have evolved high CO₂ affinity carboxylase (Table I, see ref. 19) as an adaptation to a decrease in atmospheric CO₂ during the evolution of photosynthesis. Inasmuch as they evolved from aquatic ancestors, the present observations suggest instead that this development may have been a prelude to or a consequence of their emergence onto land. The C₄ plants, however, if they originated from C₃ ancestors, not only evolved a CO₂-concentrating mechanism, but also reverted to RuBP carboxylase with lower CO₂ affinity (Table I, see ref. 19). This lower affinity enzyme is also different in kinetics from the C₃ enzyme in that high inorganic carbon levels ($[\text{CO}_2 + \text{HCO}_3^-] > 10 \text{ mM}$, pH 8.0) inhibit the C₃ enzyme but not the C₄ enzyme (data not shown). Thus, the C₄ enzyme may remain fully active at high CO₂ levels encountered in the bundle sheath. Plants with CAM pathway, which are also postulated to have evolved directly from C₃ ancestors and which behave like normal C₃ plants during the day, also have RuBP carboxylases consistent with those of C₃ plants (Table I).

It is meaningless to characterize submerged aquatic angiosperms as C₃ or C₄ photosynthetic types on the basis of leaf anatomical features of terrestrial plants (10, 11), and the same arguments

Table III. $K_m(\text{RuBP})$ Values of RuBP Carboxylase from Grasses

Species	$K_m(\text{RuBP})$ μM	Species	$K_m(\text{RuBP})$ μM
Bamboo		Panicoids sensu lato	
<i>Arundinaria</i> sp.	44 \pm 5	Eu-panicoids:	
Oryzoids		<i>Entolasia stricta</i>	64 \pm 5
<i>Oryza sativa</i> cv. Baru	39 \pm 4	<i>Isachne globosa</i>	85 \pm 5
<i>Oryza sativa</i> cv. Calrose	43 \pm 3	<i>Oplismenus aemulus</i>	51 \pm 4
Pooids		<i>Panicum bisulcatum</i>	45 \pm 11
Triticeae:		<i>Panicum milioides</i>	96 \pm 2
<i>Hordeum vulgare</i>	25 \pm 3	<i>Axonopus compressus</i>	46 \pm 11
<i>Secale cereale</i>	57 \pm 4	<i>Brachiaria lorentziana</i>	56 \pm 8
<i>Triticum aestivum</i>	31 \pm 4	<i>Echinochloa crus-galli</i>	38 \pm 1
Bromeae:		<i>Panicum antidotale</i>	56 \pm 6
<i>Bromus arenarius</i>	76 \pm 3	<i>Panicum decompositum</i>	51 \pm 7
<i>Bromus unioloides</i>	81 \pm 2	<i>Panicum lanipes</i>	31 \pm 4
Agrostideae:		<i>Panicum maximum</i>	33 \pm 4
<i>Anthoxanthum odoratum</i>	25 \pm 2	<i>Panicum miliaceum</i>	56 \pm 7
<i>Deyeuxia quadriseta</i>	47 \pm 7	<i>Panicum stapfianum</i>	82 \pm 10
<i>Holcus lanatus</i>	40 \pm 6	<i>Pennisetum typhoides</i>	40 \pm 4
<i>Lagurus ovatus</i>	39 \pm 3	<i>Setaria geniculata</i>	26 \pm 1
<i>Phalaris brachystachya</i>	36 \pm 5	<i>Spinifex hirsutus</i>	57 \pm 4
<i>Polypogon monspeliensis</i>	35 \pm 6	Andropogonoids	
Aveneae:		<i>Bothriochloa macra</i>	42 \pm 4
<i>Amphibromus neesii</i>	51 \pm 13	<i>Cymbopogon refractus</i>	28 \pm 3
<i>Avena sativa</i>	60 \pm 6	<i>Imperata cylindrica</i>	29 \pm 4
Meliceae:		<i>Sorghum bicolor</i>	21 \pm 1
<i>Glyceria declinata</i>	49 \pm 3	<i>Themeda australis</i>	26 \pm 3
Poeae:		<i>Zea mays</i>	18 \pm 2
<i>Cynosurus echinatus</i>	40 \pm 3	Isolated genera and small groups of doubtful affinities	
<i>Festuca arundinacea</i>	56 \pm 7	Arundineae:	
<i>Festuca littoralis</i>	35 \pm 6	<i>Arundo donax</i>	61 \pm 5
<i>Lolium perenne</i>	53 \pm 10	<i>Phragmites australis</i>	64 \pm 5
<i>Poa helmsii</i>	49 \pm 8	Stipeae:	
Danthonioids		<i>Anisopogon avenaceus</i>	42 \pm 3
<i>Cortaderia selloana</i>	39 \pm 7	<i>Nassella trichotoma</i>	49 \pm 2
<i>Danthonia pallida</i>	56 \pm 3	<i>Stipa mollis</i>	136 \pm 8
<i>Triraphis mollis</i> ^a	49 \pm 8	"Oddments":	
Chloridoids		<i>Microlaena stipoides</i>	25 \pm 3
<i>Chloris truncata</i>	17 \pm 1	<i>Tetrarrhena juncea</i>	27 \pm 3
<i>Eleusine coracana</i>	23 \pm 2		
<i>Eragrostis curvula</i>	42 \pm 6		
<i>Sporobolus virginicus</i>	33 \pm 1		
<i>Zoysia macrantha</i>	15 \pm 2		

^a C_4 species are indicated by bold face.

would apply to algae and bryophytes. Some of the aquatics exhibit biochemical responses typical of C_4 terrestrial plants, without satisfying the criteria of C_4 anatomy, while others combine biochemical features of C_3 and C_4 species, depending on the environmental conditions (5, 7, 12). RuBP carboxylases of aquatic plants, including algae, bryophytes, and spermatophytes, are here shown all to have $K_m(\text{CO}_2)$ values similar to those of C_4 terrestrial plants (cf. Tables I and II). Obviously, the presence of RuBP carboxylase with low CO_2 affinity does not indicate possession of a C_4 photosynthetic system similar to that of C_4 terrestrial plants. Nevertheless, it does hint at the possession of CO_2 -concentrating mechanisms.

C_4 plants seem to have evolved from C_3 forms independently in several lines of descent; and if the various groups of aquatic angiosperms also evolved independently from C_3 terrestrial forms, as seems probable, then one must conclude that the low CO_2 affinity RuBP carboxylases have arisen, perhaps in association with CO_2 -concentrating mechanisms, on numerous occasions in different taxonomic groupings and in different physiological contexts. The predictability of $K_m(\text{CO}_2)$ values across our wide sample

of plants in terms of photosynthetic pathways and the presence or absence of possible CO_2 -concentrating mechanisms, suggests that simple correlation with ploidy levels reported in *Lolium* cultivars (9, 15) represents either a rare phenomenon or one which has not figured prominently in the evolution of this enzyme. All available evidence points to the fact that the $K_m(\text{CO}_2)$ of RuBP carboxylase may be amenable to artificial genetic manipulation.

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