Phytochemistry, Vol 28, No 4, pp 1043–1046, 1989
Printed in Great Britain

0031-9422/89 $3 00+0 00

VARIATIONS IN SUBUNIT AMINO ACID COMPOSITIONS AND KINETICS OF RuBP CARBOXYLASE AMONG CASSAVA CULTIVARS

KALANETHEE PAUL and HOCK-HIN YEOH*

Department of Botany, National University of Singapore, Kent Ridge, Singapore 0511

(*Received* 15 *August* 1988)

Key Word Index—*Manihot esculenta*, Euphorbiaceae, cassava, RuBP carboxylase, amino acid compositions, K_m values

Abstract—Variations were observed in the large and small subunit amino acid compositions of RuBP carboxylase of 15 cassava cultivars The K_m (CO_2) values ranged from 12.2 to 18 7 μM, while the K_m (RuBP) values ranged from 11 8 to 55 6 μM.

INTRODUCTION

Variations in the subunit amino acid compositions and kinetic properties of RuBP carboxylase have been reported from taxonomically diverse plants, upto the species level [1–5]. Below the species level, there are only a few reports on the variations in kinetic properties of the enzyme [6, 7]. These variations have been taken to reflect evolutionary changes and/or adaptation to changing carbon dioxide concentrations in the vicinity of the enzyme. Nonetheless, among grasses, differences in subunit amino acid compositions of this enzyme have also shown some correlation with their tribal groupings [4] while variations in K_m (CO_2) values largely parallel with the photosynthetic types [2].

This paper describes the extent of variation in the amino acid compositions and K_m values of RuBP carboxylase from 15 cassava (*Manihot esculenta* Crantz) cultivars. We also discuss whether variations in amino acid compositions of the enzyme may be related to the kinetic properties

RESULTS AND DISCUSSION

RuBP carboxylase of different cassava cultivars exhibited M_rs ranging from 532 000 to 590 000, their mean $\pm$ s d. being 560 000 $\pm$ 17 000. The native enzyme could be dissociated into two subunit types, the large subunit (M_r 52 000 to 58 000) and the small subunit (M_r 15 000 to 16 000). The large subunit also differed in amino acid composition from its small subunit, the former having higher levels of Asp, Thr, Gly, Ala, Val and Arg and lower levels of Ser, Glu, Pro, Leu, Tyr, Phe and Trp (Tables 1 and 2) The differences between the large and small subunit types in terms of the gross molecular feature and amino acid compositions reflect the molecular make-up common to all higher plant RuBP carboxylases.

The amino acid compositions of the large subunits of cassava RuBP carboxylase were closely similar to one another (Table 1). Nonetheless, variations were detectable and both principle component analysis and Ward's minimum variance cluster analysis revealed the presence of two basic patterns; those of Merah Bercabang, Black Twig, Buloh, Su-ting, Kunyit and Yellow Twig against those of Green Twig, Tiga Bulan, Pulut, Merah Jambu, Brazil, C-5, Lohot, Putih and Peranchis by their distinctively lower Asp level In addition, the first group also tended towards lower Thr and Ser and higher Ala, Val, Leu and Arg levels

Among the large subunits of RuBP carboxylase from Merah Bercabang, Black Twig, Buloh, Su-ting, Kunyit and Yellow Twig, those of Buloh, Su-ting, Kunyit and Yellow Twig gave very similar amino acid compositions. Merah Bercabang showed higher levels of Gly and Trp and lower levels of Ala, Val and Arg while Black Twig gave higher Trp level. The large subunits of both Merah Bercabang and Black Twig gave lower Asp compared to those of Kunyit and Yellow Twig.

Some variations in amino acid compositions were also observed among the more closely related large subunits of Green Twig, Tiga Bulan, Pulut, Merah Jambu, Brazil, C-5, Lohot, Putih and Peranchis Those of Green Twig, Tiga Bulan, Pulut, Merah Jambu and Brazil showed lower Asp and Ser and higher Thr, Pro and Arg compared to those of C-5, Lohot, Putih and Peranchis

There were more varied differences in amino acid compositions of the small subunits of cassava RuBP carboxylase (Table 2) Within this small sample size, cluster analyses showed several related groups of small subunit patterns, those of Buloh, Merah Bercabang and Black Twig, Pulut, Merah Jambu, Brazil and Green Twig; Peranchis and Lohot, and Yellow Twig and Kunyit Small subunit amino acid compositions of Putih, Tiga Bulan, C-5 and Su-ting, on the other hand, showed more varied compositions

Small subunit amino acid compositions of Buloh, Merah Bercabang and Black Twig were characterized by low Asp level As a group they differed from those of Pulut, Merah Jambu, Brazil and Green Twig in the levels of Ser, Pro, Val and Ile, and from those of Yellow Twig and Kunyit in Thr, Pro, Ile, Leu and Arg levels They were also distinguishable from those of Peranchis, Lohot,

*Author to whom correspondence should be addressed
Abbreviation· RuBP ribulose-1,5-bisphosphate.

Table 1. Amino acid composition of the large subunit of RuBP carboxylase from cassava cultivars

Cultivars	Amino acid composition (mol % total amino acids)															
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
Merah Bercabang	95	64	33	106	58	119	98	58	16	31	90	37	42	28	49	19	58
Black Twig	95	62	34	105	55	113	104	61	16	30	88	37	42	27	50	19	64
Buloh	97	61	33	104	55	115	104	62	15	30	90	38	43	27	48	13	65
Su-ting	97	63	34	107	54	114	102	64	16	32	90	35	42	26	49	13	62
Kunyit	99	61	35	107	53	115	104	64	15	32	91	36	42	26	46	12	63
Yellow Twig	99	63	34	107	58	111	105	62	15	30	92	36	42	26	47	12	63
Green Twig	110	70	37	109	61	112	98	57	16	29	86	37	40	25	45	12	58
Tiga Bulan	110	70	37	112	63	109	101	58	16	30	85	36	39	24	46	08	58
Pulut	110	71	35	111	66	108	101	58	16	29	85	35	38	24	46	12	56
Merah Jambu	112	69	39	109	58	112	99	57	16	29	86	37	40	25	45	10	58
Brazil	112	69	36	110	60	110	101	58	16	29	86	35	39	24	46	13	57
C-5	113	68	40	107	53	114	98	57	16	28	89	34	40	24	49	16	54
Lohot	114	69	38	109	56	110	98	58	16	29	87	36	40	24	47	14	56
Putih	115	67	46	108	55	108	96	56	17	32	86	35	40	23	51	14	52
Peranchis	116	67	41	108	53	112	100	56	16	28	88	36	41	24	47	12	56

Table 2. Amino acid composition of the small subunit of RuBP carboxylase from cassava cultivars

Cultivars	Amino acid composition (mol % total amino acids)															
	Asp	Thr	Ser	Glu	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
Buloh	75	48	52	122	75	99	59	45	16	39	108	58	52	26	52	27	48
Merah Bercabang	76	51	53	121	75	99	55	42	16	41	105	56	51	26	54	31	47
Black Twig	77	50	53	122	72	96	60	44	17	39	106	57	52	26	53	29	48
Yellow Twig	80	42	49	121	67	105	63	46	17	43	117	58	54	25	53	31	34
Kunyit	83	39	54	120	66	102	64	49	13	46	120	54	51	24	49	24	43
Su-ting	87	46	54	119	57	103	67	52	22	41	104	50	50	24	52	45	28
Pulut	83	50	58	120	79	95	59	37	18	33	103	55	51	26	52	36	46
Merah Jambu	85	53	58	120	81	98	58	34	17	34	102	54	51	24	50	32	49
Brazil	85	51	59	121	80	100	58	34	17	34	102	53	52	25	51	29	49
Green Twig	86	52	60	120	75	99	58	34	17	34	102	55	52	25	51	31	50
Tiga Bulan	85	47	62	117	83	97	48	36	16	33	102	50	51	23	53	56	42
C-5	92	50	66	120	76	100	33	39	15	34	101	46	49	22	56	56	44
Peranchis	85	51	62	119	69	99	58	35	17	33	101	52	51	25	53	46	46
Lohot	90	52	62	122	61	99	53	36	17	34	105	54	53	24	55	38	46
Putih	95	54	68	116	62	105	60	38	16	35	100	46	49	23	58	36	39

Putih, Tiga Bulan and C-5 by their Ser, Trp, Val, Ile, Leu and Tyr levels. Comparison between those of Pulut, Merah Jambu, Brazil and Green Twig and those of Yellow Twig and Kunyit showed the former group exhibited higher levels of Thr, Ser, Pro and Arg and lower levels of Gly, Ala, Val, Ile and Leu

It is noteworthy that if the RuBP carboxylase large subunits are structurally more closely related to one another, then the small subunits from the same source are likely to show the same tendency For example, RuBP carboxylases of cultivars Merah Bercabang, Black Twig and Buloh exhibited closely related large and small subunit amino acid compositions, respectively.

Despite variations in amino acid compositions of subunits from different cassava RuBP carboxylase the proportion of the neutral, hydrophobic and hydrophilic amino acids remained rather constant for each subunit type This hints at amino acid substitution being confined to those of similar physico-chemical properties so that the overall enzyme active site property may be preserved. This observation has been reported for RuBP carboxylase from grasses [4]

The K_m (CO_2) varied from 12.2 to 18.7 μM, with a mean $\pm$s.d of 15.4$\pm$2.0 μM whereas the K_m (RuBP) values ranged from 11.8 to 55 6 μM and the mean $\pm$s.d. was 27.2$\pm$12.8 μM (Table 3) Both K_m (CO_2) and K_m (RuBP) values differ slightly from those reported using partially purified enzymes [7] These differences possibly indicate modifications associated with the catalytic sites during purification However, the affinity for CO_2 was least affected as compared to that for RuBP, and K_m (CO_2) values reported here still fall within the range for C_3 plants [2, 3]

RuBP carboxylase from several cultivars showing closely similar amino acid compositions in both the large and small subunits gave a similar range of K_m (CO_2) values For example, those of Black Twig and Buloh exhibited K_m values of 16.9$\pm$0 8 and 16.8$\pm$2 9 μM, respectively, those of Green Twig and Tiga Bulan gave values of 17 3$\pm$2 7 and 18 7$\pm$4 5 μM, respectively, and those of Pulut, Merah Jambu and Brazil were within the range 13 1 to 14.4 μM. On the other hand, RuBP carboxylases of Kunyit and Yellow Twig, which were closely related by their large and small subunit amino acid compositions, exhibited different K_m values, i.e. 18 1$\pm$1 5 and 14.5$\pm$0.7 μM, respectively. RuBP carboxylase demonstrating very similar large subunit compositions alone, such as Kunyit and Su-ting, also gave contrasting K_m values. Does this then reflect a possible catalytic role for the small subunit? As has been reported for other RuBP carboxylase from other species, perhaps variations in kinetic properties of cassava RuBP carboxylase may be readily related to differences in the amino acid sequences of the active site region [8].

The structural composition and kinetic properties of the cassava RuBP carboxylase, as expected, are similar to those of higher plants. The overall similarity in the large subunit composition of the enzyme re-affirms early suggestions on the evolutionary conservation of this subunit which contains the catalytic site. On the other hand, the more variable nature of the small subunit composition may be seen as a tolerance of the protein to changes during evolution and may be attributed to its indirect role in catalysis. Nonetheless, it is noteworthy that within this small sample size, the diversity in structure and kinetics of cassava RuBP carboxylase is readily detectable The existence of such variability within the cassava gene pool reflects a great potential for the development of new cultivars having RuBP carboxylase with higher affinity for carbon dioxide

EXPERIMENTAL

Plant materials Cuttings of cassava (*Manihot esculenta* Crantz) were grown in the gardens of the Botany Department, National University of Singapore

Enzyme purification and characterization Cassava leaf RuBP carboxylase was purified as described in ref. [9] Purified enzyme was dissociated into its two subunit types by SDS and separated by Sephadex G-100 chromatography according to ref [10]. Amino acid analyses of the enzyme subunits were carried out according to ref [10] using an LKB Alpha Plus amino acid analyser.

Table 3 K_m values of RuBP carboxylase from cassava cultivars

Cultivars	K_m (CO_2) (μM)	Cultivars	K_m (RuBP) (μM)
Su-ting	12 2+1 3*	Green Twig	11 8+2 5*
Putih	12 8+1 8	Yellow Twig	15 2+1 3
Pulut	13 1+2 8	Kunyit	15 4+2 4
Brazil	13 8+1 3	C-5	16 0+2 5
Merah Bercabang	14 4+0 8	Su-ting	17 4+1 0
Yellow Twig	14 5+0 7	Merah Bercabang	19 7+4 5
Lohot	14 5+2 8	Buloh	22.8+2 0
Merah Jambu	14 7+2 1	Peranchis	22.8+2 0
C-5	16 4+1 6	Putih	26.4+3 5
Buloh	16 8+2 9	Black Twig	25 8+1 3
Black Twig	16 9+0 8	Lohot	31 0+2 9
Peranchis	17 2+1 5	Brazil	37 9+2.8
Tiga Bulan	17 3+2 7	Pulut	42 1+3 8
Kunyit	18 1+1 5	Tiga Bulan	48 6+4 4
Green Twig	18 7+4 5	Merah Jambu	55 6+7 7

*Mean$\pm$s e

Enzyme assay and kinetics For monitoring RuBP carboxylase activity during purification, the spectrophotometric (340 nm) procedure was used [10] For K_m determinations, purified enzymes were first activated according to Paul and Yeoh [7] K_m values were then determined by measuring the fixation of $H^{14}CO_3^-$ [7] and were calculated according to Wilkinson [11] Protein content was determined according to the modified Lowry's method [12]

Acknowledgements—We wish to thank the Universiti Pertanian Malaysia for the cassava cultivars and Kath Britt of the Research School of Biological Sciences, The Australian National University for carrying out the amino acid analyses This work was supported by NUS research grant RP131/85

REFERENCES

1 Takabe, T and Akazawa, T (1975) *Plant Cell Physiol* **16**, 1049
2 Yeoh, H H, Badger, M R and Watson, L (1980) *Plant Physiol* **66**, 1110
3 Yeoh, H H, Badger, M R and Watson, L (1981) *Plant Physiol* **67**, 1151
4 Yeoh, H H, Stone, N E and Watson, L (1982) *Phytochemistry* **21**, 71
5 Yeoh, H H and Hattersley, P (1985) *Phytochemistry* **18**, 561
6 Makino, H, Mae, T and Ohira, K (1987) *Plant Cell Physiol* **28**, 799
7 Paul, K and Yeoh, H H (1987) *Phytochemistry* **26**, 1965
8 Parry, M A J, Schmidt, C N G, Cornelius, M J, Millard, B N, Burton, S., Gutteridge, S, Dyer, T A and Keys, A J (1987) *J Expt Botany* **38**, 1260
9 Paul, K and Yeoh, H H (1988) *Plant Physiol Biochem* (in press)
10 Yeoh, H H, Stone, N E, Creaser, E H and Watson, L (1979) *Phytochemistry* **18**, 561
11 Wilkinson, G N (1961) *Biochem J* **80**, 324
12 Miller, G L (1959) *Analyt Chem* **31**, 964