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Species-Dependent Variation in the Interaction of Substrate-Bound Ribulose-1,5-Bisphosphate Carboxylase/Oxygenase (Rubisco) and Rubisco Activase

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ABSTRACT

Purified spinach (*Spinacea oleracea* L.) and barley (*Hordeum vulgare* L.) ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) activase supported 50 to 100% activation of substrate-bound Rubisco from spinach, barley, wheat (*Triticum aestivum* L.), soybean (*Glycine max* L.), pea (*Pisum sativum* L.), *Arabidopsis thaliana*, maize (*Zea mays* L.), and *Chlamydomonas reinhardtii* but supported only 10 to 35% activation of Rubisco from three Solanaceae species, tobacco (*Nicotiana tabacum* L.), petunia (*Petunia hybrida* L.), and tomato (*Lycopersicon esculentum* L.). Conversely, purified tobacco and petunia Rubisco activase catalyzed 75 to 100% activation of substrate-bound Rubisco from the three Solanaceae species but only 10 to 25% activation of substrate-bound Rubisco from the other species. Thus, the interaction between substrate-bound Rubisco and Rubisco activase is species dependent. The species dependence observed is consistent with phylogenetic relationships previously derived from plant morphological characteristics and from nucleotide and amino acid sequence comparisons of the two Rubisco subunits. Species dependence in the Rubisco-Rubisco activase interaction and the absence of major anomalies in the deduced amino acid sequence of tobacco Rubisco activase compared to sequences in non-Solanaceae species suggest that Rubisco and Rubisco activase may have coevolved such that amino acid changes that have arisen by evolutionary divergence in one of these enzymes through spontaneous mutation or selection pressure have led to compensatory changes in the other enzyme.

The isolation and characterization of an *Arabidopsis thaliana* mutant provided the first evidence for the identification of Rubisco activase (18), a regulatory element of Rubisco activation state in vivo (8). The mutant required high CO_2 for growth because Rubisco activity in illuminated leaves of the mutant was much lower than that of wild type, even though the properties of Rubisco isolated from the mutant were indistinguishable from those of the wild type. Two chloroplast stromal polypeptides were found to be missing in the mutant (15), and these polypeptides were subsequently shown to activate Rubisco at physiological CO_2 and RuBP[2] concentrations (8).

Catalysis of ATP hydrolysis is an intrinsic and essential attribute of Rubisco activase (10, 19). The hydrolysis of ATP by Rubisco activase does not require the presence of Rubisco, and the reaction is highly specific for ATP and Mg^{2+} (10). Changing the conserved Lys^{111} residue at the consensus ATP-binding site in spinach Rubisco activase to Arg, Ile, or Thr by directed mutagenesis abolished both ATP hydrolysis and Rubisco activation activities in the recombinant protein (17). Thus, the ATPase activity is required for Rubisco activation even though it does not appear tightly coupled to Rubisco activation. Furthermore, the activation of RuBP-bound Rubisco by Rubisco activase was accompanied by the same extent of carbamylation as occurred in spontaneous activation (22), indicating that carbamylation is still another attribute of the Rubisco activase mechanism.

RuBP, a potent inhibitor of spontaneous Rubisco activation (4, 12, 13, 17, 19, 22), is removed from the enzyme during activation by Rubisco activase (7). Rubisco activase also restores activity to carbamylated Rubisco that has been pretreated with the endogenous inhibitor CA1P (9). The reversal of CA1P inhibition requires ATP but does not involve the metabolism of the CA1P by Rubisco activase. Finally, Rubisco activase also prevents the frequently observed time-dependent decline in Rubisco activity in vitro (11), which is caused by the isomerization of RuBP on the carbamylated enzyme during catalysis (1). These results indicate that Rubisco activase interacts with both carbamylated and noncarbamylated forms of Rubisco, releasing various sugar phosphate inhibitors from substrate-binding sites. The catalytic ability of Rubisco activase observed in vitro provided a new way to rationalize the biochemical basis of the light regulation of Rubisco activity in leaves. A working model was proposed (7) in which Rubisco activase promotes the release of RuBP from the inactive Rubisco-RuBP complex, allowing CO_2 and Mg^{2+} more rapid access for carbamylation and activation of Rubisco. Such a mechanism requires an intimate protein-protein interaction between Rubisco and Rubisco activase.

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[2] Abbreviations: RuBP, ribulose-1,5-bisphosphate; BTP, 1,3-bis[tris(hydroxymethyl)-methylamino]propane; CA1P, carboxyarabinitol-1-phosphate; LSU, Rubisco large subunit; SSU, Rubisco small subunit.

The primary structure of Rubisco activase from spinach (*Spinacia oleracea* L.) (24), *Arabidopsis* (23), and barley (*Hordeum vulgare* L.) (14) shows a high degree of sequence identity, as is found with Rubisco LSU (2, 5) and SSU (2) from these and other higher plant species. Less well conserved is the Rubisco activase from *Chlamydomonas reinhardtii* (13). However, we found that *Chlamydomonas* Rubisco-RuBP was readily activated by spinach Rubisco activase (13). In subsequent experiments, however, it became apparent that Rubisco and Rubisco activase from different species were not always compatible. A preliminary survey of the Rubisco-Rubisco activase interaction for several species was thus undertaken.

MATERIALS AND METHODS

Source of Plant Material

Spinach (*Spinacia oleracea* L. cv American Hybrid 424) was grown at 20°C in hydroponic nutrient culture with an 11-h photoperiod at 300 μmol photons m^{-2} s^{-1}. Tobacco (*Nicotiana tabacum* L. cv Xanthi), wheat (*Triticum aestivum* L.), petunia (*Petunia hybrida* L.), pea (*Pisum sativum* L.), and *Arabidopsis thaliana* were grown in Jiffy Mix (Florist Products Inc.[3]) in a growth chamber under the same conditions as for spinach. Leaves of tomato (*Lycopersicon esculentum* L.), soybean (*Glycine max* L. cv Williams), and maize (*Zea mays* L. cv Pioneer 3379) were obtained from plants grown in the greenhouse. *Chlamydomonas reinhardtii* strain 2137A$^+$ cells were grown as described earlier (13).

Purification of Rubisco Activase

All purification procedures were conducted at 4°C. Leaves (50–100 g) were deribbed and ground into a fine powder in liquid nitrogen. The frozen leaf powder in liquid N_2 was added to 150 mL of extraction buffer (20 mM BTP [pH 7.0], 5 mM $MgCl_2$, 1 mM EDTA, 0.4 mM ATP, 10 mM DTT, 1 mM PMSF, 1 mM benzamidine, and 0.01 mM leupeptin) with continuous stirring until the temperature increased above 0°C, indicating that the ice had melted and the solution could be filtered. The leaf slurry was filtered through two layers of cheesecloth and one layer of Miracloth and centrifuged for 15 min at 30,000*g*. Solid $(NH_4)_2SO_4$ was added slowly to the supernatant fluid with continuous stirring to 35% saturation. After 30 min, the precipitate was collected by centrifugation for 10 min at 8000*g*. The pellet was resuspended in 5 mL of 20 mM BTP (pH 7.0), 10 mM DTT, 0.2 mM ATP, and 5 mM $MgCl_2$ (buffer A), and 50% (w/v) PEG-10,000 was added to bring the final concentration to 18%. After the suspension was stirred for 10 min, the precipitate was collected by centrifugation for 10 min at 8000*g*. The pellet was dissolved in 10 mL of buffer A and centrifuged for 10 min at 20,000*g*.

The supernatant fluid was loaded onto a 20-mL Q-Sepharose column and eluted with 30 mL of 20 mM BTP (pH 7.0) at a flow rate of 1 mL min^{-1} before continuing with 120 mL of a linear gradient from 0 to 0.5 M NaCl in the same buffer. Fractions (2 mL) were collected, and those with high Rubisco activase and ATP hydrolysis activity were pooled and stored at −80°C. A similar procedure was used with tobacco and petunia leaves except that 1.5% (w/v) insoluble PVP and 0.5 mg mL^{-1} of 2-mercaptobenzothiazole were added to the extraction buffer, and 0.33 mg mL^{-1} of 2-mercaptobenzothiazole was added to buffer A for resuspension of the 35% $(NH_4)_2SO_4$ precipitate.

Barley Rubisco activase was purified from *Escherichia coli* cells transformed with an expression vector encoding barley Rubisco activase. A pellet of *E. coli* cells (4-L culture, courtesy of R.E. Zielinski) was resuspended in 25 mL of 40 mM BTP (pH 7.0), 5 mM $MgCl_2$, 0.5 mM ATP, 5 mM DTT, 2 mM benzamidine, 2 mM PMSF, 0.01 mM leupeptin, and 10 μg mL^{-1} of antipain (buffer B). Then, 50,000 units mL^{-1} of lysozyme, 20 units mL^{-1} of RNase, and 4.5 mL of glycerol were added, and the solution was stirred for 20 min at 4°C. A crude lysate was obtained by sonication (two 30-s periods) and centrifugation at 30,000*g* for 15 min. A saturated $(NH_4)_2SO_4$ solution was added to the supernatant fluid to 40% saturation. After the solution was stirred for 30 min, the precipitate was collected by centrifugation at 10,000*g* for 8 min. The pellet was dissolved in 6 mL of buffer B and precipitated by addition of PEG-10,000 to 17%. The precipitate was collected by centrifugation at 10,000*g* for 8 min and redissolved in 10 mL of buffer B. The procedures used for further purification on the Q-Sepharose column were the same as those described above.

Purification of Rubisco

The 0 to 35% $(NH_4)_2SO_4$ supernatant fraction from the Rubisco activase purification described above for spinach, tobacco, and petunia was brought to 55% $(NH_4)_2SO_4$ saturation, and the precipitate was collected by centrifugation for 10 min at 10,000*g*. The pellet was dissolved in 10 mL of 40 mM Tris (pH 7.5), 10 mM $NaHCO_3$, 10 mM $MgCl_2$, and 10 mM DTT (buffer C), and PEG-10,000 was added to a final concentration of 17%. The resulting precipitate was collected by centrifugation for 10 min at 10,000*g*, resuspended in 10 mL of buffer C, and loaded onto a 100-mL Q-Sepharose column equilibrated with 20 mM Tris (pH 7.5), 10 mM $MgCl_2$, and 10 mM $NaHCO_3$. The column was eluted with 150 mL of the same buffer containing 0.1 M NaCl at a flow rate of 2 mL min^{-1} before continuing with 400 mL of a linear gradient from 0.1 to 0.5 M NaCl. Fractions with a Rubisco purity of >95%, as determined by SDS-PAGE, were pooled and stored at −80°C. The same purification procedure was used for the other species except that ATP was not present in the extraction buffer. Barley Rubisco was kindly provided by R.E. Zielinski.

Assay of Total Rubisco Activity

Rubisco (stored at −80°C) was thawed, DTT was added to 10 mM, and the solution was incubated at 45°C for 10 min in a sealed vial. The enzyme was then deactivated by passage over a 20-mL Sephadex G-50 column equilibrated with 40 mM Tricine (pH 8.0), and 0.5 mM EDTA. The deactivated

[3] Mention of a trademark, proprietary product, or vendor does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products or vendors that may also be suitable.

enzyme (10–30 μg) was preincubated for 10 min at 25°C in an activation mixture containing 50 mM Tricine (pH 8.0), 10 mM $MgCl_2$, and 10 mM [^{14}C]$NaHCO_3$ (0.15–0.20 Ci mol^{-1}) in a closed 7-mL scintillation vial. The 0.5-mL reaction was initiated with 0.4 mM RuBP and terminated after 30 s at 25°C by injection of 180 μL of 4 M formic acid in 1 N HCl. The vial was dried at 65°C, the residue resuspended in 0.5 mL of 0.1 N HCl, and then 4.5 mL of scintillation fluid added. The ^{14}C incorporated into acid-stable products was determined by liquid scintillation spectroscopy.

Assay of Rubisco Activation by Rubisco Activase

Deactivated Rubisco (2–3 mg mL^{-1}, prepared as above) was incubated with 0.2 mM RuBP at room temperature for at least 1 h. Then, the inactive Rubisco-RuBP complex (120 μg of protein) was added to the 0.5 mL of activation medium containing 50 mM Tricine (pH 8.0), 40 mM KCl, 10 mM $NaHCO_3$, 10 mM $MgCl_2$, 4 mM RuBP, 2 mM ATP, 1 mM phosphocreatine, 30 units mL^{-1} of phosphocreatine kinase, and Rubisco activase (40–80 μg) at 25°C. Rubisco activity was determined by transferring 50-μL aliquots of the mixture at the times indicated in Figure 1, and after 6 min as shown in Figure 2, to 450 μL of 50 mM Tricine (pH 8.0), 10 mM [^{14}C]$NaHCO_3$ (containing 0.15–0.25 Ci mol^{-1}), 10 mM $MgCl_2$, and 0.4 mM RuBP and terminating the reaction after 30 s at 25°C by addition of 180 μL of 4 M formic acid in 1 N HCl. The samples were dried, and the incorporation of $^{14}CO_2$ into acid-stable products was determined as above.

Tobacco Rubisco Activase Deduced Amino Acid Sequence

A putative full-length cDNA encoding Rubisco activase from the tobacco cultivar SR1 was isolated from a Stratagene (Stratagene Cloning Systems, La Jolla, CA) Lambda ZAP II cDNA library following the manufacturer's directions for antibody and ^{32}P-labeled probe screening procedures. The

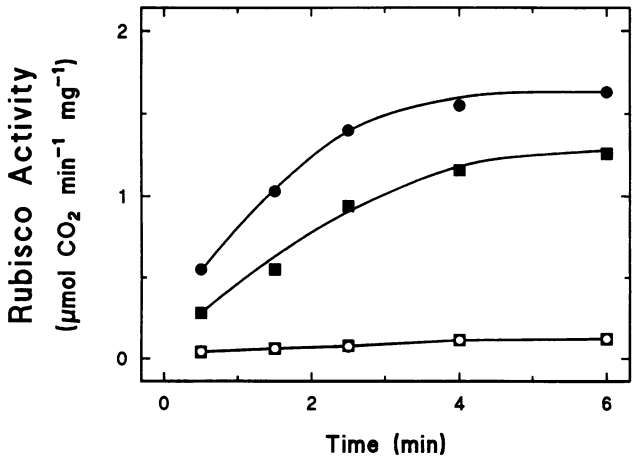

Figure 1. Time course of Rubisco activation by Rubisco activase. Rubisco-RuBP from spinach (●, □) or tobacco (○, ■) was preincubated in activation mixtures at 25°C containing Rubisco activase from spinach (●, ○) or tobacco (■, □) for the indicated time periods. Rubisco activity was then assayed for 30 s at 25°C.

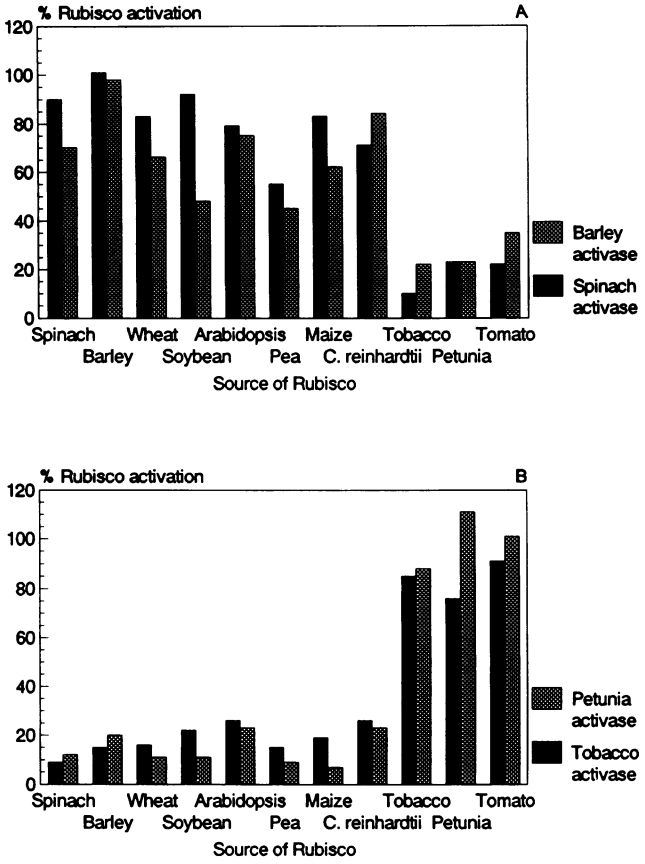

Figure 2. Species dependence of Rubisco activation by Rubisco activase. Rubisco-RuBP from the indicated species was preincubated for 6 min at 25°C in activation mixtures containing Rubisco activase from (A) spinach or barley and (B) tobacco or petunia, followed by a 30-s Rubisco assay. The data are presented as the percentage of Rubisco activity after 6 min of incubation with Rubisco activase compared to full activation by preincubation with 10 mM $NaHCO_3$ and 10 mM $MgCl_2$ at pH 8.0 and 25°C.

cDNA, approximately 1.6 kb in length, was subcloned into the *Hin*dIII and *Eco*RI sites of M13mp18 and 19 (6), and single-stranded templates were prepared from the vectors. Both strands of the cDNAs were then sequenced using the chain termination method (16) following the protocol provided by the manufacturer (United States Biochemical Corp., Cleveland, OH) with Sequenase (20). SR1-specific oligonucleotide primers were synthesized by the University of Illinois Biotechnology Center. Rubisco activase cDNA from the tobacco cv Xanthi was then amplified from total mRNA using a polymerase chain reaction method similar to one previously published (3), and the polymerase chain reaction product was cloned and sequenced as described above. The transit peptide cleavage site was established by sequence analysis of the amino terminus of the mature protein, performed by the University of Illinois Biotechnology Center.

RESULTS AND DISCUSSION

Rubisco activase catalyzes the activation of RuBP-bound Rubisco (Rubisco-RuBP) in a time-dependent manner (12).

Spinach Rubisco reached about 90% of full activation in 4 min at 25°C when incubated with spinach Rubisco activase and ATP (Fig. 1). Tobacco Rubisco-RuBP was similarly activated in the presence of tobacco Rubisco activase. However, spinach Rubisco activase activated tobacco Rubisco-RuBP only poorly, and the same result was found with the combination of tobacco Rubisco activase and spinach Rubisco-RuBP. These observations indicate a distinct specificity in the Rubisco-Rubisco activase interaction for these two species. This result contrasts with a previous experiment in which spinach Rubisco activase readily activated *Chlamydomonas* Rubisco-RuBP (13).

A preliminary survey was undertaken to determine the extent of the specificity in the Rubisco-Rubisco activase interaction (Fig. 2). Rubisco activase isolated from spinach and barley effectively activated Rubisco-RuBP from a diverse collection of species, including spinach, barley, wheat, maize, soybean, *Chlamydomonas*, pea, and *Arabidopsis* (Fig. 2A). In contrast, spinach and barley Rubisco activase supported only limited activation of Rubisco-RuBP from the three Solanaceae species, tobacco, petunia, and tomato. Rubisco activase from tobacco and petunia did, however, catalyze high activation rates of Rubisco-RuBP isolated from the Solanaceae species but were not very effective in activating the non-Solanaceae Rubisco-RuBP complexes (Fig. 2B). Hence, this initial survey defined two general groups with respect to Rubisco-Rubisco activase interaction, Solanaceae species and non-Solanaceae species. A wider survey may well define additional groups or identify exceptions to the two groups described in this experiment.

The observed variations in the Rubisco-Rubisco activase interaction suggested that significant structural dissimilarities might exist between Solanaceae and non-Solanaceae Rubisco activase, because the amino acid sequences of Rubisco LSU and SSU are very similar for these various species (2, 5). To determine whether this might be the case, the amino acid sequence of tobacco Rubisco activase was deduced from a cDNA sequence and compared (Fig. 3) to the other known Rubisco activase sequences from spinach (24), *Arabidopsis* (23), barley (14), and *Chlamydomonas* (13), which are all non-Solanaceae species. This comparison identified several residues of dissimilarity with the non-Solanaceae species, but, except for the N terminus, these dissimilarities occur in those regions where there is also no sequence consensus in the non-Solanaceae species.

A more detailed analysis, classifying residues in one of four groups, either nonpolar, polar/uncharged, acidic, or basic, revealed that tobacco Rubisco activase had 10 residues uniquely different in comparison to the other polypeptides. These differences, indicated by arrows in Figure 3, included the presence of four basic residues at Lys^{72}, Lys^{96}, Lys^{360}, and Lys^{363}, two acidic residues at Asp^{316} and Glu^{364}, three polar/uncharged residues at Asn^{166}, Asn^{376}, and Asn^{377}, and one nonpolar residue replacing a basic residue at Phe^{322}. By comparison, *Arabidopsis* Rubisco activase had seven changes of the kind described above for tobacco; there were 20 in

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Nt      EEKDADP-KKQTDSDRWKGL-V-QDFSDDQQDITRGKGMVDSLFQAPTGT-GTHHAVLQSYEYVS  61
At   V--KEDK-------QTDGDRWRGL-A-YDTSDDQQDITRGKGMVDSVFQAPMGT-GTHHAVLSSYEYVS
Hv   AENIDEK------RNTD--KWKGL-A-YDISDDQQDITRGKGIVDSLFQAPTGH-GTHEAVLSSYEYVS
So   AEN-EEK-------NTD--KWAHL-A-KDFSDDQLDIRRGKGMVDSLFQAPADA-GTHVPIQSSFEYES
Cr   V-------A-PSRKQM-G-RWRSIDAGVDASDDQQDITRGREMVDDLFQGGFGAGGTHNAVLSSQEYLS

                ↓                              ↓
Nt   QGLRQYNLDNKLDGFYIAPAFMDKLVVHITKNFLKLPNIKVPLILGIWGGKGQGKSFQCELVFRKMGIN 130
At   QGLRQYNLDNMMDGFYIAPAFMDKLVVHITKNFLTLPNIKVPLILGIWGGKGQGKSFQCELVMAKMGIN
Hv   QGLRKYDFDNTMGGFYIAPAFMDKLVVHLSKNFMTLPNIKIPLILGIWGGKGQGKSFQCELVFAKMGIN
So   QGLRKYDIDNMLGDLYIAPAFMDKLVVHITKNFLNLPNIKIPLILGVWGGKGQGKSFQCELVFAKLGIN
Cr   QSRASFN--NIEDGFYISPAFLDKMTIHIAKNFMDLPKIKVPLILGIWGGKGQGKTFQCALAYKKLGIA

                                       ↓
Nt   PIMMSAGELESGNAGEPAKLIRQRYREAAEIIRKGNMCCLFINDLDAGAGRMGGTTQYTVNNQMVNATL 199
At   PIMMSAGELESGNAGEPAKLIRQRYREAADLIKKGKMCCLFINDLDAGAGRMGGTTQYTVNNQMVNATL
Hv   PIMMSAGELESGNAGEPAKLIRQRYREAADMIKKGKMCCLFINDLDAGAGRMGGTTQYTVNNQMVNATL
So   PIMMSAGELESGNAGEPAKLIRQRYREAADLIAKGKMCALFINDLEPGAGRMGGTTQYTVNNQMVNATL
Cr   PIVMSAGELESGNAGEPAKLIRTRYREASDIIKKGRMCSLFINDLDAGAGRMGDTTQYTVNNQMVNATL

Nt   MNIADNPTNVQLPGMYNKQENARVPIIVTGNDFSTLYAPLIRDGRMEKFYWAPTREDRIGVCTGIFRTD 268
At   MNIADNPTNVQLPGMYNKEENARVPIICTGNDFSTLYAPLIRDGRMEKFYWAPTREDRIGVCKGIFRTD
Hv   MNIADAPTNVQLPGMYNKRENPRVPIVVTGNDFSTLYAPLIRDGRMEKFYWAPTRDDRIGVCKGIFQTD
So   LNIADNPTNVQLPGMYNKQDNARVPIIVTGNDFSTLYAPLIRDGRMEKFYWAPTREDRIGVCTGIFKTD
Cr   MNIADNPTNVQLPGVYKNEEIPRVPIVCTGNDFSTLYAPLIRDGRMEKYYWNPTREDRIGVCMGIFQED

                                                  ↓     ↓
Nt   NVPAEDVVKIVDNFPGQSIDFFGALRARVYDDEVRKWVSGTGIEKIGDKLLNSFDGPPTFEQPKMTIEK 337
At   KIKDEDIVTLVDQFPGQSIDFFGALRARVYDDEVRKFVESLGVEKIGKRLVNSREGPPVFEQPEMTYEK
Hv   NVCDESVVKIVDTFPGQSIDFFGALRARVYDDEVRKWVGSTGIENIGKRLVNSRDGPVTFEQPKMTVEK
So   KVPAEHVVKLVDAFPGQSTDFFGALRARVYDDQVRKWVNSVGVDNVGKKLVNSKDGPPVFEQPEMTLQK
Cr   NVQRREVENLVDTFPGQSIDFFGALRARVYDDMVRQWITDTGVDKIGQQLVNARQKVAM-PKVSMDLNV

                              ↓  ↓↓          ↓↓
Nt   LLEYGNMLVQEQENVKRVQLADKYLKEAALGDANADAINNGSFFAS                        383
At   LMEYGNMLVMEQENVKRVQLAETYLSQAALGDANADAIGRGTFYGKTEEKEPSK
Hv   LLEYGHMLVQEQDNVKRVQLADTYMSQAALGDANQDAMKTGSFYG
So   LMEYGNMLVQEQENVKRVQLADQYMSSAALGDANKDAIDRGTFFG
Cr   LIKYGKSLVDEQENVKRVQLADAYLSGAELAGHGGSSLPEAYSR
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Figure 3. Comparison of Rubisco activase amino acid sequences deduced from cDNA sequences for tobacco (*Nt*), *Arabidopsis* (*At*; 23 and subsequent corrections, unpublished), barley (*Hv*; 14), spinach (*So*; 24 and subsequent corrections, unpublished), and *Chlamydomonas* (*Cr*; 13). Regions of identity and homology are shaded, and the 10 uniquely different residues in the tobacco sequence are indicated by arrows (see text for discussion).

spinach and four in barley. In contrast, *Chlamydomonas* Rubisco activase had about 54 such changes. Nonetheless, *Chlamydomonas* Rubisco-RuBP was readily activated by spinach and barley Rubisco activase but not by Rubisco activase from tobacco and petunia (Fig. 2). The species dependence in the Rubisco-Rubisco activase interaction is thus not obviously apparent from the deduced sequences and probably arises from subtle changes in the tertiary structures of one or both enzymes that may involve charge redistribution or charge reversals at sites of interaction with Rubisco.

The Rubisco activase mechanism of action is not known, but several partial reactions of the physiological activation process have been determined. The known activities catalyzed by Rubisco activase include the release of RuBP and other sugar phosphates from Rubisco (7), ATP binding (21) and hydrolysis (10, 19), and carbamylation of Rubisco (22). These reactions, except for ATP binding and hydrolysis, require an interaction between Rubisco activase and Rubisco, and the data presented here indicate that this interaction is a very intimate one and is likely influenced by small changes in protein shape or charge distribution. The results also indicate that, in several species of at least one identifiable group of plants, the Solanaceae, Rubisco, and Rubisco activase have coevolved to the extent that these two important regulatory enzymes of photosynthesis recognize only poorly their counterparts from non-Solanaceae species.

From analyses of nucleotide changes in Rubisco SSU genes (5) and amino acid changes in Rubisco LSU and SSU (2), phylogenetic trees have been constructed and used to determine the evolutionary divergence of plant species. The Solanaceae were shown to diverge from other plants in all cases, consistent with phylogenetic analysis of morphological traits (5). However, the multiple SSU genes in each species were more similar to each other than to any SSU genes from other species (5). Such conservatism in the SSU evolutionary process could be explained by the necessity of compensatory mutations in LSU or other proteins with which SSU or Rubisco might interact (5). The requisite interaction between Rubisco and Rubisco activase, and the species dependence in this interaction, as reported here, indicates a strong likelihood that the mutation rates of the genes for LSU, SSU, and Rubisco activase are highly dependent upon the rate of compensatory mutations in the other two genes or gene families. Nucleotide sequences for the Rubisco activase gene from several additional species will be needed to determine whether the rate of change in the amino acid sequence for this enzyme is markedly less than observed for polypeptides that do not interact so intimately with other proteins.

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