Phytochemistry, Vol. 24, No. 10, pp. 2277–2279, 1985.
Printed in Great Britain.

0031–9422/85 \$3.00 + 0.00

K_m (CO_2) VALUES OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE IN GRASSES OF DIFFERENT C_4 TYPE

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(*Received* 15 *January* 1985)

Key Word Index—Grasses; Gramineae; ribulose-1,5-bisphosphate carboxylase; C_4 types; photosynthesis; systematics.

Abstract—Statistical analysis of K_m (CO_2) values of ribulose-1,5-bisphosphate (RuBP) carboxylase from 35 C_4 grass species shows that the mean value for PEP-carboxykinase (PCK) type C_4 species (41.4 ± s.e. 2.2 μM CO_2) is significantly different from that of NAD-malic enzyme (NAD-ME) type species (55.3 ± 3.1 μM CO_2) or NADP-malic enzyme (NADP-ME type species (52.5 ± s.e. 2.0 μM CO_2). These C_4 type differences remain detectable within both the eu-panicoid and chloridoid grass subfamilies. By contrast, no between-subfamily differences were found within C_4 types. Variation in K_m (CO_2) values of RuBP carboxylase may be related to *in vivo* differences in CO_2 concentration at the enzyme site, mediated perhaps by differences in CO_2-leakiness of C_4 leaf 'photosynthetic carbon reduction' (PCR or 'Kranz') tissue.

INTRODUCTION

Studies of the kinetic properties of RuBP carboxylase from taxonomically diverse plants, including grasses, show that differences in K_m (CO_2) values are correlated with variation in photosynthetic pathway, namely C_3 versus C_4 [1, 2]. The C_4 plant enzyme (especially from grasses) was found to have a lower affinity than that from C_3 terrestrial plants, perhaps because RuBP carboxylase is confined to the CO_2-tight 'photosynthetic carbon reduction' (PCR or 'Kranz') tissue in C_4 plant leaves.

Within grasses, the data also suggested K_m (CO_2) value differences between C_4-acid decarboxylation types [1], viz. NADP-malic enzyme (NADP-ME), NAD-malic enzyme (NAD-ME), or PEP-carboxykinase (PCK) type [3]. The species sample, however, was too small to yield a conclusive result on this point. We have therefore determined K_m (CO_2) values for 13 additional species, deliberately selected to supplement the original data for 24 C_4 grasses [1], in order to clarify whether K_m (CO_2) values are most highly correlated with C_4 type or grass subfamily.

RESULTS AND DISCUSSION

Table 1 shows the K_m (CO_2) values of RuBP carboxylase from 35 C_4 grass species, a sample comprising 13 PCK-type, 13 NADP-ME, and 8 NAD-ME type species. Within each C_4 type, the major grass subfamilies in which the type occurs, are about equally represented (cf. [4]). Of the additional species now sampled (Table 1), typical C_3 K_m (CO_2) values are exhibited by the two C_3 species included for comparison, and values for the C_4 species are also consistent with the earlier results for that type [1].

The results clearly show that the mean K_m (CO_2) value of PCK-type C_4 grasses (41.4 ± s.e. 2.2 μM CO_2; excluding *Triraphis mollis*) is significantly different from the mean value of NAD-ME type species (55.3 ± s.e. 3.1 μM CO_2; $P < 0.002$) and from that of NADP-ME type species (52.5 ± s.e. 2.0 μM CO_2; $P < 0.01$). NADP-ME and NAD-ME type means do not differ significantly, even at the 10% probability level.

T. mollis (chloridoid or danthonioid) can be tentatively classed as PCK-type, on the basis of its leaf anatomy, and including it in the analysis does not alter the probability levels. *Neurachne munroi* is of unknown C_4 type, and belongs in an endemic Australian genus containing C_3, C_4 and C_3–C_4 intermediate species [5]. Even in this genus, K_m (CO_2) values for a C_3 species (*N. alopecuroidea*) and a close C_3 relative (*Thyridolepis mitchelliana*) are characteristically different from that of the C_4 species, *N. munroi* (Table 1).

Within subfamilies, the sample sizes are too small for valid statistical analysis. Nevertheless, differences between C_4 types are detectable even here, viz. comparing PCK-type eu-panicoids (40.2 ± s.e. 3.2 μM CO_2) with NAD-ME eu-panicoids (57.4 ± s.e. 3.2 μM CO_2) and with NADP-ME eu-panicoids (52.3 ± s.e. 3.3 μM CO_2), and comparing PCK-type chloridoids (42.7 ± s.e. 3.3 μM CO_2) with NAD-ME type chloridoids (51.7 ± s.e. 6.7 μM CO_2). Within C_4 types, on the other hand, there is no obvious difference in K_m (CO_2) values between eu-panicoids and chloridoids for either PCK or NAD-ME type C_4 species (Table 1). Similarly, within the NADP-ME type, there is no significant difference ($P > 0.10$) between NADP-ME eu-panicoids (52.3 ± s.e. 3.3 μM CO_2) and NADP-ME andropogonoids (52.7 ± s.e. 2.4 μM CO_2). Although the mean K_m (CO_2) value for total eu-panicoids (49.7 ± s.e. 2.5 μM CO_2; $n = 18$) is higher than that for total chloridoids (45.7 ± s.e. 3.2 μM CO_2; $n = 9$), the difference is not significant even at the 10% probability level; the higher eu-panicoid mean is consequent upon the fact that, of these two subfamilies, only

Table 1. K_m (CO_2) values of ribulose-1,5-bisphosphate carboxylase for grasses of different C_4 type

Photosynthetic pathway	Grass subfamily	[Species]	$K_m(CO_2) \pm$ s.e. (μM)
C_4 PCK	chloridoid	*Chloris truncata* R. Br.	34 ± 2
		Eragrostis chloromelas Steud.	46 ± 3
		**E. philippica* Jedw.	46 ± 2
		**Sporobolus elongatus* R. Br.	55 ± 9
		S. africanus (Poir.) Robyns et Tournay	41 ± 7
		Zoysia macrantha Desv.	34 ± 4
		Triraphis mollis R. Br.	39 ± 5
	eu-panicoid	**Brachiaria foliosa* (R. Br.) Hughes	44 ± 5
		B. lorentziana (Mez) Parodi	28 ± 2
		**Eriochloa meyeriana* (Nees) Pilg.	45 ± 5
		**Panicum laevifolium* Hack.	50 ± 4
		P. maximum Jacq.	37 ± 5
		**Rhynchelytrum repens* (Willd.) C. E. Hubbard	37 ± 6
C_4NADP-ME	andropogonoid	*Bothriochloa macra* (Steud.) S. T. Blake	51 ± 5
		Cymbopogon refractus (R. Br.) A. Camus	52 ± 11
		Imperata cylindrica (L.) Beauv.	62 ± 8
		Sorghum bicolor (L.) Moench	50 ± 4
		Themeda australis (R. Br.) Stapf	45 ± 9
		Zea mays L.	56 ± 5
	eu-panicoid	*Axonopus compressus* (Swartz) Beauv.	61 ± 15
		Echinochloa crus-galli (L.) Beauv.	57 ± 21
		Panicum antidotale Retz.	53 ± 3
		**P. bulbosum* H.B.K.	56 ± 1
		Pennisetum typhoides (Burm.) Stapf & Hubb.	54 ± 3
		Setaria geniculata (Lam.) Beauv.	51 ± 2
		Spinifex hirsutus Labill.	34 ± 9
C_4NAD-ME	chloridoid	**Buchloë dactyloides* (Nutt.) Engelm.	50 ± 8
		Eleusine coracana (L.) Gaertn.	41 ± 5
		**E. indica* (L.) Gaertn.	64 ± 4
	eu-panicoid	**Panicum capillare* L.	62 ± 3
		P. decompositum R. Br.	59 ± 5
		P. lanipes Mez	45 ± 1
		P. miliaceum L.	58 ± 6
		P. stapfianum Fourc.	63 ± 8
C_4, unknown type	eu-panicoid	**Neurachne munroi* (F. Muell.) F. Muell.	32 ± 3
C_3	eu-panicoid	**Neurachne alopecuroidea* R. Br.	19 ± 1
		**Thyridolepis mitchelliana* (Nees) S. T. Blake	23 ± 3

Asterisked species are those for which original data are presented; other data from [1]. Refer to ref. [3] for biochemical basis of C_4 acid decarboxylation types. Two C_3 species are included for comparison.

eu-panicoids contain NADP-ME type C_4 species.

Since variation in K_m (CO_2) values of grass RuBP carboxylases is predictable via C_4 type irrespective of high level taxonomic groups, the correlations with C_4 type may be of functional significance, related to differences in CO_2 concentration at the site of RuBP carboxylase, within the PCR (or 'Kranz') compartment in C_4 plant leaves. This may in turn be a direct consequence of general differences between C_4 types in degree of 'CO_2-tightness' of the PCR compartment. The latter have already been inferred from the known differences between C_4 type in structure [6], $\delta^{13}C$ values [7], and absorbed quantum yield for CO_2 uptake [8]. It has been suggested [6, 7] that NADP-ME species have the most CO_2-tight PCR compartment, and NADP-ME type species the least, with PCK-type species being intermediate. The difference in mean K_m (CO_2) value between NADP-ME and PCK type species is qualitatively compatible with such a hypothesis. However, the similar mean K_m (CO_2) values for NAD-ME and NADP-ME types are inconsistent with the notion that the former have the least CO_2-tight PCR compartment. Rather, they indicate that alternative or additional explanations suggested for variation in, for example, $\delta^{13}C$ values [7] may hold. There remains the possibility therefore that variation in K_m (CO_2) values of RuBP carboxylase for different C_4 types, may also reflect differences in features other than (or additional to) 'CO_2-leakiness'. Variation in the catalytic capacity (kcat) of RuBP carboxylase between grasses has been suggested as perhaps functionally more pertinent than variation in K_m (CO_2), though these two kinetic parameters seem to be mechanistically related [9].

EXPERIMENTAL

Plant material. Plants were grown from seeds in a greenhouse at NUS. Species identify was checked with reference to appropriate regional floristic works, and vouchers retained.

Enzyme preparation and assay. RuBP carboxylase was extracted in 100 mM Bicine–NaOH buffer pH 8, containing 25 mM $MgCl_2$ and 5 mM DTT and partially purified by elution through Sephadex G-25 in the same buffer. The enzyme was preactivated in 10 mM $NaHCO_3$ and then assayed by measuring the fixation of (^{14}C) bicarbonate in a CO_2-free system according to ref. [1]. The CO_2 concn was then calculated from the pH and HCO_3^- concentration using the Henderson–Hasselbach equation and the pK' value of 6.37 at 25° [2, 10].

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