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K_m VALUES OF RIBULOSE-1,5-BISPHOSPHATE CARBOXYLASE OF CASSAVA CULTIVARS

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Key Word Index—*Manihot esculenta*; Euphorbiaceae; cassava; RuBP carboxylase; K_m values; plant systematics.

Abstract—Ribulose-1,5-bisphosphate (RuBP) carboxylase activities of two cassava cultivars increased with leaf age but their $K_m(CO_2)$ and K_m(RuBP) values remained relatively constant. $K_m(CO_2)$ values of 16 cassava cultivars ranged from 7.8 to 14.0 μM CO_2, while K_m(RuBP) values varied from 7.5 to 24.8 μM RuBP. Differences in the K_m values could not be attributed to different physiological ages of plant material or to intra-varietal variation, and are more likely to have been inherited. The results also showed that K_m values have potential applications in cassava systematics.

INTRODUCTION

Cassava, *Manihot esculenta* Crantz, is cultivated in many parts of the tropics for its storage roots. Among other factors, root development is dependent on the photosynthetic rate of the plants [1]. While the photosynthetic responses of cassava cultivars have received attention [2–4], little is known of ribulose-1,5-bisphosphate (RuBP) carboxylase (EC 4.1.1.39), the key enzyme of photosynthesis, which comprises up to 60% of the soluble leaf proteins in C_3 plants [5, 6]. The affinity of this enzyme for CO_2 is recognized as a factor limiting photosynthetic carbon assimilation [7]. Thus, it seems logical that efforts to selectively modify this enzyme so as to increase cassava productivity should perhaps be directed towards screening plants having carboxylase mutants with a high affinity for CO_2. Also, prior knowledge of this property within a plant population may be important as a source of data for selection of crosses in breeding experiments. Kinetic studies on RuBP carboxylases from taxonomically diverse plants have revealed variation in K_m (CO_2) values associated primarily with differences in photosynthetic pathway, and correlated to some extent with taxonomic groupings [8–10].

In this paper, we studied the kinetic parameters of RuBP carboxylases from 16 cassava cultivars with a view to discovering the extent of variation in the affinity of the enzyme for its two substrates, CO_2 and RuBP, and whether differences in K_m values are of potential use in cassava systematics.

RESULTS AND DISCUSSION

Variations in $K_m(CO_2)$ values of RuBP carboxylase from some plants are associated with the different stages of plant development [11]. Therefore, it is desirable to determine initially whether variations in both $K_m(CO_2)$ and K_m(RuBP) values of the enzyme from cassava are associated with such factors. Two cultivars, Putih and Merah Bercabang, grown in the same experimental plot were used. The results in Table 1 show that the levels of carboxylase activities for both cultivars increased with leaf age. Slight variations in the $K_m(CO_2)$ and K_m(RuBP) values of RuBP carboxylase were also observed. For cultivar Putih, the carboxylase activity increased from 151 nmol HCO_3^-/min/mg protein in the 5th visible leaf to 234 nmol HCO_3^-/min/mg protein in the 14th visible leaf whereas $K_m(CO_2)$ and K_m(RuBP) values remained relatively constant, with mean ± s.d. values of 15.1 ± 1.1 μM CO_2 and 10.8 ± 1.6 μm RuBP, respectively. In the case of Merah Bercabang the carboxylase activity increased from 95 nmol/HCO_3^-/min/mg protein in the 5th visible leaf to 174 nmol HCO_3^-/min/mg protein in the 11th visible leaf. A lower carboxylase activity was observed in the 14th visible leaf. The $K_m(CO_2)$ and K_m(RuBP) values of the enzyme from this cultivar averaged 10.1 ± 0.8 μM CO_2 and 12.0 ± 1.2 μM RuBP, respectively. Differences in levels of carboxylase activities and K_m values were observed for the two cultivars (Table 1). $K_m(CO_2)$ and K_m(RuBP) values of RuBP carboxylase determined for different plants belonging to the same cultivar also gave almost identical values. The present data suggest that differences in K_m values of the enzyme among cassava cultivars are unlikely to be influenced to any great extent by the physiological age of leaves or by intra-varietal variation.

Table 2 shows the $K_m(CO_2)$ and K_m(RuBP) values of RuBP carboxylase from 16 cassava cultivars. The $K_m(CO_2)$ values ranged from 7.8 μM CO_2 to 14.0 μM CO_2, with a mean ± s.d. of 10.8 ± 2.1 μM CO_2. It is not surprising that these values are similar to those reported for C_3 terrestrial plants [8, 9, 12, 13] since cassava plants have been suggested as having the C_3 pathway [14]. However, it is interesting that within a single species, the highly conserved carboxylase enzyme exhibited different levels of affinities for CO_2. Cultivars Buloh and Tiga Bulan gave RuBP carboxylase with the highest affinity for CO_2 (K_m values for both cultivars being about 8 μM CO_2) while cultivar Kunyit yielded enzyme having the lowest affinity for CO_2 (the K_m value being about 14 μM CO_2). K_m (RuBP) values of RuBP

Table 1. RuBP carboxylase activity and its K_m values from leaves of different ages for two cassava cultivars

Cultivar	Carboxylase activity (nmol HCO_3^-/min/mg protein) (mean ± s.e.)	$K_m(CO_2)$ (μM CO_2) (mean ± s.e.)	K_m(RuBP) (μM RuBP) (mean ± s.e.)
Putih			
Leaf 5	151 ± 4	13.3 ± 1.1	10.6 ± 1.3
Leaf 8	175 ± 4	15.6 ± 0.8	12.9 ± 1.2
Leaf 11	222 ± 5	15.2 ± 1.2	11.0 ± 0.9
Leaf 14	234 ± 7	16.2 ± 1.7	8.5 ± 0.9
Merah Bercabang			
Leaf 5	95 ± 1	11.0 ± 0.6	10.9 ± 0.9
Leaf 8	127 ± 1	9.6 ± 0.2	12.7 ± 0.9
Leaf 11	174 ± 3	9.1 ± 0.6	13.5 ± 0.3
Leaf 14	145 ± 2	10.7 ± 0.5	10.8 ± 0.8

The youngest visible leaf is termed leaf 1, the next oldest being leaf 2 and so on, counting from the top of the plant downwards.

Table 2. K_m values of RuBP carboxylase from cassava cultivars

Cultivar	$K_m(CO_2)$ (μM) (mean ± s.e.)	Cultivar	K_m(RuBP) (μM) (mean ± s.e.)
Buloh	7.8 ± 0.4	Su-ting	7.5 ± 0.2
Tiga Bulan	7.9 ± 0.6	C-5	8.4 ± 0.9
Peranchis	8.5 ± 0.4	C-Segamat	9.7 ± 1.7
Merah Bercabang	8.8 ± 0.2	Lohot	10.5 ± 0.7
Lohot	9.0 ± 0.6	Putih	11.0 ± 0.9
Yellow Twig	9.4 ± 1.4	Kunyit	11.3 ± 0.7
Brazil	9.7 ± 0.6	Yellow Twig	11.7 ± 0.8
C-5	10.3 ± 0.6	Black Twig	13.0 ± 0.8
Su-ting	11.4 ± 1.0	Green Twig	13.3 ± 1.3
C-Segamat	11.7 ± 1.5	Merah Bercabang	13.5 ± 0.3
Pulut	12.0 ± 1.3	Merah Jambu	13.5 ± 1.6
Black Twig	12.2 ± 0.7	Tiga Bulan	13.9 ± 0.4
Putih	12.9 ± 1.5	Brazil	19.6 ± 1.3
Merah Jambu	13.3 ± 1.1	Buloh	19.9 ± 1.7
Green Twig	13.5 ± 1.3	Pulut	24.1 ± 2.1
Kunyit	14.0 ± 1.0	Peranchis	24.8 ± 1.6

$K_m(CO_2)$ values were calculated using a pK_a value of 6.18 at 25°, corrected for ionic strength of the assay buffer.

carboxylase also showed variations, ranging from 7.5 to 24.8 μM RuBP, with a mean ±s.d. of 14.1 ± 5.2 μM RuBP, and are comparable in values to those reported for other plant species [8, 9]. Cultivars Su-ting, C-5 and C-Segamat gave carboxylases having lower K_m(RuBP) values (ranging from 7.5 to 9.7 μM RuBP) compared to those from cultivars Pulut and Peranchis (K_m(RuBP) values being *ca* 25 μM RuBP). There is no correlation between K_m(RuBP) and $K_m(CO_2)$ for the 16 cultivars studied ($r = -0.304$; $p = 0.05$).

Ward's minimum variance cluster analysis shows four possible clusters of cassava cultivars in terms of the $K_m(CO_2)$ and K_m(RuBP) values (labelled as A, B, C and D; Fig. 1). Cluster A is shown to be distinct from clusters B, C and D, whereas the last three clusters are more closely associated with one another. This similarity among various cultivars in terms of the enzyme kinetic properties perhaps reflects their close taxonomic affinities. It also suggests close similarity in the enzyme structure since the overall composition of the enzyme dictates the nature of kinetics observed. Since the cultivars examined here represent only a fraction of available cultivars grown in this region, one may expect to discover more variant forms of this enzyme. It seems likely that information on RuBP carboxylase kinetics and structure may prove useful as a tool in cassava systematics.

Variations in $K_m(CO_2)$ values of RuBP carboxylase from taxonomically diverse plants and representing dif-

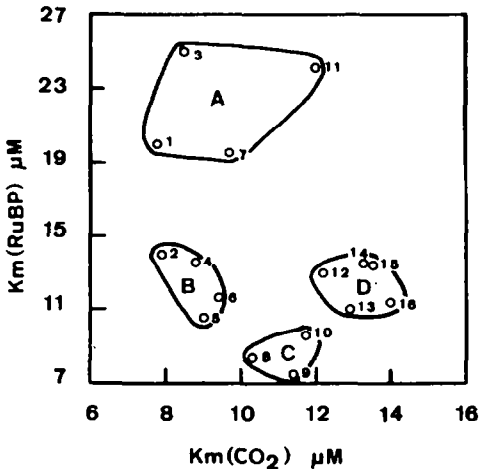

Fig. 1. Relationship among cassava cultivars in terms of RuBP carboxylase K_m values. Numerals represent different cultivars: Buloh (1), Tiga Bulan (2), Peranchis (3), Merah Bercabang (4), Lohot (5), Yellow Twig (6), Brazil (7), C-5 (8), Su-ting (9), C-Segamat (10), Pulut (11), Black Twig (12), Putih (13), Merah Jambu (14), Green Twig (15), Kunyit (16). Four clusters (A, B, C and D) were produced by Ward's minimum variance cluster analysis.

ferent photosynthetic types have been ascribed to evolutionary pressures caused by variations in the CO_2 concentration at the site of carboxylation [9, 15]. Likewise, the variations in $K_m(CO_2)$ values observed here could be attributed to minor variations in the internal CO_2 concentrations [10]. On the other hand, the lack of functional correlation for variations in K_m(RuBP) may reflect that it is relatively unimportant in relation to the enzyme concentration found *in vivo* [16]. However, it is interesting that this flexibility in K_m values, particularly $K_m(CO_2)$, demonstrated by the enzyme during its evolution and which has been also observed in other single plant species [11], is again manifested in cassava plants. Certainly, the existence of kinetic variability within the cassava gene pool makes possible the development of new cultivars having RuBP carboxylase with higher CO_2 affinities than the present ones.

EXPERIMENTAL

Plant materials. Cuttings of cassava (*Manihot esculenta* Crantz) cultivars were obtained from Universiti Pertanian Malaysia and grown in the gardens of Botany Department, NUS.

Enzyme preparation and assay. All extraction and partial purification procedures were carried out at 4°. RuBP carboxylase was extracted in 100 mM Bicine–NaOH buffer pH 8, containing 25 mM $MgCl_2$, 5 mM DTT and 1% (w/v) PVP-10 from freshly harvested and deveined leaves (1 g fr. wt/5 ml buffer), and partially purified by elution through Sephadex G-25 in 100 mM Bicine buffer pH 8, containing 5 mM $MgCl_2$ and 5 mM DTT [9]. The enzyme was preactivated in 5 mM $NaHCO_3$ at 25° for 10 min, and then assayed by measuring the fixation of [^{14}C]bicarbonate in a CO_2-free system containing 100 mM Bicine–NaOH pH 8 and 5 mM $MgCl_2$ according to ref. [9]. $K_m(CO_2)$ and K_m(RuBP) values were determined according to ref. [9], and were calculated following the method of Wilkinson [17]. The CO_2 concn was then calculated from the pH and HCO_3^- concn using the Henderson–Hasselbach equation and the pK' value of 6.18 at 25°, which had been corrected for the ionic strength of the assay buffer [12, 18]. Protein content was determined according to the modified Lowry's method [19].

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