Glycine 176 Affects Catalytic Properties and Stability of the Synechococcus sp. Strain PCC6301 Ribulose-1,5-bisphosphate Carboxylase/Oxygenase*

Stephanie A. Smith and F. Robert Tabita‡

From the Department of Microbiology and Plant Molecular Biology/Biotechnology Program, The Ohio State University, Columbus, Ohio 43210-1292

A previously described system for biological selection of randomly mutagenized ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) employing the phototrophic bacterium Rhodobacter capsulatus (Smith, S. A., and Tabita, F. R. (2003) J. Mol. Biol. 331, 557–569) was used to select a catalytically altered form of a cyanobacterial (Synechococcus sp. strain PCC6301) enzyme. This mutant Rubisco, in which conserved glycine 176 was replaced with an aspartate residue, was not able to support CO2-dependent growth of the host strain. Site-directed mutant proteins were also constructed, e.g. asparagine and alanine residues replaced the native glycine with the result that these mutant proteins either greatly reduced the ability of R. capsulatus to support growth or had little effect, respectively. Growth phenotypes were consistent with the Rubisco activity levels associated with these proteins, and this was also borne out with purified recombinant proteins. Despite being catalytically challenged, the G176D and G176N mutant proteins were found to exhibit a more favorable interaction with CO2 than the wild type protein but exhibited a reduced affinity for the substrate ribulose 1,5-bisphosphate. The G176A enzyme differed little from the wild type protein in these properties. None of the mutants had CO2/O2 specificities that differed markedly from the wild type. Further studies taken from the known structure of the Synechococcus Rubisco indicated that substitutions at Gly-176 affected associations between large subunits. Supporting experimental data included an unusual protein concentration-dependent effect on in vitro activity, differences in thermal stability relative to the wild type protein, and aberrant migration on nondenaturing polyacrylamide gels. From these results, it is apparent that residues not directly located within the active site but near large subunit interfaces can affect key kinetic properties of Rubisco. These results suggest that further bioselection protocols (using these proteins as starting material) might yield novel mutant forms of Rubisco that relate to key functional properties.

Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), the key enzyme of the Calvin-Benson-Bassham reductive pentose phosphate pathway, catalyzes the fixation of CO2 onto the five-carbon substrate ribulose 1,5-bisphosphate (RuBP) yielding two molecules of 3-phosphoglycerate. Rubisco also catalyzes a competing monooxygenase reaction, whereby RuBP reacts with molecular O2 yielding one molecule of 3-phosphoglycerate and one molecule of 2-phosphoglycolate. The rates at which Rubisco catalyzes the competing carboxylase and oxygenase reactions are determined by the inherent substrate specificity factor of the protein, , and may be expressed by: \[ \frac{V_{c}}{K_{c}} = \frac{V_{o}}{K_{o}} \] In this definition, \[ \frac{V_{c}}{K_{c}} \] and \[ \frac{V_{o}}{K_{o}} \] represent the Michaelis constants for carboxylation and oxygenation (c and o, respectively). The production of 2-phosphoglycolate is the first reaction of a respiratory pathway, which ultimately leads to the loss of carbon from cells. It is not understood why different yet structurally similar Rubisco proteins possess inherent and variable catalytic efficiencies and substrate selectivities (1, 2). The inherent kinetic properties of this bifunctional enzyme, particularly , and the relative affinities for its gaseous substrates constitute a major limiting factor governing biomass yields of many industrially and ecologically significant microorganisms that fix CO2 as well as several economically important plants. For these reasons, there is considerable interest in elucidating the molecular factors that influence the variability in kinetic properties exhibited by structurally similar Rubisco enzymes. There have been multiple attempts to change or engineer Rubisco; however, no alterations have significantly influenced the physiology of the host organism so that some distinct benefit might be achieved.

Recently a system was described for directed evolution of prokaryotic Rubisco genes using the purple, nonsulfur, phototrophic bacterium Rhodobacter capsulatus as a selective host (3). R. capsulatus is capable of aerobic growth using organic carbon (chemoheterotrophy) and can synthesize its own organic carbon through CO2 fixation via the Calvin-Benson-Bassham cycle either photosynthetically (photoautotrophy) or during dark aerobic growth (chemoautotrophy). The strain of R. capsulatus used in this system, strain SBI-II, is a Rubisco deletion strain incapable of either photosynthetic/chemoautotrophic or photoheterotrophic growth (4) unless complemented in trans by exogenous Rubisco genes. Indeed, this system has the inherent advantage that growth of strain SBI-II under these conditions is directly dependent on the properties of the Rubisco gene(s) employed for complementation. Thus, by expressing heterologous Rubisco genes in strain SBI-II followed by complementation to CO2-dependent growth, the enzyme from the cyanobacterium Synechococcus PCC6301 was chosen to: \[ K_{c}, K_{o}, k_{cat}, \text{turnover number} \]

Received for publication, February 6, 2004, and in revised form, March 31, 2004

Published, JBC Papers in Press, April 2, 2004, DOI 10.1074/jbc.M401360200

* This work was supported by Grant GM24497 from the National Institutes of Health. The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

‡ To whom correspondence should be addressed: Dept. of Microbiology, The Ohio State University, 484 W. 12th Ave., Columbus, OH 43210-1292. Tel.: 614-292-4297; Fax: 614-292-6337; E-mail: tabita.1@osu.edu.

1 The abbreviations used are: Rubisco, ribulose-1,5-bisphosphate carboxylase/oxygenase; RuBP, ribulose 1,5-bisphosphate; , specificity factor; , turnover number; for carboxylase; for oxygenase; for ribulose 1,5-bisphosphate; , , for carboxylase; , for oxygenase; for carboxylase; for oxygenase.

Downloaded from http://www.jbc.org/ by guest on July 24, 2018
as a useful target to initiate bioselec tive studies. A positive selection condition was initiated in which the wild type Synec choccus Rubisco genes were unable to support growth at levels of atmospheric CO₂ lower than 1.5% but could support growth at concentrations of CO₂ greater that 5%. Using this selection system a variety of mutant Rubisco proteins were isolated (some better able to support growth at low CO₂ levels), and enzymes with inherent difficulties to support growth at higher levels of CO₂ were also obtained (3). In addition to the phenotype that Synecchoccus Rubisco genes confer to R. capsulatus SBI-II, these genes may also be conveniently expressed to produce recombinant enzyme in Escherichia coli. The previously solved x-ray structure (5) then allows one to better understand the positioning of mutated residues in the cyanobacterial enzyme. Furthermore, the cyanobacterial (Synec choccus) enzyme is a “green-like” Rubisco with high sequence and structural identity to plant enzymes, making it a suitable model to understand plant and eukaryotic Rubisco (1).

Using the bacterial selection system, potentially interesting but defective enzymes were identified by their inability to complement R. capsulatus SBI-II under conditions where the wild type enzyme was fully competent (3). One particular residue, glycine 176 (Gly-176), was identified through this selection system. Specifically, a mutation that involved a change of Gly-176 to aspartate (G176D) resulted in an enzyme that was unable to complement strain SBI-II to photoautotrophic growth in the presence of high levels of CO₂. However, in a previous study G176D had not been shown to influence catalysis to any great extent (6). The G176D enzyme and the newly constructed G176N and G176A enzymes were used to study the influence of residue Gly-176 in greater depth. In this report we show that the kinetic properties (Kₑ and Kₑapp) of the G176D and G176N mutant proteins were greatly altered. In addition, the altered enzymes exhibited various degrees of reduced thermal stability and to differing extents showed an unusual protein concentration-dependent alteration in specific activity. These latter properties along with the potential for this residue to influence important associations of catalytic subunits suggest that the Gly-176 mutant proteins might serve as starting points for further mutagenesis and selection.

EXPERIMENTAL PROCEDURES

Strains, Plasmids, and Growth—Construction of the Rubisco deletion strain of R. capsulatus strain SBI-II was described previously (4). To maintain cultures, R. capsulatus was grown aerobically on peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast extract plates (7) or in a broth containing Ormerod’s basal salts supplemented with 1 μg/ml nicotinic acid and 1 μg/ml thiamine hydrochloride. Antibiotics in peptone yeast...
assay times were necessary to stay within the linear range of enzyme assayed for 1 min; the other mutants were assayed for 5 min. Different the standard protocol. The wild type, K11R, and G176A enzymes were

strain background of SBI-II

reconstructed the G176D mutation to ensure that none of the

residue further; additional site-directed mutants, G176A and G176N, were prepared to assist in these studies. We also

G176D and G176N enzymes supported growth although with
doubling times nearly 3-fold higher than the wild type enzyme.
The G176A enzyme was much less affected in its ability to
complement strain SBI-II \* under these growth conditions (Table I). Furthermore, Rubisco specific activity levels in crude
extracts of photoautotrophically grown cells seemed to reflect
the growth rates with the G176D and G176N mutants having
activities that were only a fraction of those measured for either
the G176A mutant or the wild type enzymes (Table I). To
ensure that these low activities were not simply the conse-
quence of poor expression of the mutant genes in \textit{R. capsulatus},
the steady state levels of large subunit polypeptides were qual-
litatively assayed on Western blots of these same extracts,
and there appeared to be no significant differences (Fig. 1). Fig. 1 also included a K11R mutant that was discovered as part of a
different study and found to have no effect on the properties of
the enzyme or the phenotype of \textit{R. capsulatus} SBI-II \* relative
to the wild type enzyme (data not shown).

 catalytic properties of the purified enzymes were studied \textit{in vitro}.
Surprisingly, the G176D and G176N mutant enzymes had a
higher affinity for CO\textsubscript{2} than either the wild type or G176A
mutant, and they had a lower affinity for the substrate RuBP
(Table II). Note that despite the roughly 2-fold difference in
the \textit{K}_{c} for these mutants relative to the wild type there was no
significant difference in specificity, at least with the precision
of the employed specificity assay, indicating that parameters
that were not measured, such as the \textit{K}_{o}, were also probably
affected.

When determining the \textit{k}_{cat} of the G176D enzyme, it was noted
that the \textit{k}_{cat} was difficult to validate at different concen-
trations of enzyme. Thus, an experiment was undertaken to
examine this property for all of the mutant enzymes. The G176D enzyme consistently showed a pattern of increasing
specific activity with respect to protein concentration up to a
concentration of 291 nM (40 \textmu g/assay). This property was not
displayed by the wild type enzyme and perhaps only mildly
exhibited by the G176N and G176A enzymes (Fig. 2). When
this experiment was repeated with a constant concentration of
Rubisco and increasing concentrations of bovine serum albu-
m in, there was no effect (data not shown). Thus, we conclude
that the protein concentration dependence effect on enzyme
activity exhibited by the G176D enzyme was a property specific
to this Rubisco and not solely the consequence of increasing
concentrations of just any protein.

protein Conformation—It is apparent that the ability of the
holoenzyme to maintain its active conformation was compro-
mised in the G176D mutant enzyme. The protein concentration
effect described above, together with analysis of the known
structure (5, 17), suggested the potential for glycine 176 to
influence large subunit associations into dimers and eventual
formation to the catalytically important octameric core; alter-
natively, Gly-176 potentially influenced the ability of the
catalytic octameric core to properly associate with small subunits to form
Table II

| Enzyme     | $k_{cat}$ | $V_c$ | $K_M$ | $t^*$ | $K_{Mn}$ |
|------------|-----------|-------|-------|-------|----------|
| Wild type  | 3.5       | 3.0   | 183   | 37.8  | 44.7     |
| G176D      | 0.3       | 0.2   | 97    | 33.4  | 100.2    |
| G176N      | 0.4       | 0.3   | 107   | 34.3  | 91.6     |
| G176A      | 1.3       | 1.1   | 237   | 37.9  | 40.0     |

$^a$ Average of at least three independent assays.

Fig. 2. Protein concentration dependence of Rubisco specific activity for the G176 mutant enzymes. The Rubisco specific activity was measured with concentrations of purified enzyme ranging from 5 to 40 μg in each assay, expressed as 36 to 291 nM here. Beyond these concentrations there was a plateau of activity. The specific activity obtained at 36 nM for each enzyme was: wild type (wt), 3.8 μmol of CO$_2$ per fixed/min/mg protein; G176D, 0.07 μmol of CO$_2$ per fixed/min/mg protein; G176N, 0.55 μmol/min/mg of protein; and G176A, 1.0 μmol/min/mg of protein. Because of the range of activities, the wild type and G176A enzymes were assayed for 1 min, and the G176D and G176N mutants were assayed for 5 min. The logarithm of the specific activity at each concentration relative to the activity in the 36 nM assay is represented by the y axis.

Fig. 3. Thermal stability of the wild type and G176 mutant enzymes. All samples were diluted to a concentration of 0.5 μg/μl in TEM + 20% glycerol (the same buffer in which the enzymes were stored). Samples were incubated at 55°C. At the indicated times, samples were withdrawn from the incubations and assayed for Rubisco activity. One assay was performed prior to incubation of the samples ($t = 0$), and this was the reference assay for calculating relative activity after incubation, represented as a logarithm on the y axis.

Fig. 4. PAGE of purified wild type Rubisco (lanes 1 and 7), G176D (lane 4), G176N (lane 5), and G176A (lane 6) enzymes in the presence (upper panel) or absence (lower panel) of SDS. Lanes 2 and 3 contain K11R and K11R/G176D mutants that were not part of the current study (under "Results"). The gel with SDS contained 15% acrylamide, and the gel without SDS contained 7% acrylamide. Approximately 2 μg of each purified sample were loaded onto the SDS gels, and 3 μg were loaded onto the nondenaturing gels. Both were stained with Coomassie Blue. LSU, large subunit; SSU, small subunit.

The development of a prokaryotic system to enable the biological selection of potentially interesting mutant forms of Rubisco was described previously (3). The inability of the Synchococcus PCC6301 Rubisco to complement R. capsulatus SBI-II - to photoautotrophic growth, predicted to be a consequence of the extremely high $K_c$ ($\sim 173 \mu M$) of this enzyme (18), was the major rationale for using this enzyme in these initial studies. Although our original hypothesis was that a system of positive selection in which mutant enzymes capable of complementing SBI-II - to photoautotrophic growth under a low CO$_2$ atmo-
G401S enzyme obtained was shown to be folding/assembly-incompetent (3). Gly-176 lies within a stretch of 7 amino acids that are completely conserved; these residues are localized in a loop preceding the first α-helix of the C-terminal domain of the enzyme near an interface between large subunits that participate in dimerization (5, 17). A broad survey of the effects of substitutions at conserved glycyl residues had previously identified Gly-176 as a potentially interesting residue (6), but the properties of the G176A enzyme prepared in this earlier work differ somewhat from the results obtained for the G176A mutant prepared in the current study. In particular, Cheng and McFadden (6) described their mutant as having ~18% wild type activity, although results described here placed it closer to 40–50% wild type activity. A possible explanation for this discrepancy is the slight concentration-dependent effect on activity shown by this protein (Fig. 2). Although the amount of enzyme used in the assays performed by Cheng and McFadden (6) was not reported, perhaps if higher concentrations had been used the reported activity would more closely resemble that which is reported here. In addition, the G176A enzyme was initially described as mildly defective in its ability to fold properly, but no data were shown. It is conceivable that the slightly altered migration on nondenaturing gels observed here might have been observed previously and leads to classification of the G176A enzyme (G179A in the Cheng and McFadden paper, Ref. 6) as defective for folding/assembly.

The protein concentration-dependent effect on maximal activity, particularly with the G176D enzyme, could be a consequence of forcing an equilibrium toward the association of large subunits into functional dimers, the fundamental units of Rubisco assembly. Interestingly, the nondenaturing gel shown in Fig. 4 may also support this notion. There appears to be significantly less protein in the mutant lanes on this gel (lanes 3–6). A possible explanation for this is that, if the association of large subunits has indeed been affected by substitutions at Gly-176, the decreased stability would result in a lower proportion of holoenzyme relative to total protein in the preparation. The dissociated subunits that may be present would migrate off of the gel, and there would appear to be a lower concentration of enzyme.

At lower protein concentrations, a tendency for weak large subunit associations could also account for the observed thermal instability of enzymes with substitutions at residue 176. If this is true, then the results of the kinetic measurements for the Gly-176 mutant proteins support the hypothesis that the $K_c$ and $K_{RuBP}$ values are determined, at least in part, by conformational properties at a tertiary and possibly quaternary level. Our previous finding that a D103V mutation located in a region near the interface between large subunits dimers had the opposite effect on the $K_c$ further supports this prediction (3). Our attempts to model the G176D mutation in silico (using the Swiss Protein Data Base viewer, version 3.7) indicate that any of the possible conformations assumed by an aspartate at this position would not be accommodated because the aspartate would contact neighboring residues. Disregarding this warning from the software because the G176D enzyme obviously reaches a conformation that has some catalytic competency and using the lowest energy conformation proposed by the software, the model produced (Fig. 5) indicated minor repositioning of some of the local residues with the most apparent change observed in threonine 72 of the opposing large subunit. This model as well as the in vitro data suggest that changing residue 176 to aspartate caused major conformational shifts in a localized region at the interface between large subunits in the dimers. It is conceivable that these changes in monomer association may subsequently be translated to larger conformational changes in the holoenzyme. The properties of decreased thermal stability and altered migration on nondenaturing gels seem to support this conclusion.

An important feature of Rubisco enzymology is the activation of large subunits by a nonsubstrate molecule of CO$_2$ resulting in the formation of a lysyl carbamate at a completely conserved lysine residue within the active site (Lys-201) (19–21). In the absence of activation by CO$_2$, RuBP and other sugar phosphates will inhibit Rubisco. Activated Rubisco is stabilized by a Mg$^{2+}$ cofactor, which is essential for the carbamate to act as a general base, abstracting a proton from the substrate RuBP and forming an unstable enedial intermediate (22). Both carboxylation and oxygenation proceed through this intermediate, and it is beyond that point that the chemistry diverges. The same active site residues, therefore, are required for either carboxylation or oxygenation. Rubisco activation was not the focus of this study, but it is worth noting that it was briefly investigated by comparison of the wild type and the original G176D mutant. No difference was noticed in this single activation assay (data not shown), but this property may be worth further investigation in future studies of this enzyme.

It is also interesting to note that the mutations we have found that affected $K_c$ (at residues Asp-103 and Gly-176) had no effect on specificity. At the outset of these studies, the $K_c$ was the presumed “target” of positive selection, because we predicted the enzyme would require a lower $K_c$ to enable $R.$ capulatus SBI-II to grow photoautotrophically with lowered CO$_2$ in the atmosphere. Furthermore, we hoped that such a mutant would have an increased specificity as a consequence of its decreased $K_c$ (see the equation in the Introduction). These results and the results of several mutant studies that precede this reflect that the specificity parameter is recalcitrant to increases even when mutations result in a lower $K_c$ as with the G176D and G176N mutants. Perhaps some of the mutant proteins described here will serve as a better starting material for further mutagenesis and selection, allowing us to obtain the long sought highly active improved specificity mutant proteins. For example, the G176D mutant has a better affinity for CO$_2$ than the wild type enzyme but less than 10% of wild type carboxylase activity. Because this enzyme already possesses one of the desirable characteristics, i.e. low $K_c$, random mutagenesis might lead to a protein with either an enhanced $k_{cat}$ and/or normal $K_{RuBP}$, which could be identified by the ability of such proteins derived from G176D to support photoautotrophic growth. It seems possible that such a mutant might retain the gains made in $K_c$. It could then be used in further rounds of selection under aerobic chemoautotrophic growth conditions
where the levels of CO₂ and O₂ may be changed. Altering the relative concentrations of these gaseous reactants may target specificity mutations more directly. The complex nature of the specificity issue certainly suggests that multiple rounds of mutagenesis and selection will be necessary; the scenario outlined here might allow for such an approach where one aspect of catalysis is targeted ($K_c$) followed by another ($k_{cat}$) and/or $K_{RuBP}$. This stepwise approach, rather than gross recombination and shuffling, will allow us to keep track of which changes and residues are important. At the very least, biological selection will allow us to learn more of the molecular determinants that influence the affinity and/or association of the enzyme with its substrates and gaseous reactants. Alternatively, isolating suppressor mutations of the G176D, G176N, and G176A enzymes directly by selecting for mutations in those genes that restore normal growth of strain SBI-II/ H11002 under photoautotrophic conditions should also prove fruitful.

Acknowledgment—We thank the staff of the Plant-Microbe Genomics Facility for automated DNA sequencing.

REFERENCES
1. Tabita, F. R. (1999) Photosyn. Res. 60, 1–28
2. Spreitzer, R. J., and Salvucci, M. E. (2002) Annu. Rev. Plant Physiol. Plant Mol. Biol. 53, 449–475
3. Smith, S. A., and Tabita, F. R. (2003) J. Mol. Biol. 331, 557–569
4. Pauli, G. C., Vichivanives, P., and Tabita, F. R. (1998) J. Bacteriol. 180, 4258–4269
5. Newman, J., and Gutteridge, S. (1993) J. Biol. Chem. 268, 25876–25886
6. Cheng, C. Q., and McFadden, B. A. (1998) Protein Eng. 11, 457–465
7. Weaver, K. E., and Tabita, F. R. (1983) J. Bacteriol. 156, 507–515
8. Ormerod, J. G., Ormerod, K. S., and Gest, H. (1981) Arch. Biochem. Biophys. 94, 449–463
9. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual, 1st Ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.
10. Yanisch-Perron, C., Vieira, J., and Messing, J. (1985) Gene (Amst.) 33, 103–119
11. Boyer, H. W., and Roulland-Dussoix, D. (1969) J. Mol. Biol. 41, 459–472
12. Figurski, D., and Helinski, D. R. (1979) Proc. Natl. Acad. Sci. U. S. A. 76, 1648–1652
13. Kovach, M. E., Elzer, P. H., Hill, D. S., Robertson, G. T., Farris, M. A., Roop, R. M., H, and Peterson, K. M. (1995) Gene (Amst.) 166, 175–176
14. Whitman, W., and Tabita, F. R. (1976) Biochem. Biophys. Res. Commun. 71, 1034–1039
15. Markwell, M., Haas, S. M., Bieber, L. L., and Tolbert, N. E. (1978) Anal. Biochem. 87, 298–310
16. Laemmli, U. K. (1970) Nature 277, 680–685
17. Knight, S., Anderson, L., and Branden, C. I. (1990) J. Mol. Biol. 215, 113–160
18. Horken, K. M., and Tabita, F. R. (1999) Arch. Biochem. Biophys. 361, 163–194
19. Lorimer, G. H., Badger, M. R., and Andrews, T. J. (1976) Biochemistry 15, 529–536
20. Miziorko, H. M. (1979) J. Biol. Chem. 254, 270–272
21. Lorimer, G. H., and Miziorko, H. M. (1980) Biochemistry 19, 5321–5328
22. Cleland, W. W., Andrews, T. J., Gutteridge, S., Hartman, F. C., and Lorimer, G. H. (1998) Chem. Rev. 98, 549–561
Glycine 176 Affects Catalytic Properties and Stability of the *Synechococcus* sp. Strain PCC6301 Ribulose-1,5-bisphosphate Carboxylase/Oxygenase
Stephanie A. Smith and F. Robert Tabita

*J. Biol. Chem.* 2004, **279**:25632-25637.
doi: 10.1074/jbc.M401360200 originally published online April 2, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M401360200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 19 references, 5 of which can be accessed free at http://www.jbc.org/content/279/24/25632.full.html#ref-list-1