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Communication

Species Variation in the Predawn Inhibition of Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase[1]

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ABSTRACT

The activity of ribulose-1,5-bisphosphate carboxylase/oxygenase was measured in extracts of leaves collected before dawn (predawn activity, pa) and at midday (midday activity, ma). Twenty-three of the 37 species examined showed a pa/ma ratio (≤0.75, while only *Capsicum frutescens*, *Cucumis sativa*, *Glycine max*, *Nicotiana tabacum*, *Vigna unguiculata*, and 3 *Solanum* species showed a pa/ma ratio ≤0.5. *Phaseolus vulgaris* consistently showed a pa/ma ratio of ≤0.1. Activities and pa/ma ratios of the same species grown in the United States and the United Kingdom were very similar. Gel filtration of extracts before assay had no effect on the observed activities and the pa/ma ratios. These data are consistent with the hypothesis that in a number of species the enzyme is partially inhibited following the night period by the presence of a tight-binding inhibitor.

In some species, RuBisCO[4] (EC 4.1.1.39) activity is lower in extracts of leaves collected before dawn (dark) than in extracts of leaves collected at midday (high light) (5, 6, 8–10). The lower activity of the enzyme in extracts of leaves of tobacco (6) and *Phaselous vulgaris* (5) collected before dawn has been attributed to the presence of a phosphorylated inhibitor which binds tightly at the enzyme's active site. The inhibitor is substantially absent from high light treated leaves.

Vu *et al.* (10) found after examining representative plants from various photosynthetic groups that, although this phenomenon was not associated with a particular photosynthetic group, the predawn inhibition of RuBisCO was large (≥50%) in only soybean, tobacco, pineapple, and *Bromelia*, while a number of other species, including wheat and maize, showed no predawn inhibition. Likewise, Seemann et al. (5) found substantial predawn inhibition of RuBisCO in only 6 of 15 species examined and in only 5 of 9 leguminous species, indicating that the phenomenon might not be associated with a particular taxonomic group. We have extended these two previous studies by examining the phenomenon among a large number of species and families, taking care to maintain the enzyme in the Mg^{2+}/CO_2-activation state during extraction to prevent loss of the inhibitor from the enzyme (6). Furthermore, the similarity of the predawn inhibition of RuBisCO was measured in the same species growing in widely different localities (United States and United Kingdom).

MATERIALS AND METHODS

Plant Material. Leaves were selected at random from either cultivated or natural populations of field plants growing in the vicinity of Blacksburg, VA, and Harpenden, Herts, UK. Leaf material (≥10 g) was collected either 1 h before dawn (predawn, <0.01 mmol photons m^{-2} s^{-1}) or during the middle of the day (midday, >1 mmol photons m^{-2} s^{-1}) from the same population of plants. Leaf material was stored in liquid N_2 and analyzed usually within 24 h of harvest

RuBisCO Activity and Protein Determinations. The leaf material was ground to a fine powder in a mortar, previously chilled with liquid N_2. One g of the leaf material was removed and homogenized in 5 ml of an ice-cold extraction medium (50 mM Bicine-KOH or Tris-HCl, pH 8.0 at 0°C, 20 mM $MgCl_2$, 10 mM $NaHCO_3$, and 50 mM 2-mercaptoethanol). The extract was clarified by centrifugation (10,000*g*, 1 min) and assayed for RuBisCO activity as described previously (4, 7). In some cases, 1 ml of the extract was centrifuge-desalted (1400*g*, 2 min at 25°C) through 5 ml of Sephadex G-25 (fine) equilibrated in extraction medium (pH 8.0, 25°C) before assay. Total protein was determined using dye-binding assays (1, 2) and purified RuBisCO as a standard.

RESULTS AND DISCUSSION

The predawn and midday activities of RuBisCO for 37 species are shown in Table 1. Ten species and one genus, *Trifolium*, were measured twice, once on plant material grown in the United States and again from plants grown in the United Kingdom. Although some of these species did not show a lower predawn activity, a very close similarity was observed in the pa/ma ratio and the actual specific activities between plants of the same species growing in quite different localities. Activities and the pa/ma ratios were changed very little by the gel filtration treatment. As shown previously for tobacco (6) and soybean (8), gel filtration neither removed the phosphorylated inhibitor nor relieved the predawn inhibition as long as the enzyme was maintained in the Mg^{2+}/CO_2-activation state.

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[4] Abbreviations: RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; pa/ma, predawn activity/midday activity.

Table I. *Species Variation in the Specific Activity of RuBisCO Measured in Extracts of Predawn and Midday Leaves*

RuBisCO activities were measured in centrifuged extracts (Ex) and in these same extracts following gel filtration (GF). Values followed by SD are the average of three separate extractions of similar leaf material.

Family Species		Predawn Activity	Midday Activity	pa/ma
		μmol CO_2 fixed/min·mg protein		*ratio*
Aizoaceae				
Mesembryanthemum crystallinum	Ex	0.63 ± 0.02	0.81 ± 0.03	0.77
Amaranthaceae				
Amaranthus tricolor	Ex	0.22 ± 0.01	0.22 ± 0.02	1.02
	GF	0.23 ± 0.01	0.26 ± 0.01	0.88
Chenopodiaceae				
Beta vulgaris	Ex	0.58 ± 0.02	0.76 ± 0.02	0.75
	Ex[a]	0.73	0.78	0.94
Chenopodium album	Ex	0.89 ± 0.05	0.78 ± 0.05	1.14
Spinacia oleracea	Ex	0.78 ± 0.02	0.63 ± 0.02	1.23
	GF	0.65 ± 0.02	0.62 ± 0.03	1.05
	Ex[a]	0.79	0.71	1.11
	GF[a]	0.77	0.75	1.03
Compositae				
Helianthus annuus	Ex[a]	0.42	0.69	0.61
Lactuca sativa	Ex	0.53 ± 0.03	0.63 ± 0.05	0.84
Taraxacum officinale	Ex[a]	0.60	0.69	0.87
Zinnia hybrida	Ex	0.49 ± 0.02	0.79 ± 0.04	0.62
Crassulaceae				
Crassula argentea	Ex[a]	0.12	0.22	0.55
Kalanchoë blossfeldiana	Ex[a]	0.18	0.10	1.8
Sedum spectable	Ex[a]	1.06	0.81	1.31
Curcurbitaceae				
Cucumis sativa	Ex[a]	0.18	0.37	0.49
Cucurbita pepo	Ex	0.27 ± 0.02	0.49 ± 0.01	0.55
	GF	0.28 ± 0.01	0.55 ± 0.01	0.50
	Ex[a]	0.23	0.32	0.73
Cyperaceae				
Cyperus esculentus	Ex	0.37 ± 0.04	0.35 ± 0.05	1.05
Gramineae				
Hordeum vulgare	Ex	0.90 ± 0.05	0.83 ± 0.01	1.08
	GF	0.82 ± 0.01	0.80 ± 0.01	1.03
	Ex[a]	0.68	0.83	0.81
Triticum aestivum	Ex	1.05 ± 0.03	0.98 ± 0.01	1.08
	Ex[a]	1.20	1.30	0.92
Zea mays	Ex	0.55 ± 0.01	0.51 ± 0.03	1.09
	GF	0.51 ± 0.05	0.50 ± 0.09	1.02
Leguminosae				
Glycine max	Ex	0.19 ± 0.01	0.58 ± 0.01	0.33
	GF	0.25 ± 0.01	0.60 ± 0.04	0.42
Lupinus polyphyllus	Ex	0.83 ± 0.06	0.82 ± 0.02	1.01
	GF	0.96 ± 0.06	1.00 ± 0.04	0.96
Phaseolus vulgaris	Ex	0.053 ± 0.004	0.52 ± 0.01	0.10
	GF	0.10 ± 0.004	0.55 ± 0.05	0.18
	Ex[a]	0.026	0.54	0.05
	GF[a]	0.027	0.65	0.04
Pisum sativum	Ex[a]	0.71	0.61	1.08
Trifolium pratense	Ex	0.40 ± 0.9	0.74 ± 0.04	0.54
	GF	0.43 ± 0.08	0.86 ± 0.04	0.50
Trifolium repens	Ex[a]	0.36	0.67	0.55
Vicia faba	Ex[a]	0.68	0.91	0.75
Vigna unguiculata	Ex	0.18 ± 0.01	0.36 ± 0.01	0.50
Portulacaceae				
Portulaca grandiflora	Ex	0.35 ± 0.01	0.47 ± 0.05	0.75
Solanaceae				
Capsicum frutecens	Ex	0.20 ± 0.01	0.42 ± 0.01	0.48
	GF	0.23 ± 0.01	0.50 ± 0.02	0.46
Lycopersicon esculentum	Ex	0.75 ± 0.05	1.09 ± 0.05	0.69
	GF	0.78 ± 0.03	1.25 ± 0.07	0.62
	Ex[a]	0.72	1.01	0.71
	GF[a]	0.64	0.94	0.70
Nicotiana tabacum	Ex	0.35 ± 0.01	0.72 ± 0.01	0.45
	GF	0.34 ± 0.03	0.77 ± 0.05	0.44
	Ex[a]	0.35	0.76	0.46
	GF[a]	0.32	0.76	0.42
Petunia hybrida	Ex	0.45 ± 0.02	0.87 ± 0.03	0.51
	Ex[a]	0.20	0.28	0.71
Physalis pruinea	Ex	0.38 ± 0.01	0.54 ± 0.02	0.70
Solanum dulcamara	Ex	0.31 ± 0.02	0.50 ± 0.02	0.61
Solanum melongena	Ex	0.22 ± 0.01	0.49 ± 0.02	0.45
	GF	0.23 ± 0.01	0.57 ± 0.02	0.41
Solanum niger	Ex	0.36 ± 0.01	0.75 ± 0.02	0.47
	GF	0.37 ± 0.01	0.83 ± 0.03	0.44
Solanum phureja	Ex	0.43 ± 0.02	0.87 ± 0.02	0.49
	GF	0.47 ± 0.01	1.02 ± 0.09	0.46
Solanum tuberosum	Ex	0.49 ± 0.01	0.85 ± 0.04	0.57
	GF	0.48 ± 0.01	0.92 ± 0.03	0.52
	Ex[a]	0.56	0.93	0.60
	GF[a]	0.52	0.79	0.66

[a] Measured at Harpenden, Herts, United Kingdom.

Twenty-three species showed a pa/ma ratio (≤0.75, while only 9 species (cowpea, cucumber, pepper, soybean, *Phaseolus vulgaris*, tobacco, and 3 *Solanum* species) showed a pa/ma ratio ≤0.50. A number of previous reports have observed similar pa/ma ratios for soybean (5, 8–10), tobacco (6, 10), and cucumber (5). *P. vulgaris* consistently showed a pa/ma ratio of ≤0.1. This curious exception was also observed by Seemann *et al.* (5). In contrast, some species, particularly spinach and those in the Gramineae, showed a pa/ma ratio ≥1. Seemann *et al.* (5) observed a pa/ma ratio for spinach of 1.2. Vu *et al.* (10) measured a pa/ma ratio in *Ananas* and *Bromelia*, both obligate CAM plants, of only 0.03. In contrast, the CAM species examined in this study had RuBisCO activities which were only moderately lower (*Mesembryanthemum* and *Crassula*) or markedly higher (*Kalanchöe* and *Sedum*) in the dark. These differences probably reflect the problems involved in extraction and assay of enzymes from these species. Furthermore, it was not determined whether the plants examined were operating in the CAM mode at the time of harvest. Further work is needed to determine whether the inhibitor is present in only certain CAM species or in CAM species operating in the CAM mode.

The predawn inhibition of RuBisCO is not strictly associated with specific plant families. For example, the members of the Leguminosae showed a wide range of variability, with *P. vulgaris* being almost completely inhibited in the dark, soybean, cowpea, and clover having an intermediate amount of inhibition, and pea and lupine showing no inhibition. However, it is interesting to note that all the members of the Chenopodiaceae and Gramineae which we examined did not show the phenomenon, but all 10 members of the Solanaceae had a pa/ma ratio between

0.70 and 0.45. Hence, it appears likely that members of a single genus or perhaps an entire family would show similar amounts of predawn inhibition.

The predawn inhibition of RuBisCO is a reproducible phenomenon occurring in only certain plant species. The phenomenon appears to be absent in some species, probably because the inhibitor is not present in these species. The possibility also exists that the inhibitor was removed from the enzyme during extraction as a result of some unknown enzyme action or intervention of substances displacing the inhibitor from the active site. Previous work (5) has shown that RuBisCO from spinach, a non-inhibited species, is able to be inhibited by the partially purified inhibitor from *P. vulgaris*. Likewise, Gutteridge *et al.* (3) have also observed that the purified inhibitor from potato is able to inhibit wheat RuBisCO more than 90%.

Most of the species showing the phenomenon were only partially inhibited indicating that on the average only a quarter to one-half of the active sites were inhibited. *P. vulgaris* was the only species we observed which showed almost complete inhibition *in vivo*. It is not known why the inhibitor is present in a higher concentration in this species.

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