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Facilitation of the Terminal Proton Transfer Reaction of Ribulose 1,5-Bisphosphate Carboxylase/Oxygenase by Active-Site Lys166†

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ABSTRACT: The terminal step in the carboxylation pathway catalyzed by ribulose 1,5-bisphosphate carboxylase/oxygenase (Rubisco) is stereospecific protonation of the C-2 *aci*-acid of 3-phosphoglycerate (PGA). X-ray crystallographic results favor the ϵ-amino group of Lys166 as the proton donor in this step [Knight et al. (1990) *J. Mol. Biol. 215*, 113]. Nonetheless, position-166 mutants are able to catalyze forward processing of isolated 2-carboxy-3-ketoarabinitol 1,5-bisphosphate (CKABP), the carboxylated reaction intermediate [Lorimer, G. H., & Hartman, F. C. (1988) *J. Biol. Chem. 263*, 6468]. Prior assays for intermediate processing relied solely on formation of acid-stable radioactivity from acid-labile [2′-^{14}C]CKABP. Therefore, PGA, the normal reaction product, may not have been distinguished from pyruvate, the product from β-elimination of phosphate from the terminal *aci*-acid intermediate [Andrews, T. J., & Kane, H. J. (1991) *J. Biol. Chem. 266*, 9447]. If Lys166 indeed serves as the terminal proton donor, mutants lacking an ionizable side chain at position 166 might process the carboxylated intermediate predominantly to pyruvate. We have thus used anion exchange chromatography and enzyme coupling to separate and identify the products from turnover of [2′-^{14}C]CKABP by wild-type, K166G, and K166S enzymes. Although PGA is the only labeled product of significance formed by wild-type enzyme, pyruvate is a major labeled product formed by the mutants. These results provide the first direct functionally-based evidence that Lys166 is crucial to the last step in Rubisco-catalyzed conversion of RuBP to PGA.

As the only significant avenue for net fixation of atmospheric CO_2, ribulose 1,5-bisphosphate carboxylase/oxygenase (EC 4.1.1.39) is indispensible for photosynthetic carbon assimilation. However, the kinetic sluggishness ($k_{cat} = 2-4$ s^{-1}) of the enzyme and nonproductive misprocessing of its labile reaction intermediates impose severe limitations on biomass yields [reviewed in Hartman and Harpel (1993, 1994), Harpel et al. (1995), Andrews and Lorimer (1987), and Andrews et al. (1994)]. In particular, the RuBP-enediol[1] (the first intermediate in catalysis) reacts with O_2, in competition with CO_2, to initiate the energetically-wasteful photorespiration pathway (Bowes et al., 1971; Lorimer et al., 1973). Other side reactions catalyzed by Rubisco not only siphon RuBP but also produce potent inhibitors of the enzyme (Andrews & Kane, 1991; Edmondson et al., 1990; Zhu & Jensen, 1991).

The carboxylative cleavage reaction catalyzed by Rubisco entails a succession of diverse partial reactions, inclusive of proton abstraction, enolization, electrophilic addition, hydra-

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[1] Abbreviations: RuBP, D-ribulose 1,5-bisphosphate; Rubisco, D-ribulose 1,5-bisphosphate carboxylase/oxygenase; CKABP, 2-carboxy-3-ketoarabinitol 1,5-bisphosphate; PGA, 3-phospho-D-glycerate; Bicine, *N*,*N*-bis(2-hydroxyethyl)glycine.

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tion, carbon−carbon cleavage, stereochemical inversion, and proton addition [reviewed in Hartman and Harpel (1993, 1994), Schloss (1990), Andrews and Lorimer (1987)]. Because these partial reactions encompass many of the fundamental reaction types, individually catalyzed by numerous and functionally diverse enzymes, Rubisco may be viewed as a paradigm for mechanistic enzymology.

The function of active-site residues of Rubisco has been probed by numerous approaches, including chemical modification, site-directed mutagenesis, and X-ray crystallography. One particularly powerful approach has been to combine site-directed mutagenesis with assays of partial reactions, as evaluated independently of net carboxylase activity. For example, inability of position-329 mutants to catalyze net carboxylation, even though competent in the enolization of RuBP and the processing of CKABP, the isolated carboxylation-reaction intermediate, pinpoints the catalytic role of Lys329[2] in facilitating CO_2 addition to enediol and in stabilizing the subsequent carboxylated intermediate (Soper et al., 1988; Hartman & Lee, 1989; Lorimer et al., 1993; Harpel & Hartman, 1994). Such a role is entirely consistent with the three-dimensional structure of Rubisco complexed with an analogue of CKABP (Knight et al., 1990; Newman & Gutteridge, 1993; Andersson, 1996). Lys166 has been similarly probed, demonstrating that it exhibits characteristics predicted for the general base that abstracts the C-3 proton of RuBP to form an enediol. These include unusual nucleophilicity/acidity (Hartman et al., 1985; Smith & Hartman, 1988) and severe impairment of position-166 mutants in both net carboxylation (Hartman et al., 1987) and enolization activities (Lorimer & Hartman, 1988; Hartman & Lee, 1989), despite retention of ability to process CKABP (Lorimer & Hartman, 1988). However, crystallographic models clearly illustrate that the ϵ-amine of Lys166 is too remote and in the wrong orientation to serve as the primary base for substrate enolization (Knight et al., 1990; Newman & Gutteridge, 1993; Andersson, 1996). Thus, facilitation of enolization by Lys166 is indirect or reflective of Lys166 serving as a secondary, rather than primary, acceptor of the C-3 proton.

Recently, we have noted the value of analyzing the products of partial reactions catalyzed by site-directed mutants, the generic assays for which do not always discriminate between normal and aberrant reaction products, to further delineate residue function. For example, whereas mutants that promote the exchange of radiolabel from [3-^{3}H]-RuBP (labeled at the position of proton abstraction) are deemed competent in enediol formation (Hartman & Lee, 1989), only after chemical identification of the products of these reactions was it revealed that some such mutants are unable to stabilize the enediol (Larimer et al., 1994; Harpel & Hartman, 1994). We now apply this approach to address another uncertainty involving Lys166. Structurally, Lys166 is the only active-site residue suitably located to serve as a proton donor for the terminal *aci*-acid intermediate of carboxylation and thereby complete formation of the second molar equivalent of PGA from RuBP (Knight et al., 1990). However, the K166G mutant catalyzes forward processing

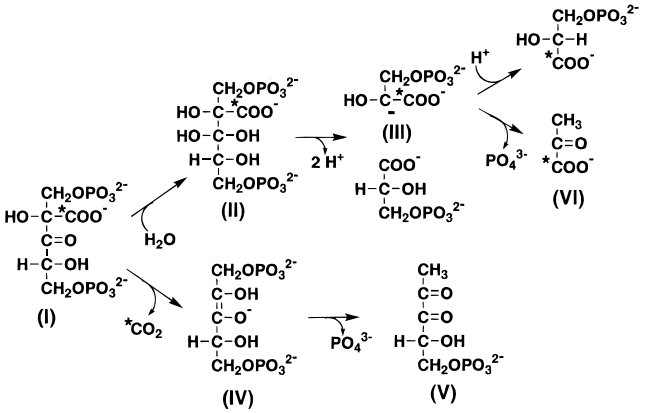

FIGURE 1: Potential fates of isolated [2′-^{14}C]CKABP (I). The upper pathway depicts the reaction catalyzed by wild-type Rubisco, which involves hydration, carbon−carbon scission of *gem*-diol (II), inversion of stereochemistry at C-2 and stereospecific protonation of the *aci*-acid (III). Pyruvate (VI), as an end-product from CKABP, derives from β-elimination of phosphate from the *aci*-acid of PGA (III), the terminal intermediate in overall Rubisco catalysis. This pathway predominates in basic solution (pH > 11) (Lorimer et al., 1986) and is now observed with position-166 mutants (see Figure 2). The lower pathway depicts decarboxylation of CKABP to form the enediolate (IV), which undergoes β-elimination of phosphate to form 1-deoxy-D-*glycero*-2,3-pentodiulose 5-phosphate (V); this pathway defines spontaneous decomposition at moderate pH and is also catalyzed by unactivated Rubisco (Pierce et al., 1986) and by some mutant Rubiscos (Gutteridge et al., 1988; Harpel et al., 1993a). The disposition of radiolabel from [2′-^{14}C]CKABP is traced with an asterisk; of the three potential products, PGA and pyruvate retain the labeled carboxyl group and are thus visualized in corresponding chromatographic profiles.

(hydration and cleavage) of CKABP (Lorimer & Hartman, 1988), implying that the terminal protonation is not compromised by the absence of a lysyl side chain at position 166. Because multiple fates are possible for this reactive intermediate (Figure 1) and the final products of its turnover by K166G were not characterized directly, product verification would appear crucial to reconcile these structural and functional observations.

Herein, we demonstrate that the major radiolabeled product derived from the processing of [2′-^{14}C]CKABP by K166G is not PGA, the normal product of this reaction, but pyruvate, the product formed from β-elimination of phosphate from the terminal *aci*-acid intermediate. Thus, in addition to its role in the initial deprotonation catalyzed by Rubisco, Lys166 also facilitates the terminal protonation step.

EXPERIMENTAL PROCEDURES

Materials. Wild-type Rubisco was isolated from *Rhodospirillum rubrum* as previously described (Schloss et al., 1982). The mutant *rbc* genes encoding K166G and K166S *R. rubrum* Rubiscos (Hartman et al., 1987) were transferred to the efficient expression vector pFL245 (Larimer et al., 1990) and expressed in *Escherichia coli* strain MV1190 (Harpel et al., 1991). Mutant proteins were purified to electrophoretic homogeneity according to the published protocols (Harpel et al., 1991; Harpel & Hartman, 1994). Stocks of purified Rubiscos (>20 mg/mL) were stored at −80 °C in a pH-8.0 buffer containing 50 mM Bicine, 66 mM $NaHCO_3$, 10 mM $MgCl_2$, 1mM EDTA, 20% glycerol, and 10 mM 2-mercaptoethanol. All other enzymes were purchased from Sigma.

[2′-^{14}C]CKABP, a gift of Dr. G. H. Lorimer (DuPont), was produced by acid-quenching a reaction mixture contain-

[2] Residue numbers refer to the sequence position in *Rhodospirillum rubrum* Rubisco. In the designations for mutants, the first letter refers to the amino acid found in the wild-type enzyme and the second letter identifies the amino acid introduced at the position numbered.

ing $^{14}CO_2$, wild-type *R. rubrum* Rubisco, and unlabeled RuBP (Pierce et al., 1986). The resulting preparation contained ~23.9 μM [2′-^{14}C]CKABP (~4300 dpm/nmol); RuBP (~160 μM; unlabeled) and [1-^{14}C]PGA (~35.5 μM; ~2150 dpm/nmol) were also present as a consequence of the synthetic strategy employed. [U-^{14}C]Lactate and [2−^{14}C]-pyruvate were obtained from DuPont-New England Nuclear. All other chemicals were procured from commercial sources at the highest purity readily available.

[2′-^{14}C]CKABP Turnover Reactions. Decarboxylation of [2′-^{14}C]CKABP (as illustrated in Figure 1) occurs spontaneously ($t_{1/2}$ = 1 h) and is also catalyzed by unactivated Rubisco (Lorimer et al., 1986; Pierce et al., 1986). Thus, all reaction mixtures for examinations of turnover of CKABP contained high enzyme:intermediate molar ratios (0.2:1 for wild-type and 7:1 for mutants), to promote rapid enzymatic processing of intermediate, and high concentrations of $NaHCO_3$ and Mg^{2+}, to ensure complete activation (*i.e.*, formation of the Mg^{2+}-stabilized carbamate of Lys191) (Lorimer, 1981; Donnelly et al., 1983). Otherwise, conditions were similar to those described for kinetic determination of reaction-intermediate processing (Pierce et al., 1986; Lorimer & Hartman, 1988).

Enzymes were pre-equilibrated for 15 min at 25 °C in 0.3 mL of a pH-8.0 buffer containing 130 mM Bicine, 13 mM $MgCl_2$, 1.3 mM EDTA, and 67 mM $NaHCO_3$. Reactions were initiated by addition of 0.1 mL of [2′-^{14}C]CKABP to give a final concentration of 6 μM; final enzyme concentrations were 0.05 mg/mL for wild-type (1 μM active site) and 2.1 mg/mL for mutants (42 μM active site). A control reaction (0.3 mL total volume) lacked enzyme but included the reaction intermediate at 6 μM. After the samples were incubated for 15 min at 25 °C, an aliquot (0.1 mL) of each was brought to 40 mM $NaBH_4$ for evaluation of the extent of CKABP consumption. The rest of each reaction mixture was then quenched with 0.2−0.25 mL of 10% acetic acid, concentrated 2−5-fold under a stream of dry N_2, adjusted to 2 mL with H_2O, and deproteinated by ultrafiltration with Amicon Centricon-10 filters in preparation for chromatographic analysis (see below).

To confirm product identities, 6 μM [2′-^{14}C]CKABP was incubated for 15 min with K166G (2.0 mg/mL, 40 μM active sites) (final volume of 0.8 mL) as described above. The unquenched reaction mixture was then subdivided for further processing. One portion (0.35 mL) was supplemented with 50 units of bovine heart lactate dehydrogenase and 1 mM NADH in order to convert pyruvate to lactate. A second portion (0.35 mL) was supplemented with 2 mM NADH, 10.7 mM ATP, PGA kinase (13 units), glyceraldehydephosphate dehydrogenase (3 units), glycerophosphate dehydrogenase (0.5 unit), and triosephosphate isomerase (5 units), for conversion of D-PGA to phosphoglycerol. The remaining portion (0.1 mL) was untreated. After an additional 15 min at 25 °C, the three samples were quenched with 1% SDS and deproteinated by ultrafiltration using Amicon Centricon-10 filters.

Chromatographic Separation of Reaction Mixtures. Deproteinated reaction mixtures were analyzed by anion-exchange chromatography (Pharmacia MonoQ HR 5/5) as previously described (Harpel et al., 1993b). Flow rate (1 mL/min) and elution gradients were controlled by a Pharmacia fast protein liquid chromatography system. The elution buffer consisted of 1 mM sodium borate (prepared by titration of 1 mM boric acid with concentrated NaOH to pH 8.0) in a gradient of NH_4Cl. Radiolabeled components were detected by in-line scintillation counting (IN/US βRAM II, 500 μL cell) at an eluate-to-scintillant (IN/US IN-FLOW ES cocktail) ratio of 1:4. Raw data were collected at 1 data point per s and averaged over 20 s intervals for graphic presentation.

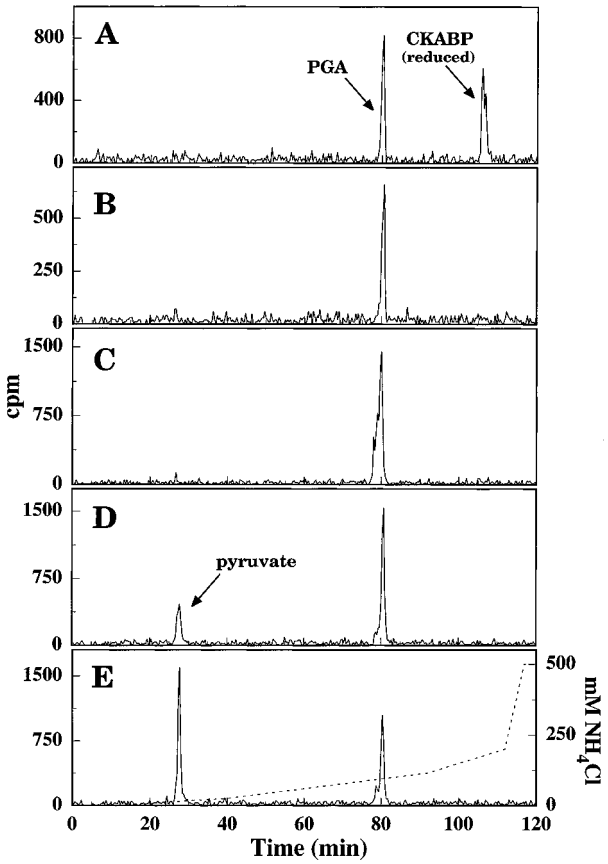

FIGURE 2: MonoQ anion-exchange analyses of radiolabeled products derived from reaction of wild-type and mutant Rubiscos with [2′-^{14}C]CKABP. Starting material (50-μL aliquot of stock solution) diluted directly into $NaBH_4$ (final concentration = 40 mM) without prior acid quenching (A). Acid-quenched control lacking enzyme (representing 50 μL of [2′-^{14}C]CKABP stock) (B). Acid-quenched reaction mixtures (each representing 75 μL of [2′-^{14}C]-CKABP stock) with wild-type (C), K166S (D) and K166G (E) Rubisco. Specific reaction conditions are given in "Experimental Procedures."

RESULTS

Anion-Exchange Analysis of CKABP Turnover. Conversion of unstable CKABP to the corresponding stable epimeric alcohols by borohydride reduction allows a direct chromatographic evaluation of the PGA:CKABP ratio (~1:1) in the preparation used in the turnover experiments (Figure 2A). Under acidic conditions, CKABP undergoes rapid decarboxylation (Lorimer et al., 1986); thus, CKABP is not observed in chromatographic profiles of acid-quenched samples, whereas [1-^{14}C]PGA (as a contaminant from starting material or as formed enzymatically) persists (Figure 2B). This differential between borohydride-stable and acid-stable radioactivity has served as the basis for determining the kinetics of CKABP utilization by wild-type and mutant Rubiscos (Pierce et al., 1986; Gutteridge et al., 1988; Lorimer & Hartman, 1988; Lee et al., 1993; Lorimer et al., 1993; Larson et al., 1995).

As concluded in prior characterizations (Pierce et al., 1986), processing of CKABP by wild-type Rubisco forms

PGA as the major product (Figure 2C); *i.e.*, when normalized to equivalent amounts of starting material, the number of counts associated with PGA approximates the combined number of counts associated with PGA and CKABP in the starting material (Figure 2A). By analogy with turnover of RuBP by wild-type Rubisco, in which pyruvate is formed from the terminal *aci*-acid intermediate via β-elimination of phosphate at a frequency of 1 per 150 molecules carboxylated (Andrews & Kane, 1991), pyruvate should also be a minor product derived from processing of CKABP. However, in the experiment represented in Figure 2C, 1% of the input of ~7000 cpm is below the threshold for detection.

In contrast to the apparent absence of pyruvate in wild-type and control reaction mixtures, a substantial amount of a labeled compound, which co-elutes with authentic pyruvate, is clearly present in reaction mixtures with K166S (Figure 2D) and K166G (Figure 2E). Prolonged incubation of [3-^{3}H]-PGA with K166G does not result in formation of chromatographically detectable levels of pyruvate (data not shown); thus, the pyruvate present in the reaction mixtures represented by Figure 2D and 2E must have arisen directly from CKABP. In the case of K166S, the amount of labeled pyruvate (~25% of total counts) is very similar to the incremental increase in labeled PGA relative to its endogenous level in the starting preparation of CKABP. These data signify a partitioning ratio of about 1 for protonation vs β-elimination of the *aci*-acid. The approximately equal values of pyruvate and PGA observed in the K166G reaction mixture, mimicking the approximately equal amounts of PGA and CKABP in the starting preparation, demonstrates that with this mutant partitioning of the *aci*-acid derived from CKABP cleavage is almost exclusively into pyruvate. These results are compatible with the previous report that K166G is competent in forward processing carboxylation-reaction intermediate (Lorimer & Hartman, 1988) but demonstrate that position-166 mutants are nevertheless impaired in the protonation of the terminal *aci*-acid intermediate.

Consumption of [2′-^{14}C]CKABP was complete in all three enzyme-containing reactions, as demonstrated by the absence of reduced [2′-^{14}C]CKABP following $NaBH_4$ treatment of an aliquot of each reaction at the time of quenching (data not shown). Furthermore, the high recoveries of initial radioactivity (>90%) discount decarboxylation of [2′-^{14}C]-CKABP as a significant pathway.

Chemical Confirmation of Chromatographic Identifications of CKABP-Derived Products. In addition to co-elution with authentic standards, the products of turnover reactions with K166G were verified as pyruvate and PGA by applicable enzymatic conversions. Pyruvate was identified by its shift in elution position to that of lactate following reduction by NADH, as catalyzed by lactate dehydrogenase (Figure 3C). Incubation of the K166G reaction mixture with ATP, NADH, and appropriate coupling enzymes for the stereospecific reduction of D-PGA to phosphoglycerol resulted in the disappearance of the peak attributed to PGA and the appearance of a similarly sized peak eluting at the position of phosphoglycerol (Figure 3D). Complete displacement of the peaks attributed to pyruvate and PGA by the action of highly specific enzymes discounts the possibility that either peak contains additional radiolabeled products. Significantly, L-PGA, the product that would arise from nonstereoselective protonation of terminal *aci*-acid, was not detected even though it has been invoked, but never verified, as a product of wild-type enzyme (Brändén et al., 1980).

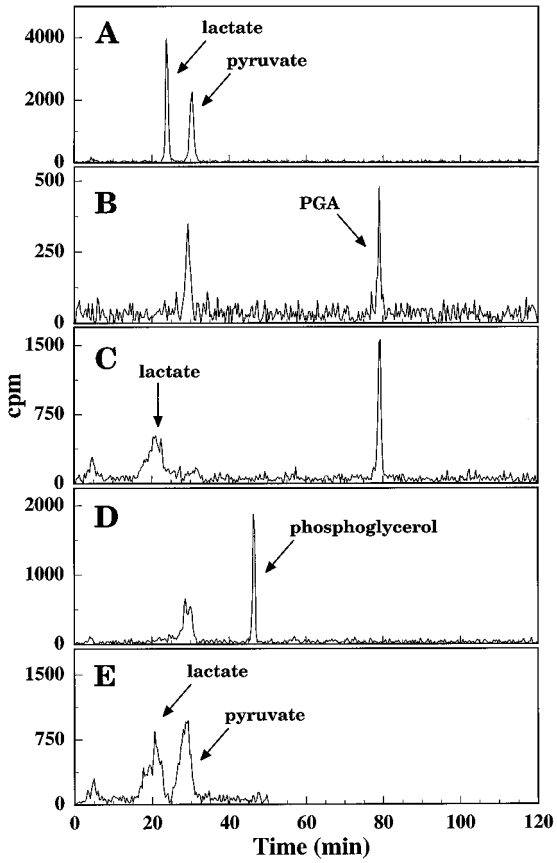

FIGURE 3: Enzymatic identification of products from the turnover of CKABP by K166G. [U-^{14}C]Lactate (0.02 nmol) and [2-^{14}C]-pyruvate (37.5 nmol) standards (A). Chromatographic analyses of samples were performed as described in the legend to Figure 2. The K166G reaction mixture after no additional treatment (B), after treatment with lactate dehydrogenase (C), and after treatment with coupling enzymes to reduce D-PGA (D). [U-^{14}C]Lactate (12.5 nmol) and [2-^{14}C]pyruvate (37.5 nmol) standards prepared in buffer including NADH and SDS (but lacking CKABP and enzymes) to more closely match the solute composition of the sample represented in panel C (E). Additional details and specific reaction conditions are given in "Experimental Procedures."

Broadening of the pyruvate and lactate peaks in panels C and D of Figure 3, relative to authentic standards (Figure 3A), is most likely due to the total solute compositions of the samples, as similar broadening is observed with pyruvate and lactate standards inclusive of the same array and concentrations of solutes (Figure 3E).

DISCUSSION

At first glance, the observed competency of position-166 mutants of Rubisco in the turnover of CKABP (Lorimer & Hartman, 1988) and the crystallographic assignment of Lys166 as the proton donor for the terminal *aci*-acid (Knight et al., 1990) would seem incompatible. However, the assay that was used to monitor CKABP processing by this enzyme is based on the contrasting acid-lability of [2′-^{14}C]CKABP (with decarboxylation and volatilization of radioactivity) and acid-stability of [1-^{14}C]PGA, the product formed by wild-type Rubisco (Pierce et al., 1986; Lorimer et al., 1986). Because the earlier characterizations of K166G predated the discovery of pyruvate as a minor side product arising from β-elimination of phosphate from the terminal *aci*-acid during processing of RuBP by wild-type Rubisco (Andrews & Kane,

1991), acid-stable radioactivity produced from [2′-^{14}C]-CKABP by K166G was equated with PGA. In retrospect, this was an unwarranted assumption, because pyruvate may have indeed survived the acidic conditions encountered during the course of the assay. Given these considerations and the mechanistic relevance of reconciling structurally- and functionally-based deductions, we have analyzed directly the distribution of products derived from the turnover of [2′-^{14}C]CKABP by position-166 mutants. Our premise underlying the experiments is straightforward: if Lys166 aids protonation of the terminal *aci*-acid intermediate, then the predominant [2′-^{14}C]CKABP-derived product with position-166 mutants may not be PGA but rather a side product reflective of misprocessing.

The obvious interpretation of pyruvate as the only significant labeled product from the turnover of [2′-^{14}C]-CKABP by K166G is that Lys166 is the proton donor for the terminal *aci*-acid. Thus, in the absence of the proton donor, the exclusive fate of the *aci*-acid is β-elimination of phosphate. This interpretation must, however, be tempered by the finding that both labeled pyruvate and PGA are formed in equal amounts from [2′-^{14}C]CKABP during processing by K166S. Even though the product profiles with both mutants argue for a key role in the terminal protonation step, the results with K166S compel a consideration of an indirect rather than a direct role.

Rapid-quenching experiments indicated that the *aci*-acid does not accumulate to substantial levels relative to other intermediates during catalysis by wild-type Rubisco (Jaworowski et al., 1984), despite the observation of solvent isotope discrimination during *aci*-acid protonation (Saver & Knowles, 1982) and the occasional formation of pyruvate by wild-type enzyme (Andrews & Kane, 1991). Presumably, the wild-type enzyme mitigates β-elimination of phosphate from this intermediate by constraining the conformer in which the bridge oxygen of the phosphate is coplanar with the *aci*-acid double bond (Rose, 1981). However, this conformer, which is most resistant to β-elimination, does not prevail automatically upon scission of the C2−C3 bond of CKABP but rather is achieved subsequent to considerable bond rotations (Larimer et al., 1994; Morell et al., 1994). Therefore, enhanced formation of pyruvate can be due to factors other than direct removal of the terminal proton donor; these include destabilization of the productive conformer of the *aci*-acid, hindrance in achieving this preferred conformation, and premature dissociation of the *aci*-acid from the active site followed by β-elimination. As an example of the former, site-directed substitutions of those residues which anchor the C1 phosphate via hydrogen-bonding interactions result in elevated levels of pyruvate from RuBP (Larimer et al., 1994; Morell et al., 1994).

None of the above reasons can be dismissed as the origin of pyruvate formed by the position-166 mutants. However, the equivalent partitioning of the *aci*-acid by K166S between PGA and pyruvate would appear to argue for significant stabilization of the intermediate by this mutant. Unquestionably, the protonation of the *aci*-acid by K166S to form PGA occurs at the active site rather than in solution following its dissociation, because the D-isomer is formed exclusively. Furthermore, as based on solution studies with glyceraldehyde-3-phosphate, β-elimination should be favored over protonation of *aci*-acid by a ratio of >100:1 (Richard, 1984). The protonation of the CKABP-derived *aci*-acid by K166S, albeit at a lesser efficiency relative to wild-type enzyme, could be explained in either of two ways: this mutant still contains the normal proton donor or the mutant "recruits" an alternate proton donor in the absence of an acid/base group at position 166, as has been invoked as explanation for the residual activities observed after substitution of an essential acid/base group in other enzymes [for examples, see Xue et al. (1989), Steyaert et al. (1990), and Knowles (1991)]. With both scenarios, the absence of any side chain at position 166 (*i.e.*, K166G) could result in a shortened residence time for the *aci*-acid at the active site whereby any opportunity for protonation is lost.

Specifics of the formation of pyruvate from RuBP, as catalyzed by wild-type enzyme (Andrews & Kane, 1991), may be relevant to the partial retention of terminal protonation activity by K166S. With wild-type enzyme the [pyruvate]/[PGA] partitioning ratio is independent of pH and independent of Rubisco source. This prompted the suggestion by Andrews and Kane (1991) of water as the terminal proton donor. In such a model, Lys166 would bind and orient the pertinent water molecule; while such a function might be partially mimicked by a seryl residue, a glycyl residue would be totally ineffective. Alternatively, if Lys166 is the direct proton donor for the *aci*-acid, the hydrogen bonding potential of the seryl substitution could facilitate recruitment of water to replace the lost function, whereas such an alternate pathway could not be offered by the glycyl substitution.

In summary, the identification of pyruvate as a major product of CKABP turnover by position-166 mutants lends strong reinforcement to the crystallographic-based deduction that Lys166 serves a role in the final protonation step of Rubisco catalysis even though our data do not unequivocally establish Lys166 as the actual proton donor. However, utilization of the acid/base properties of the lysyl side chain is an especially attractive tenet in view of the importance of Lys166 to the first and last steps of the overall carboxylation pathway, both of which necessitate proton transfers.

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