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TAXONOMIC VARIATION IN THE SUBUNIT AMINO ACID COMPOSITIONS OF RuBP CARBOXYLASES FROM GRASSES

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Key Word Index—Poaceae; grasses; ribulose-1,5-bisphosphate carboxylase; amino acid compositions; taxonomic variation.

Abstract—Subunits of purified RuBP carboxylase extracted from 44 grass species (39 genera) have been analysed and compared in terms of MWs, amino acid compositions and tryptic peptide maps. The large and small subunits have an average MW of 54 500 and 13 000, respectively, and there is no structural resemblance between them. Variations in the amino acid compositions of the large and small subunits are consistent with taxonomic groupings. Those of the pooids (as a whole) are distinguishable from those of the chloridoids, eu-panicoids and andropogonoids while those of a bamboo, *Oryza*, and Stipeae show closer resemblance to those of the pooids. Despite differences in amino acid compositions of the subunits of RuBP carboxylase from taxonomically diverse grasses, the proportions of the neutral, hydrophilic and hydrophobic amino acid residues seem relatively constant throughout. The amino acid compositions of the subunits do not resemble the total leaf protein amino acid patterns, and there is no detectable variation in amino acid compositions consistent with observed differences in kinetics between C_3 and C_4 versions of the enzyme.

INTRODUCTION

Taxonomic patterns have been detected in the total leaf protein amino acid profiles of grasses [1], and it seems desirable to find out whether they are attributable to any particular protein. Ribulose 1,5-bisphosphate (RuBP) carboxylase has seemed a logical first choice for study in this context, because it is the major soluble protein fraction in all plant leaves [2, 3], and as such is likely to contribute significantly to the total leaf protein. In addition, scrutiny of information on the enzyme for diverse plants and for photosynthetic bacteria suggested the existence of taxonomic differences in amino acid composition [4], subunit polypeptide composition [5–8] and kinetic constants at various taxonomic hierarchical levels [9, 10]. In any case, RuBP carboxylase holds great interest in its own right, through its central position in the control of photosynthesis and photorespiration [11], and the possibility of contributing new information to current discussion of its function and evolution was attractive [4–10]. In connection with grasses, our earlier studies on the kinetic properties of RuBP carboxylase have hinted at the existence of structural variation, associated with the clear functional differences between C_3 and C_4 versions of the enzyme and also with taxonomy [9, 10]. Amino acid compositions of the native enzyme have in fact provided evidence of taxonomic patterns [18]. However, given that the subunits of the RuBP carboxylase molecule are encoded by different genomes, i.e. chloroplastic and nuclear [2], one would expect to obtain better taxonomic and/or structural–functional information from separate analyses of the subunits.

Here, we compare the subunits of RuBP carboxylases from 44 grass species (39 genera) in terms of MWs, amino acid compositions and tryptic–peptide maps. The sample of grasses has been chosen to cover all the main taxonomic groups of the family (cf. ref. [12, 13]), with the aim of assessing (1) the extent to which taxonomic pattern is detectable in the subunit compositions of the enzyme, (2) whether the amino acid profiles of the subunits correlate with total leaf protein amino acid patterns and (3) whether any structural variation detected correlates with observed differences in function between C_3 and C_4 versions of the enzyme.

RESULTS AND DISCUSSION

Purity of preparations

RuBP carboxylase was eluted as a single peak in a Sephadex G-200 column and was homogeneous by criteria of electrophoresis in different gel concentrations. The dissociated enzyme showed two protein peaks when separated both on an SDS–Sephadex G-100 column, and by SDS–polyacrylamide gel electrophoresis. Calculated MWs of RuBP carboxylases from 39 grass species (35 genera) averaged $546\,000 \pm 15\,000$, representing two subunits of $54\,500 \pm 1700$ and 13 000 (the observed variation is not significant and correlates neither with taxonomic groups nor with different photosynthetic pathways).

Amino acid compositions of the large subunits

The amino acid compositions of large subunits from 44 grass species (39 genera) given in Table 1 and summarized in Table 2, are arranged under major groupings ($\simeq$subfamilies) and tribes according to current information on taxonomic relationships [12, 13]. The large subunit forms the bulk of the RuBP carboxylase molecule in terms of its molecular size, and might be expected to show amino acid composition (and variation) similar to that of the native enzyme. It is not surprising,

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

Species*	Amino acid composition (mol % total amino acids)																
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
POOIDS																	
Triticeae																	
Hordeum vulgare	9.9	6.4	4.3	9.9	6.0	10.7	9.4	6.0	2.1	3.4	8.6	3.4	4.3	2.6	5.2	1.3	6.4
Secale cereale	9.7	6.6	4.0	9.7	6.2	11.0	9.7	6.2	1.8	3.5	7.9	3.5	4.4	2.6	5.3	1.3	6.6
Triticum aestivum	9.7	5.8	4.4	9.7	5.8	10.7	9.2	6.3	1.9	4.4	8.3	2.9	4.9	2.9	5.3	1.5	6.3
Bromeae																	
Bromus arenarius	10.9	5.2	5.2	10.5	5.7	9.6	9.6	6.6	2.2	3.9	8.3	3.5	4.4	2.6	5.7	0.9	5.2
Bromus molliformis	10.6	6.2	4.4	10.6	6.2	10.6	10.1	5.7	2.2	3.1	7.9	3.5	4.4	2.6	5.3	0.4	6.2
Bromus unioloides	10.0	6.5	3.3	10.9	6.1	10.4	10.4	6.1	1.3	3.0	8.3	3.5	4.3	2.6	5.2	0.9	6.1
Agrostideae																	
Anthoxanthum odoratum	10.0	6.4	4.4	10.4	5.6	10.4	10.4	6.0	2.0	3.6	8.8	3.2	4.0	2.4	4.8	1.6	6.0
Deyeuxia quadriseta	10.0	6.4	3.2	10.0	5.9	10.0	10.5	6.4	1.8	4.1	8.2	3.7	4.6	2.7	5.0	1.4	5.9
Holcus lanatus	9.7	6.2	4.0	10.1	5.7	11.0	11.0	5.7	2.2	2.6	8.4	3.5	4.4	3.1	4.8	0.9	6.6
Lagurus ovatus	10.0	6.1	3.5	10.0	5.6	10.4	10.4	6.1	2.2	3.9	8.7	3.5	4.3	2.6	4.8	1.7	6.5
Phalaris arundinacea	10.7	6.2	3.6	9.8	5.8	10.2	10.2	6.2	2.2	4.0	8.0	3.6	4.4	2.7	5.3	1.3	5.8
Phalaris brachystachya	9.9	6.4	4.3	10.7	5.6	10.3	10.3	6.0	1.7	3.0	8.6	3.4	4.3	2.6	5.2	1.3	6.4
Polypogon monspeliensis	10.5	5.9	5.5	10.1	5.9	9.3	9.3	6.3	2.5	4.2	8.4	3.4	4.2	2.1	5.9	1.3	5.1
Aveneae																	
Amphibromus neesii	10.4	6.6	3.7	10.8	5.8	10.4	10.8	5.4	1.7	2.9	8.3	3.3	4.1	2.9	5.4	1.2	6.2
Avena sativa	10.0	6.6	3.5	10.5	5.7	10.5	10.0	6.1	2.2	3.5	7.9	3.5	4.4	3.1	4.8	1.3	6.6
Poeae																	
Briza maxima	9.6	6.5	3.9	10.0	6.1	10.9	10.0	5.7	2.2	3.5	7.8	3.5	4.3	2.6	5.7	2.2	5.7
Festuca arundinacea	10.4	6.5	3.9	10.0	5.7	10.4	10.4	6.1	2.2	3.5	7.8	3.5	4.3	3.0	4.8	1.3	6.1
Lolium perenne	9.9	5.9	4.5	10.8	5.9	10.8	10.4	5.9	2.3	3.2	8.1	3.6	4.5	2.3	5.0	1.4	5.9
Poa helmsii	9.8	6.7	3.1	10.3	6.3	10.7	10.7	5.8	1.8	3.1	8.0	3.6	4.5	2.7	5.4	1.3	6.3
BAMBOOS, BAMBUSOIDS, etc.																	
Bamboo																	
Arundinaria sp.	10.5	6.2	4.3	10.5	6.2	9.5	9.5	5.2	1.9	3.3	8.6	3.3	4.8	2.9	5.2	1.0	7.1
Oryzoids																	
Oryza sativa cv Baru	9.9	6.3	3.6	9.9	5.9	9.9	9.9	6.3	2.3	3.6	8.6	3.6	4.5	2.7	5.4	1.4	6.3
Oryza sativa cv Calrose	9.6	5.9	3.8	10.5	5.4	10.5	10.5	5.0	2.1	3.3	9.2	3.3	4.2	2.1	6.3	1.3	7.1

Table 1—*continued*

Species*	Amino acid composition (mol % total amino acids)																
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
STIPEAE																	
Nassella trichotoma	10.0	6.9	3.5	10.8	5.6	10.0	10.0	5.6	2.2	3.0	8.7	3.5	4.3	3.5	5.6	0.9	6.1
Stipa falcata	9.4	6.1	5.3	11.0	5.7	9.4	9.4	6.1	2.4	4.5	8.2	3.7	4.1	2.4	4.9	1.2	6.1
DANTHONIOIDS																	
Arundo donax	9.6	7.1	4.2	10.0	6.3	10.5	10.5	5.9	2.5	2.5	7.5	3.8	4.2	2.1	4.6	2.1	6.7
Cortaderia selloana	9.7	6.3	4.6	10.1	5.9	10.9	10.1	5.9	2.1	2.9	8.4	3.8	4.2	2.5	5.0	1.3	6.3
Danthonia pallida	10.0	7.1	3.9	10.8	6.2	10.0	9.1	5.8	2.1	2.9	8.3	3.7	4.1	2.5	5.8	1.7	6.2
TRIODIEAE																	
Triodia irritans†	10.6	6.3	4.3	10.6	6.3	9.8	10.6	5.5	2.0	3.5	8.3	3.5	3.9	2.8	5.5	0.4	5.9
CHLORIDOIDS																	
Chloris gayana†	10.2	5.7	4.9	10.6	5.7	8.6	9.8	6.5	2.0	4.5	9.0	3.3	4.1	2.4	5.7	1.2	5.7
Eleusine coracana†	10.6	6.1	4.5	9.8	5.7	9.4	10.2	5.7	2.0	4.1	8.6	3.7	4.1	2.4	5.3	1.6	6.1
Eleusine indica†	9.9	6.5	3.9	10.3	5.6	10.3	9.9	6.0	2.2	3.9	8.2	3.4	4.3	3.0	4.7	1.3	6.5
Sporobolus virginicus†	10.8	6.4	5.6	10.0	5.6	10.0	10.4	5.6	2.0	3.6	8.8	3.6	4.0	2.0	5.2	1.2	5.2
Zoysia macrantha†	10.2	5.7	5.7	11.0	6.1	8.3	9.5	6.1	2.3	3.8	9.5	3.4	3.8	2.3	6.1	0.8	5.7
PANICOIDS *sensu lato*																	
Eu-panicoids																	
Echinochloa crus-galli†	10.8	5.7	5.2	11.6	4.4	8.8	9.2	5.6	2.0	4.4	9.6	3.2	4.0	2.0	6.4	0.8	6.0
Oplismenus aemulus	10.4	5.9	5.9	10.4	6.3	9.3	10.4	5.6	2.2	3.7	8.6	3.3	3.7	2.2	6.3	0.7	4.8
Panicum milioides	9.7	5.5	4.6	11.4	5.9	8.4	9.3	6.3	2.1	4.2	9.3	3.4	4.2	2.5	5.5	1.3	6.3
Pennisetum typhoides†	10.0	5.8	5.4	11.6	6.2	9.1	9.5	5.0	2.1	3.3	9.1	3.7	4.1	2.5	5.4	1.2	5.8
Setaria geniculata†	9.8	5.6	4.7	10.7	6.0	9.0	9.4	6.0	2.1	4.3	9.4	3.4	4.3	2.6	5.6	1.3	6.0
Spinifex hirsutus†	10.2	5.5	4.2	11.0	5.9	9.7	9.3	5.5	2.1	4.2	9.3	3.4	4.2	2.5	5.5	1.3	5.9
Andropogonoids																	
Hemarthria uncinata†	9.9	5.7	6.7	11.7	6.0	8.5	9.6	5.3	2.1	3.9	9.9	3.2	3.5	1.8	6.0	1.1	5.0
Imperata cylindrica†	9.7	5.5	6.2	11.7	6.2	7.9	9.0	5.2	2.1	3.8	11.4	3.1	3.4	1.7	6.6	1.0	5.5
Sorghum bicolor†	10.3	5.7	5.7	11.1	6.1	8.8	9.5	5.7	1.9	3.8	9.9	3.4	3.8	1.9	6.1	0.8	5.3
Themeda australis†	10.0	6.2	3.7	9.5	6.2	10.8	10.8	5.8	2.1	3.3	9.1	3.7	4.1	2.5	5.0	1.2	5.8
Zea mays†	10.0	6.2	5.0	10.0	6.2	10.0	10.0	5.8	2.1	3.3	8.7	3.7	4.1	2.5	5.4	1.2	5.8

* Taxonomic groupings after Watson and Dallwitz [13] and MacFarlane and Watson ([12]; for pooids).

† C_4 species.

Table 2. Amino acid composition of the large subunit of RuBP carboxylase from grasses: taxonomic group means of data in Table 1

Major groups/tribes (no. spp./no. genera)	Amino acid composition (mol % total amino acids)																
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
POOIDS (19/16)	10.1	6.3	4.1	10.3	5.9	10.5	10.1	6.0	2.0	3.5	8.2	3.5	4.4	2.7	5.2	1.3	6.1
Triticeae (3/3)	9.8	6.3	4.2	9.8	6.0	10.8	9.4	6.2	1.9	3.8	8.3	3.3	4.5	2.7	5.3	1.4	6.4
Bromeae (3/1)	10.5	6.0	4.6	10.7	6.0	10.2	10.0	6.1	1.9	3.3	8.2	3.5	4.4	2.6	5.4	0.7	5.8
Agrostideae (7/6)	10.1	6.2	4.1	10.2	5.7	10.2	10.3	6.1	2.1	3.6	8.4	3.5	4.3	2.6	5.1	1.4	6.0
Aveneae (2/2)	10.2	6.6	3.6	10.7	5.8	10.5	10.4	5.8	2.0	3.2	8.1	3.4	4.3	3.0	5.1	1.3	6.4
Poeae (4/4)	9.9	6.4	3.9	10.3	6.0	10.7	10.4	5.9	2.1	3.3	7.9	3.6	4.4	2.7	5.2	1.6	6.0
BAMBOO, BAMBUSOIDS, etc.																	
Bamboo (1/1)	10.5	6.2	4.3	10.5	6.2	9.5	9.5	5.2	1.9	3.3	8.6	3.3	4.8	2.9	5.2	1.0	7.1
Oryzoids (2/1)	9.8	6.1	3.7	10.2	5.7	10.2	10.2	5.7	2.2	3.5	8.9	3.5	4.4	2.4	5.9	1.4	6.7
STIPEAE (2/2)	9.7	6.5	4.4	10.9	5.7	9.7	9.7	5.9	2.3	3.8	8.5	3.6	4.2	3.0	5.3	1.1	6.1
DANTHONIOIDS (3/3)	9.8	6.8	4.2	10.3	6.1	10.5	9.9	5.9	2.2	2.8	8.1	3.8	4.2	2.4	5.1	1.7	6.4
TRIODIEAE (1/1)	10.6	6.3	4.3	10.6	6.3	9.8	10.6	5.5	2.0	3.5	8.3	3.5	3.9	2.8	5.5	0.4	5.9
CHLORIDOIDS (5/4)	10.3	6.1	4.9	10.3	5.7	9.3	10.0	6.0	2.1	4.0	8.8	3.5	4.1	2.4	5.4	1.2	5.8
PANICOIDS *sensu lato* (11 11)	10.1	5.8	5.2	10.8	6.0	9.1	9.6	5.6	2.1	3.8	9.5	3.4	4.0	2.3	5.8	1.1	5.7
Eu-panicoids (6/6)	10.2	5.7	5.0	10.8	5.8	9.1	9.5	5.7	2.1	4.0	9.2	3.4	4.1	2.4	5.8	1.1	5.8
Andropogonoids (5 5)	10.0	5.9	5.5	10.8	6.1	9.2	9.8	5.6	2.1	3.6	9.8	3.4	3.8	2.1	5.8	1.1	5.5

therefore, to find that the amino acid compositions of the large subunits closely resemble those of the native enzyme [1]. Comparisons among the large subunits from pooids, chloridoids and panicoids show that the large subunit of pooid RuBP carboxylase differs from that of panicoids in its higher levels of Thr, Gly, Ala, Val, Phe, His and Arg and lower levels of Ser, Glx, Ile, Leu and Lys (the differences being significant at the 5% probability level). The close taxonomic affinity between the eu-panicoids and andropogonoids, i.e. among panicoids *sensu lato* [13], is reflected in the closely similar amino acid compositions of their large subunits; in terms of the present sample, they differ only in Ser and Leu. The chloridoids, which morphologically, anatomically and in taxonomic schemes occupy a somewhat intermediate position between the pooids and panicoids [13], have yielded a large subunit pattern that closely resembles the panicoid pattern (high Ser, Ile and Leu and low Gly, His and Phe).

The large subunit of RuBP carboxylase from three danthonioid genera (*Arundo*, *Danthonia* and *Cortaderia*) has given high Thr and Gly, and low His, Ile, Leu and Lys; i.e. reflecting some features of both the pooid and the panicoid enzyme pattern. On the other hand, the large subunits from a bamboo, *Oryza*, and Stipeae show closer resemblance to the pooid pattern (i.e. higher Gly and Phe and lower Ile and Leu) than to chloridoids or panicoids; and that of *Triodia* has exhibited low Phe, a feature of the chloridoid–panicoid pattern.

Among the Pooideae, the large subunits of enzyme from the Agrostideae, Aveneae and Poeae seem indistinguishable in amino acid compositions. Large subunits from the Triticeae, however, have yielded relatively low Glx and Ala, and those from *Bromus* have relatively high Asx, compared with the rest of the Pooideae (they also differ from one another, in Asx, Glx, Gly, Ile and Arg).

Amino acid compositions of the small subunits

The amino acid compositions of the small subunits of RuBP carboxylase from 44 grass species (39 genera) are given in Table 3 and the taxonomic group mean values in Table 4. It is clear from Tables 2 and 4 that amino acid compositions of small subunits do not bear any resemblance to those of large subunits for the same species: the former are consistently higher in Ser, Glx, Pro, Tyr and Lys and lower in Thr, Gly, Ala, Leu and Arg.

Small subunit profiles are more diverse than those of large subunits, showing greater variations in Asx, Ser, Glx, Pro, Gly, Ala, Val, Tyr, Phe and Lys (contrast Tables 1 and 3). Nevertheless, some taxonomic pattern is detectable in small subunit compositions among grasses. Comparisons of the small subunits from pooids, chloridoids, eu-panicoids and andropogonoids, clearly show that those of the pooid tribes are distinguishable from those of the eu-panicoids and andropogonoids by having higher Glx, Pro, Phe, Lys and Trp and lower Asx, Ala, Leu and Arg (these differences being significant at the 5% probability level). The close taxonomic affinity between the eu-panicoids and andropogonoids is reflected again, in that the patterns from the two tribes differ only in Ser and Ile contents. The small subunit of RuBP carboxylase from the chloridoids, like that of the large subunit, has provided a pattern closely similar to that of panicoids (i.e. higher Asx, Leu and Arg and low Glx, Pro, Phe and Lys).

Comparisons among the amino acid compositions of the small subunits of danthonioids, pooids and panicoids indicate that the danthonioid version (as represented by *Arundo*, *Danthonia* and *Cortaderia*) conforms with that of the large subunit in occupying an intermediate position between those of the pooids and panicoids (see Table 4 for Asx, Ser, Glx, Ala, Leu, Phe and Lys). Unlike the corresponding large subunits, however, small subunits of RuBP carboxylase from a bamboo, *Oryza*, and Stipeae have also shown patterns with intermediate features (resembling pooids in high Glx and Pro, and resembling panicoids in high Leu and low Phe). The amino acid pattern of the small subunit of *Triodia* is closer to that of the panicoids than is the large subunit, having high Asx, Ala, Leu and Arg and lower Val, Phe and Lys.

Even among the pooid tribes and in spite of the small sample sizes, there are considerable variations in profiles of the small subunit of RuBP carboxylase. The Triticeae small subunit exhibits high levels of Thr, Ala and Arg and low levels of Ile and Phe, while that of *Bromus* has shown high Asx and Ser and low Pro and Phe; however, both Triticeae and *Bromus* (i.e. Triticanae [12]) feature lower Phe than the rest. Although the small subunits of RuBP carboxylases from the Agrostideae, Poeae and Aveneae sampled are quite similar in their amino acid compositions, small subunits from the four Poeae have given high Pro and low Val in the context of pooids as a whole, whereas those of the two Aveneae (*Amphibromus* and *Avena*) show high Glx and Pro and low Val.

Proportions of neutral, hydrophilic and hydrophobic residues

The small subunits of grass RuBP carboxylases show greater variations in their amino acid compositions than do the large subunits, in common with the situation in other plants and bacteria (cf. ref. [4]). Nevertheless, and interestingly, the proportions of the neutral, hydrophilic and hydrophobic amino acid residues in both subunits, at least in grasses, are relatively constant (Table 5), the one known exception being the small subunit of *Oryza*. This suggests that evolutionary changes in amino acid residues of the enzyme may have been restricted to those of similar physico-chemical properties (cf. ref. [14]), and perhaps to regions whose precise structural configurations are not vital to the correct functioning of the enzyme.

Tryptic–peptide maps of subunits

The primary structure of the large and small subunits of RuBP carboxylase from 24 grass species (23 genera) was investigated by tryptic–peptide mapping (data not shown). Differences in peptide map patterns would imply differences in amino acid sequences, and could be taken to indicate phylogenetic divergence in subunit structure; similarity in patterns would denote homologous amino acid sequence and conservation of structure of the subunit during evolution. In the event, the complexity of these peptide maps and problems of interpretation render detection of taxonomic patterns, or distinguishing C_3 and C_4 types, an unlikely proposition; and in fact no such distinctions have been observed. The results merely illustrate, in a subjective way, that the large subunits from different grass species have an overall similarity in their primary structure. Also, even the most different pairs of small subunit peptide maps (e.g. *Bromus*/*Zea*) show at least some peptides in common, providing good evidence of phylogenetic homology among these small subunits.

Table 3. Amino acid composition of the small subunit of RuBP carboxylase from grasses

Species*	Amino acid composition (mol % total amino acids)																
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
POOIDS																	
Triticeae																	
Hordeum vulgare	7.9	5.3	6.3	12.7	9.0	7.9	6.3	6.9	2.1	3.2	7.9	5.3	5.3	1.1	6.9	2.1	3.7
Secale cereale	8.1	5.6	6.1	12.1	6.6	9.6	8.1	6.1	2.0	3.5	8.1	4.5	5.1	2.5	6.6	1.0	4.5
Triticum aestivum	8.2	5.6	6.2	11.8	8.2	9.2	7.7	6.2	2.1	3.1	7.7	4.6	5.1	1.5	6.2	2.1	4.6
Bromeae																	
Bromus arenarius	8.9	4.7	8.9	11.7	6.1	10.3	7.0	6.1	1.9	4.2	7.5	4.2	4.7	2.3	6.5	1.4	3.7
Bromus molliformis	8.2	4.4	8.2	13.1	7.7	8.7	6.0	5.5	2.7	3.8	7.7	4.4	5.5	2.2	7.1	1.1	3.8
Bromus unioloides	9.5	4.2	7.9	12.7	6.9	8.5	6.3	6.3	1.6	3.7	7.9	4.2	5.3	1.6	7.4	1.6	4.2
Agrostideae																	
Anthoxanthum odoratum	11.3	6.2	5.6	12.4	7.3	9.6	5.1	7.3	1.1	3.4	6.8	4.0	5.6	2.8	6.8	1.1	3.4
Deyeuxia quadriseta	6.6	3.9	5.9	13.2	7.9	8.6	5.3	7.2	1.3	5.9	7.2	4.6	6.6	1.3	7.9	2.6	3.9
Holcus lanatus	7.8	4.5	6.5	13.0	9.7	9.1	5.8	5.8	1.9	3.9	7.1	4.5	6.5	1.9	7.1	0.6	3.9
Lagurus ovatus	6.6	3.7	5.9	14.0	8.1	8.1	5.9	8.1	1.5	5.1	7.4	4.4	7.4	0.7	8.1	1.5	3.7
Phalaris arundinacea	6.4	3.8	5.7	12.7	7.6	8.9	6.4	7.0	1.9	5.1	7.6	4.5	6.4	1.9	7.6	2.5	3.8
Phalaris brachystachya	8.8	4.1	7.1	12.9	7.6	8.8	7.1	6.5	1.2	4.7	7.1	4.1	5.9	1.8	7.6	1.2	3.5
Polypogon monspeliensis	9.4	6.4	6.4	12.4	5.9	9.9	6.9	6.4	1.5	4.5	7.9	4.5	5.0	1.5	6.9	1.0	3.5
Aveneae																	
Amphibromus neesii	6.1	4.1	6.1	13.5	8.8	8.1	6.1	6.1	1.4	4.7	8.1	4.7	6.8	1.4	8.1	2.0	4.1
Avena sativa	7.0	4.5	6.4	14.0	8.9	8.9	5.7	5.7	1.9	4.5	7.0	4.5	6.4	1.3	7.6	1.9	3.8
Poeae																	
Briza maxima	7.2	4.8	7.2	12.7	8.4	9.0	6.6	5.4	2.4	4.2	7.8	4.2	6.0	1.2	7.2	1.8	3.6
Festuca arundinacea	7.1	5.2	5.8	13.0	9.1	8.4	6.5	5.8	1.9	3.9	7.8	4.5	6.5	1.3	7.1	1.9	3.9
Lolium perenne	8.5	4.9	6.1	12.8	7.9	9.8	7.3	4.9	1.8	4.3	7.3	4.3	6.1	1.2	6.7	1.8	4.3
Poa helmsii	6.5	4.5	6.5	13.6	9.1	8.4	5.2	5.8	1.9	3.9	7.1	4.5	6.5	1.3	9.1	2.6	3.2
BAMBOO, BAMBUSOIDS, etc.																	
Bamboo																	
Arundinaria sp.	10.1	5.3	5.7	11.8	7.0	10.5	8.8	6.1	1.8	3.5	7.9	3.5	4.4	2.2	4.8	0.9	5.7
Oryzoids																	
Oryza sativa cv Baru	7.8	4.1	4.7	13.0	8.3	7.8	4.7	6.7	1.6	4.1	9.8	6.2	5.2	1.0	7.8	2.6	4.7
Oryza sativa cv Calrose	8.3	3.9	5.8	13.1	8.3	7.8	4.9	6.3	1.5	3.9	9.7	6.3	4.9	1.0	7.8	2.4	4.4

Table 3—*continued*

Species*	Amino acid composition (mol % total amino acids)																
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
STIPEAE																	
Nassella trichotoma	4.5	3.9	6.1	12.8	8.9	7.8	9.5	6.1	1.7	3.4	8.4	5.6	5.6	1.1	8.9	2.2	3.4
Stipa falcata	9.5	5.6	6.5	11.7	7.4	8.7	7.8	5.6	1.7	3.5	9.1	4.3	4.3	1.7	6.5	1.3	4.8
DANTHONIOIDS																	
Arundo donax	7.5	5.0	5.5	13.1	8.0	9.0	7.5	4.5	2.0	4.5	8.5	5.5	5.0	1.0	7.0	1.5	4.5
Cortaderia selloana	8.2	5.1	6.2	12.3	8.7	6.7	7.2	5.1	2.1	4.6	8.2	6.2	5.1	1.0	7.7	2.1	3.6
Danthonia pallida	9.5	6.0	6.5	11.4	8.0	8.5	7.5	7.0	2.0	3.0	8.5	5.5	5.0	1.0	5.0	2.0	4.0
TRIODIEAE																	
Triodia irritans†	10.6	6.0	6.5	12.0	7.4	8.3	7.9	5.6	1.9	4.6	8.3	4.2	4.6	0.9	5.6	1.4	4.2
CHLORIDOIDS																	
Chloris gayana†	9.9	5.6	5.6	11.2	6.5	8.2	7.8	7.3	1.7	4.3	8.2	4.3	4.3	1.3	6.9	2.2	4.7
Eleusine coracana†	12.3	3.9	6.9	11.8	6.9	6.9	6.4	5.4	1.5	4.9	9.4	4.9	4.9	1.0	6.4	2.0	4.4
Eleusine indica†	8.0	5.3	5.9	12.2	7.4	9.6	7.4	5.3	2.7	5.3	8.0	4.3	5.3	1.6	6.4	2.1	4.3
Sporobolus virginicus†	11.7	6.1	8.5	11.3	6.1	9.4	6.6	5.2	1.9	3.3	7.5	4.2	4.7	2.3	6.6	0.9	3.8
Zoysia macrantha†	10.3	5.4	6.7	11.2	5.8	10.3	8.5	5.8	1.8	3.6	8.5	3.6	4.5	2.2	6.3	0.9	4.5
PANICOIDS *sensu lato*																	
Eu-panicoids																	
Echinochloa crus-galli†	7.9	5.6	6.0	10.2	5.1	9.3	9.3	6.5	1.4	4.7	9.8	4.2	4.7	2.3	7.0	0.9	5.1
Oplismenus aemulus	9.3	5.5	5.3	10.5	5.9	10.1	10.1	6.3	1.7	5.1	8.4	4.2	4.2	2.1	6.8	0.8	3.4
Panicum milioides	11.1	6.0	4.6	12.0	5.6	9.3	8.3	5.1	1.4	4.2	8.3	4.2	4.6	2.3	6.5	1.4	5.1
Pennisetum typhoides†	11.7	5.4	5.9	10.4	6.8	9.0	7.7	5.4	1.8	3.6	8.6	5.0	4.5	1.8	6.3	1.8	4.5
Setaria geniculata†	9.8	3.8	5.4	12.5	8.2	7.6	7.1	6.0	1.6	4.9	8.2	5.4	5.4	1.1	7.1	1.6	4.3
Spinifex hirsutus†	9.8	4.6	5.7	12.1	8.0	8.0	6.3	5.7	1.7	4.6	8.0	5.2	5.7	1.7	6.9	1.7	4.0
Andropogonoids																	
Hemarthria uncinata†	5.6	4.8	6.7	12.3	5.9	14.1	9.3	5.6	1.5	3.7	8.9	3.3	3.7	2.6	6.7	0.7	4.5
Imperata cylindrica†	8.3	6.0	7.4	11.5	6.9	11.1	7.4	5.5	1.8	3.7	7.8	3.7	4.6	2.3	6.9	1.4	3.7
Sorghum bicolor†	11.7	6.2	5.8	11.7	5.8	8.6	8.9	6.6	1.6	3.5	8.6	4.7	3.9	1.2	6.2	0.8	4.3
Themeda australis†	6.4	4.3	6.9	13.3	8.0	9.0	7.4	6.9	2.1	3.7	8.0	5.3	5.3	1.6	6.9	1.1	3.7
Zea mays†	11.5	6.0	6.5	10.5	7.5	7.5	6.5	5.0	2.0	3.5	9.0	6.5	5.0	1.0	6.5	1.5	4.0

* Taxonomic groupings after Watson and Dallwitz [13] and MacFarlane and Watson ([12]; for pooids).

† C_4 species.

Table 4. Amino acid composition of the small subunit of RuBP carboxylase from grasses: taxonomic group means of data in Table 3

Major groups/tribes (no. spp./no. genera)	Amino acid composition (mol % total amino acids)																
	Asx	Thr	Ser	Glx	Pro	Gly	Ala	Val	Met	Ile	Leu	Tyr	Phe	His	Lys	Trp	Arg
POOIDS (19/16)	7.9	4.8	6.6	12.9	7.9	8.9	6.4	6.3	1.8	4.2	7.5	4.5	5.9	1.6	7.9	1.7	3.9
Triticeae (3/3)	8.1	5.5	6.2	12.2	7.9	8.9	7.4	6.4	2.1	3.3	7.9	4.8	5.2	1.7	6.6	1.7	4.3
Bromeae (3/1)	8.9	4.4	8.3	12.5	6.9	9.2	6.4	6.0	2.1	3.9	7.7	4.3	5.2	2.0	7.0	1.4	3.9
Agrostideae (7/6)	8.1	4.7	6.2	12.9	7.7	9.0	6.1	6.9	1.5	4.7	7.3	4.4	6.2	1.7	7.4	1.5	3.7
Aveneae (2/2)	6.6	4.3	6.3	13.8	8.9	8.5	5.9	5.9	1.7	4.6	7.8	4.6	6.6	1.4	7.9	2.0	4.0
Poeae (4/4)	7.3	4.9	6.4	13.0	8.6	8.9	6.4	5.5	2.0	4.1	7.5	4.4	6.3	1.3	7.3	2.0	3.8
BAMBOO, BAMBUSOIDS, etc.																	
Bamboo (1/1)	10.1	5.3	5.7	11.8	7.0	10.5	8.8	6.1	1.8	3.5	7.9	3.5	4.4	2.2	4.8	0.9	5.7
Oryzoids (2/1)	8.1	4.0	5.3	13.1	8.3	7.8	4.8	6.5	1.6	4.0	9.8	6.3	5.1	1.0	7.8	2.5	4.6
STIPEAE (2/2)	7.0	4.8	6.3	12.3	8.2	8.3	8.7	5.9	1.7	3.5	8.8	5.0	5.0	1.4	7.7	1.8	4.1
DANTHONIOIDS (3/3)	8.4	5.4	6.1	12.3	8.5	8.1	7.4	5.5	2.0	4.0	8.4	5.7	5.0	1.0	6.6	1.9	4.0
TRIODIEAE (1/1)	10.6	6.0	6.5	12.0	7.4	8.3	7.9	5.6	1.9	4.6	8.3	4.2	4.6	0.9	5.6	1.4	4.2
CHLORIDOIDS (5/4)	10.4	5.3	6.7	11.5	6.5	8.9	7.3	5.8	1.9	4.1	8.3	4.3	4.7	1.7	6.5	1.6	4.3
PANICOIDS (*sensu lato*) (11/11)	9.4	5.3	6.1	11.6	6.7	9.4	8.0	5.9	1.7	4.1	8.5	4.7	4.7	1.8	6.7	1.3	4.2
Eu-panicoids (6/6)	9.9	5.2	5.5	11.3	6.6	8.9	8.1	5.8	1.6	4.5	8.9	4.7	4.9	1.9	6.8	1.4	4.4
Andropogonoids (5/5)	8.7	5.5	6.7	11.9	6.8	10.1	7.9	5.9	1.8	3.6	8.5	4.7	4.5	1.7	6.6	1.1	4.0

Table 5. Proportion of neutral, hydrophilic and hydrophobic amino acids in RuBP carboxylase from grasses: data from Tables 1 and 3

	Amino acids (mol %)					
	Large subunit			Small subunit		
	Neutral*	Hydrophilic†	Hydrophobic‡	Neutral	Hydrophilic	Hydrophobic
POOIDS	36.8	34.3	28.9	34.6	33.5	31.8
BAMBOO, BAMBUSOIDS, etc.						
Bamboo	35.7	36.2	28.1	37.3	34.6	28.1
Oryzoids	35.9	34.9	29.4	30.2	34.5	35.6
STIPEAE	36.0	34.9	29.2	36.1	32.5	31.4
DANTHONIOIDS	37.5	34.0	28.7	35.5	32.3	32.5
TRIODIEAE	37.0	35.4	27.1	36.1	33.3	30.6
PANICOIDS *sensu lato*						
Eu-panicoids	35.0	35.2	29.6	34.8	34.3	31.4
Andropogonoids	36.2	34.3	29.4	36.9	33.0	30.1

* Thr, Ser, Pro, Gly and Ala.
† Asx, Glx, His, Lys and Arg.
‡ Val, Met, Ile, Leu, Tyr, Phe and Trp.

Comparisons of amino acid compositions of RuBP carboxylase subunits with those of total leaf proteins

The amino acid compositions of the large subunits of RuBP carboxylase consistently differ from those of total grass leaf proteins, in terms of their higher Thr, Val, Met, Tyr, His and Arg and lower Ser levels; and those of the small subunits are also very different from those of total grass leaf proteins, the former being consistently higher in Val, Ile, Tyr, Phe and Lys and lower in Ser, Gly and Ala (cf. ref. [1]).

RuBP carboxylase structure and photosynthetic pathways

Variation in the amino acid compositions of grass RuBP carboxylase and its subunits correlates significantly in the statistical sense with differences in photosynthetic pathway, in that those of the bamboo, *Oryza*, pooids, C_3 danthonioids and Stipeae (all C_3 grasses) can be distinguished as a group from those of *Triodia*, chloridoids, C_4 eu-panicoids and andropogonoids (all C_4) (see Tables 2 and 4). However, it seems that this correlation merely reflects a situation in which amino acid compositions and photosynthetic pathways are independently correlated with taxonomy. The situation is illustrated more clearly in our data on native enzyme preparations [18], but Tables 1 and 3 show a C_3 eu-panicoid (*Oplismenus aemulus*) and a C_3–C_4 intermediate species (*Panicum milioides*) having RuBP carboxylases with amino acid compositions indistinguishable from those of their C_4 eu-panicoid relatives, and quite unlike those of C_3 grass species from elsewhere in the family.

This study of RuBP carboxylase has provided no detectable evidence of structural differences consistent with the observed functional differences between C_3 and C_4 versions of the enzyme (cf. ref. [9]). Assuming these to exist (perhaps at the active site of the molecule), they are being masked by taxonomic variations in the rest of the molecule, and their detection will depend upon amino acid or DNA sequencing.

EXPERIMENTAL

Plant material. Grasses were grown in the greenhouse or collected from the field, identities being checked with reference to regional Floras.

Enzyme preparation. Leaf blades (50–100 g) excised from their sheaths at the ligule, were finely cut up and ground in a mortar with sand if they were tough or fibrous (*Stipa*, *Triodia*, *Poa*, *Spinifex*, *Imperata*, etc.), otherwise in liquid N_2 (*Anthoxanthum*, *Lagurus*, *Holcus*, *Oplismenus* etc.). The ratio of the leaf material (g) to extraction buffer (ml), 0.2 M Tris–SO_4, 10 mM EDTA and 5 mM DTT pH 8, varied from 1:2 to 1:5 depending upon the nature of the leaf samples; for tough or fibrous material, an increase in vol. of the buffer seemed to facilitate the extraction process.

RuBP carboxylase was then isolated according to the procedure described in [15] with one modification, namely, the pooled fractions from the DEAE–cellulose chromatography were eluted through a column (2.5 × 45 cm) of Sephadex G-200, equilibrated with 0.2 M Tris–SO_4, 10 mM EDTA and 5 mM DTT pH 8. Fractions containing RuBP carboxylase were collected near the void vol. of the column.

Separation of subunits was carried out using a Sephadex G-100 column as described in [15].

Electrophoresis. Analytical PAGE [16] was used to check for purity of preparation and to determine the MW of the native enzyme. SDS–PAGE was carried out as described in [15].

Amino acid analysis. The purified enzyme and subunits (*ca* 0.5 mg) were ppted by addition of cold Me_2CO to 80% and then washed with cold 80% Me_2CO. The ppt. was then recovered by centrifugation and hydrolysed in 150 μl 3 N mercaptoethanesulphonic acid in a sealed tube at 100° for 22 hr [15]. After hydrolysis, 150 μl 2 M NaOH was added to the hydrolysate, followed by 200 μl H_2O. 100 μl of the filtered hydrolysate was used for amino acid analysis on a Beckman amino acid analyser 119CL. Cys was not determined.

Beckman amino acid calibration mixtures (25 nmol each amino acid) were used for standardizing the analyser column, prior to analysing the samples. The accuracy of the method was checked by employing standard amino acid mixtures, the results proving reproducible. Duplicate analyses were run on each protein sample, and yielded consistent results. In addition, the

accuracy of the subunit amino acid analyses were counter-checked using available information from MWs and tryptic peptide maps of the subunits, plus information on the MWs and amino acid compositions of the native enzyme [1].

Tryptic digestion and mapping. The procedure was carried out as described in [15] with one modification, namely, the TLC plate was run at 50 V/cm for 25 min. It was then stained for total peptides with fluorescamine [17].

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