Inhibition by Dithiothreitol of the Utilization of Glutamine by Carbamyl Phosphate Synthetase

EVIDENCE FOR FORMATION OF HYDROGEN PEROXIDE*

(Received for publication, September 14, 1973)

PAUL P. TROTTA, LAWRENCE M. PINKUS, AND ALTON MEISTER

From the Department of Biochemistry, Cornell University Medical College, New York, New York 10021

SUMMARY

The glutaminase activity of glutamine-dependent carbamyl phosphate synthetase (Escherichia coli) and that of the separated light subunit of this enzyme, as well as the glutamine-dependent synthetase activity catalyzed by the native enzyme, are inactivated by incubation in air with relatively low concentrations (0.025 to 1 mM) of dithiothreitol; oxidized dithiothreitol does not inhibit. Other mercaptans (glutathione, 2-mercaptoethanol, dithioerythritol) also inhibit. Low concentrations of dithiothreitol under nitrogen and high concentrations (5 to 25 mM) of dithiothreitol in air do not inactivate the glutamine-related functions of the separated light subunit or of the native enzyme. However, enzyme preparations that have been inhibited by low concentrations of dithiothreitol in air are not readily reactivated by treatment with high concentrations of dithiothreitol. Incubation of the native enzyme with hydrogen peroxide (0.2 mM) also inhibits the glutamine-dependent activities. In distinction, treatment of the enzyme with either hydrogen peroxide or low concentrations of dithiothreitol in air does not affect either the ammonia-dependent synthetase activity or the synthesis of ATP from ADP and carbamyl phosphate, while the bicarbonate-dependent hydrolysis of ATP is stimulated moderately by both of these reagents. The addition of catalase as well as the addition of 1-albizzini, 1-glutamine, EDTA, or a mixture of ATP, magnesium ions, bicarbonate, and 1-ornithine protect against inhibition of the glutamine-dependent synthetase activity by dithiothreitol. Neither 1-ornithine (a positive allosteric effector) nor UMP (a negative allosteric effector) significantly affects the inhibition while a high protein concentration (10 mg per ml) protects against inhibition. The glutaminase activity of the separated light subunit, like that of the intact enzyme, is inhibited by the glutamine analog 1-2-amino-4-oxo-5-chloropentanoic acid (chloroketone); 1-glutamine or 1-albizzini can protect against this inhibition. 

The present communication describes additional studies on the structure and function of the light subunit. We have found that low concentrations of dithiothreitol lead to inactivation of the glutamine-related functions of the enzyme. This effect of dithiothreitol, which contrasts with its more usual action as a stabilizer of enzyme activity, was examined in detail. The experimental observations lead to the conclusion that dithiothreitol in the presence of oxygen can produce hydrogen peroxide which oxidizes an amino acid residue (or residues) on the light subunit which participates in catalyzing the hydrolysis of glutamine.
EXPERIMENTAL PROCEDURES

Materials

The enzyme was isolated from E. coli B as previously described (7). The light subunit was isolated by Sephadex G-200 chromatography in potassium thiocyanate (8).

Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—Materials—材

Methods

Assays—Glutaminase activity was measured by incubating the enzyme at 37°C for 10 to 30 min with 20 mM L-glutamine (native enzyme) or with 150 to 180 mM L-glutamine (light subunit) in 150 mM potassium phosphate buffer (pH 7.8) containing 0.5 mM EDTA. L-Glutamine was determined as previously described (8, 9). Glutamine-dependent carbamyl phosphate synthetase activity was determined by incubating the enzyme at 37°C for 5 to 15 min with 20 mM ATP, 20 mM MgCl₂, 20 mM L- glutamine, 100 mM KCl, 60 mM Tris-HCl (pH 7.7), and 20 mM NaH¹⁴CO₃ (30,000 to 50,000 cpm per μmol); the [¹⁴C]carbamyl phosphate formed was determined after conversion to [¹⁴C]urea (10). The other enzyme activities were determined as described previously (2).

Sulfhydryl Titration—The sulfhydryl content of the dithiothreitol solution was determined by titration with 5,5′-dithiobis(2-nitrobenzoic acid) essentially as described by Ellman (11).

RESULTS

Inhibition of Glutaminase Activity of Separated Light Subunit by Dithiothreitol by and by L-2-Amino-4-oxo-5-chloropentanoic Acid—When the separated light subunit was preincubated with relatively low concentrations of dithiothreitol (0.025 to 1 mM), there was dramatic inhibition of glutaminase activity (Fig. 1). However, at higher concentrations of dithiothreitol (5 to 25 mM), activation occurred which leveled off at about twice the initial activity. Such activation seems to reflect the reversal of oxidative changes which may have occurred during preparation of the light subunit in potassium thiocyanate. A similar interpretation may explain observations on the native enzyme in which it was found that high concentrations of dithiothreitol also activated, but only to the extent of 20 to 25% (see below).

Inhibition of the glutaminase activity of the separated light subunit was also observed after preincubation with 0.1 mM L-2-amino-4-oxo-5-chloropentanoic acid (chloroketone). This inhibition, similar to that found after preincubation of the native enzyme with the chloroketone (5), was markedly reduced when either L-glutamine or L-albizziin was added during preincubation. Although the glutaminase activity of the separated light subunit may be inhibited by preincubation with either the chloroketone or low concentrations of dithiothreitol, the data (see below) indicate that these reagents inhibit by different mechanisms.

Effect of Dithiothreitol on Various Enzymatic Activities Exhibited by Native Enzyme—In previous studies it was found that treatment of the enzyme with L-2-amino-4-oxo-5-chloropentanoic acid led to virtually complete inhibition of carbamyl phosphate synthesis from glutamine (but not from ammonia) and to a substantial increase in the carbon dioxide-dependent ATPase activity (3, 6). Treatment of the enzyme with chloroketone greatly decreased its ability to hydrolyze both γ-glutamyl hydroxamate and glutamine, but did not affect the activity responsible for the synthesis of ATP from ADP and carbamyl phosphate. In the present work it was found that incubation of the native enzyme with dithiothreitol led to effects which are similar to those observed after treatment of the enzyme with the chloroketone. As indicated in Fig. 2, incubation of the native enzyme with low concentrations of dithiothreitol led to substantial inhibition of the glutaminase activity (as was observed in similar studies on the separated light subunit (Fig. 1)). Higher concentrations of dithiothreitol produced moderate activation of the glutaminase activity. When the native enzyme was treated with 1.5 mM dithiothreitol for 16 hours at 23°C there was marked inhibition of glutamine-dependent carbamyl phosphate synthetase activity, glutaminase activity, and ability of the enzyme to hydrolyze γ-glutamyl hydroxamate (Fig. 3). On the other hand, there

1 Schroeder et al. (12) were apparently the first to use albizziin as a glutamine analog; their studies showed that albizziin inhibited formylglycinamid ribonucleotide amidotransferase.

2 It is of interest that the apparent Kᵣ value for L-glutamine for the separated light subunit is 130 to 180 mM, while the corresponding value for the native enzyme is about 1 mM (8). On the other hand, 0.1 mM chloroketone inhibits the separated light subunit and the native enzyme about equally well. This apparent difference between the binding of glutamine and that of the chloroketone may be due to the irreversible formation of a covalent linkage in the binding of the chloroketone.
The glutaminase assay was started by addition of 0.06 ml of 250 mM potassium phosphate adjusted to pH 8.6 by addition of Veronal and 0.5 mM EDTA was added to 0.02 ml of a solution containing 27 μg of the enzyme in the same buffer at 37°C. After 15 min, the glutaminase assay was started by addition of 0.06 ml of 0.5 mM L-glutamine (in 150 mM potassium phosphate buffer containing 0.5 mM EDTA). The remainder of the assay was performed as described in Fig. 1.

The addition of 1.0 mM dithiothreitol did not inhibit the glutamine-dependent carbamyl phosphate synthetase activity. One interpretation of this finding is that a high protein concentration may remove traces of traces observed with dithiothreitol (Fig. 3). Similar results were obtained when the enzyme was incubated with the chloroketone (3, 6), these are included in Fig. 3 for comparison.

As indicated in Fig. 4 (control), the glutamine-dependent carbamyl phosphate synthetase is lost rapidly when the native enzyme is incubated at 37°C in the presence of 1 mM dithiothreitol. (It was previously observed that some preparations of the enzyme exhibited transient moderate activation when studied under similar conditions (13); as stated above, such stimulation of activity may reflect the reversal of sulfhydryl group oxidation which probably occurred during or after preparation of the enzyme.) The addition of a low concentration of catalase virtually completely protected against inhibition. This result strongly implies the intermediate formation of hydrogen peroxide in the mechanism of inhibition. The addition of L-albizzinn also completely protected against inhibition. Similarly, L-albizzinn was found to protect against the inhibition observed upon the direct addition of hydrogen peroxide which is demonstrated in Fig. 3. Addition of glutamine decreased the rate at which inhibition occurred, and when the enzyme was incubated with dithiothreitol in the presence of ATP, magnesium ions, and bicarbonate, there was some initial stimulation of activity followed by inhibition. It is notable that ATP, magnesium ions, and bicarbonate also protect against chloroketone inhibition of the enzyme (3, 6). Neither the addition of L-ornithine (a positive effector) nor of UMP (a negative effector) significantly affected the rate of inhibition. No inhibition was observed in other experiments in which the dithiothreitol was first oxidized by prolonged incubation at 37°C in the presence of oxygen prior to incubation with the enzyme.

When the enzyme concentration was increased to 10 mg per ml, the addition of 1.0 mM dithiothreitol did not inhibit the glutamine-dependent synthetase activity. One interpretation of this finding is that a high protein concentration may remove traces...
1918

of metal ions which can catalyze the production of hydrogen peroxide. This hypothesis is supported by the observation that an increase of the EDTA concentration from 0.5 to 50 mM completely protects against dithiothreitol inhibition (Fig. 4). This result must reflect an interference with the production of hydrogen peroxide since the same concentration of EDTA has no effect on the inhibition if hydrogen peroxide is added directly to the enzyme.

Studies with other mercaptans gave substantially similar results. As indicated in Fig. 5, glutathione inhibited about 100 min of incubation. 2-Mercaptoethanol and dithioerythritol also inhibited; the time-course with dithioerythritol was about the same as observed with dithiothreitol. Experiments in which the native enzyme was incubated with dithiothreitol at several values of pH are described in Fig. 6. It is notable that inhibition was much greater at pH 8.6 than at pH 7.8, and that little inhibition occurred at pH 6.8. In distinction, addition of hydrogen peroxide readily inhibits at pH 6.8. Thus, the failure of dithiothreitol to inhibit at pH 6.8 is probably related to insufficient production of hydrogen peroxide rather than to a titration of residues on the protein.

Effect of Exclusion of Air on Inactivation of Glutamine-dependent Carbamyl Phosphate Synthetase Activity—When dithiothreitol alone was incubated at 37° at pH 7.8 under the conditions used in the studies on the enzyme, there was a marked decrease in its sulfhydryl titer (Fig. 7, inset); when an effort was made to exclude air by flushing with nitrogen, the sulfhydryl titer was lost much less rapidly. This oxidation is presumably associated with the formation of hydrogen peroxide. In a comparable experiment in which the enzyme was incubated with 1 mM dithiothreitol under nitrogen, the glutamine-dependent enzymatic activity was affected only slightly, while a marked reduction in activity was observed when air was not excluded (Fig. 7). In

Fig. 5. Effect of glutathione, 2-mercaptoethanol, and dithioerythritol on glutamine-dependent carbamyl phosphate synthetase. Enzyme (0.53 mg per ml in 150 mM potassium phosphate buffer (pH 7.8) containing 0.5 mM EDTA) was preincubated at 37° with 1.0 mM of the indicated mercaptan (or without mercaptan). At intervals 0.02 ml aliquots were withdrawn and assayed in a final volume of 0.5 ml for the formation of [14C]carbamyl phosphate from NaH14CO3 (see "Methods"). The effect of the various mercaptans on the activity during assay was found to be negligible.

Fig. 6. Effect of dithiothreitol concentration on glutamine-dependent carbamyl phosphate synthetase at several values of pH. Enzyme (0.53 mg per ml) was incubated at 37° with various concentrations of dithiothreitol in three buffers: pH 6.8, 150 mM potassium phosphate containing 0.5 mM EDTA; pH 7.8, 150 mM potassium phosphate containing 0.5 mM EDTA; pH 8.6, 150 mM potassium phosphate and 25 mM Veronal-HCl containing 0.5 mM EDTA. After preincubation for 10 min, 0.02-ml aliquots were assayed in a final volume of 0.5 ml for glutamine-dependent carbamyl phosphate synthetase.

Fig. 7. Effect of dithiothreitol under nitrogen on glutamine-dependent carbamyl phosphate synthetase activity. Enzyme (0.53 mg per ml in 150 mM potassium phosphate buffer (pH 7.8) containing 0.5 mM EDTA) was incubated with 1.0 mM dithiothreitol at 37°. The buffer was previously flushed with moist nitrogen gas and was frequently flushed with nitrogen throughout incubation. A control experiment was also carried out in air (O2). Aliquots (0.02 ml) were assayed for glutamine-dependent carbamyl phosphate synthetase. Inset, effect of incubation at 37° on the sulfhydryl content of dithiothreitol solutions in the presence and absence of air. Dithiothreitol (1.0 mM) in 150 mM potassium phosphate buffer (pH 7.8) containing 0.5 mM EDTA was incubated at 37°. A second sample was treated in the same way except that the solution was flushed with moist nitrogen during incubation. Aliquots (0.03 ml) were withdrawn at intervals and the sulfhydryl content was determined by titration with 5,5′-dithiobis(2-nitrobenzoic acid) according to Ellman (11).
another experiment, in which the enzyme was treated with 5 mM dithiothreitol for 150 min under nitrogen and then exhaustively dialyzed under nitrogen to remove the dithiothreitol, virtually no glutamine-dependent carbamyl phosphate synthetase activity was lost. Exposure of the enzyme to air at this point did not lead to inhibition, but addition of 1 mM dithiothreitol in the presence of air produced marked inhibition. The findings thus indicate that inhibition of the enzyme requires the simultaneous presence of both molecular oxygen and dithiothreitol. This result is consistent with the conclusion that it is the formation of hydrogen peroxide from the reaction between molecular oxygen and dithiothreitol which causes the inhibition.

The data given in Fig. 2 show that enzymatic activity is maintained in the presence of high concentrations of dithiothreitol, while inactivation occurs with low concentrations of dithiothreitol. Experiments were carried out to determine whether or not the inhibition produced by low concentrations of dithiothreitol is reversible. Thus, an enzyme preparation was inhibited by incubation with 1 mM dithiothreitol in the presence of air; about 75% inhibition was observed in this study (Fig. 8). When a high concentration (60 mM) of dithiothreitol was then added, there was only a small increase in enzymatic activity, indicating that the dithiothreitol-induced inhibition of the enzyme cannot be readily reversed (although it can be prevented; see Fig. 2) by high concentrations of dithiothreitol.

Experiments were also carried out to ascertain whether the inhibition observed in the presence of low concentrations of dithiothreitol in air might involve intermolecular or intersubunit disulfide bond formation. Polyacrylamide gel electrophoresis (14) at pH 8.0 in the presence of 0.1% sodium dodecyl sulfate gave no indication of cross-linking between subunits. Similarly, polyacrylamide gel electrophoresis of the dithiothreitol-treated enzyme in 0.1 mM Tris-acetate (pH 8.0) at 23° (conditions under which the 7.8 S monomer exists) also indicated no substantial cross-linking between monomers. This result was confirmed by a sedimentation velocity study in the analytical ultracentrifuge before and after treatment with inhibiting concentrations of dithiothreitol. The monomer sedimentation coefficient (s20, w 7.8 S) in 50 mM Tris-HCl (pH 7.6) containing 100 mM sodium chloride was virtually unaffected by preincubation with the dithiothreitol.

Effect of Dithiothreitol on Binding of [14C]Chloroketone—In order to determine whether or not the inhibition of the glutamine-dependent carbamyl phosphate synthetase activity by dithiothreitol involves the sulfhydryl group that is modified by treatment of the enzyme with the chloroketone (6), an experiment was performed in which the native enzyme and the dithiothreitol-treated enzyme were treated with [14C]chloroketone in the presence and absence of glutamine (Table I). The data show that both enzyme preparations bound equivalent amounts of labeled chloroketone; in addition, glutamine protected each of the enzyme preparations against the binding of about 1 mole of chloroketone per mole of protein. These findings indicate that inhibition of the enzyme by dithiothreitol does not involve oxidation of the sulfhydryl group that interacts with the chloroketone and suggest that the inhibition by dithiothreitol is related to an oxidation of another group (or groups) which may be involved in catalysis rather than in the binding of glutamine or of the chloroketone.

It was of interest to ascertain whether the sulfhydryl group modified by the chloroketone or the oxidation associated with inactivation by dithiothreitol was related to the sulfhydryl group reported by Foley and co-workers (15) to react with N-ethylmaleimide in the presence of ATP, magnesium chloride, and bicarbonate; reaction of this sulfhydryl group (located on the light subunit) is associated with substantial loss of the glutamine-dependent synthetase activity. In contrast, the glutaminase activity of N-ethylmaleimide-treated enzyme is greatly stimulated. This enhanced glutaminase activity can be completely abolished by incubation with 0.25 mM chloroketone, and such inhibition is virtually completely prevented by 100 mM L-albizzin (5). We have also found that incubation with 1 mM dithiothreitol in air inactivated the enhanced glutaminase activity of the N-ethylmaleimide-treated enzyme. The findings thus indicate that the glutamine-dependent synthetase activity of the enzyme can be inhibited by three separate modifications of the light subunit.4

V. P. Wellner and A. Meister, manuscript in preparation.

4 Treatment of the enzyme with cyanate also leads to inactiva-
Incorporation of [3H]Dithiothreitol into Enzyme—In order to determine whether inactivation of the enzyme is associated with the binding of dithiothreitol, the enzyme was incubated with 1 mM [3H]dithiothreitol. After complete inactivation, the enzyme was separated from unreacted reagent by dialysis and found to contain the equivalent of about 0.6 mole of [3H]dithiothreitol per mole of enzyme. However, when the same experiment was performed in the presence of 100 mM 2-mercaptoethanol, which completely protected against inactivation, the incorporation of tritium into the enzyme was about the same (about 0.7 mole per mole of enzyme). The findings indicate that incorporation of [3H]dithiothreitol into the enzyme occurs at a site (or sites) different from the glutamine binding site; such incorporation is apparently not directly related to the dithiothreitol-induced inactivation of glutamine-dependent activities.

**DISCUSSION**

The findings indicate that the glutamine-related activities exhibited by carbamyl phosphate synthetase (glutaminase, hydrolysis of γ-glutamyl hydroxamate, and glutamine-dependent carbamyl phosphate synthesis) are inhibited by low concentrations of dithiothreitol or other mercaptans in the presence of air. This effect of dithiothreitol contrasts with its more usual role as a stabilizer of enzymatic activity. The ammonia-dependent carbamyl phosphate synthetase activity and the ability of the enzyme to synthesize ATP from carbamyl phosphate and ADP are unaffected while the bicarbonate-dependent ATPase activity is moderately activated. The glutaminase activity of the isolated light subunit is also subject to the same type of inhibition by dithiothreitol observed in studies on the native enzyme. Thus, it may be concluded that the events associated with inhibition by dithiothreitol occur exclusively within the light subunit; the present findings offer no evidence for the formation of intersubunit or intermolecular disulfide bonds.

The effect of catalase in abolishing the inhibition by dithiothreitol indicates that hydrogen peroxide is the actual oxidizing agent:

\[
\begin{align*}
H_2C-SH & \quad \text{H-C-OH} \\
+ O_2 & \quad \text{H-C-} + \text{H}_2O_2 \\
\text{metal ions} & \quad \text{HO-C-H} \\
& \quad \text{H-C-SH} \\
& \quad \text{S} \\
\end{align*}
\]

( Hydrogen peroxide might also be produced in the formation of mixed disulfides between dithiothreitol and sulfhydryl groups on the protein.) Consistent with this concept is the finding that addition of hydrogen peroxide to the enzyme duplicates all of the inhibitory effects observed with dithiothreitol. Thus, the failure of high concentrations of dithiothreitol to inhibit can be explained by preferential reaction of the hydrogen peroxide formed with the excess dithiothreitol present rather than with a residue on the protein. Another interpretation is that high concentrations of dithiothreitol removes traces of metal ions, which can catalyze the autoxidation of thiols (18). The participation of metal ions in the oxidation of dithiothreitol reported here seems highly probable since EDTA or high protein concentrations, both of which can remove traces of metal ions, offer virtually complete protection against inactivation by dithiothreitol. The conclusions outlined above are consistent with the findings and interpretations of Cavallini et al. (20), who demonstrated that the luminal chemiluminescence excited by autoxidizing thiols (in alkaline solution in the presence of Cu²⁺) is related to the formation of hydrogen peroxide.

Hydrogen peroxide is a relatively nonspecific oxidizing agent; sulfur-containing amino acids are possible candidates for its site of reaction (21) but others (e.g. histidine) must be considered. The nature of the modified residue is currently being studied. The data presented here indicate that the group oxidized in the presence of dithiothreitol is neither the sulfhydryl group modified by the chloroketone (6) nor the sulfhydryl group which reacts with N-ethylmaleimide in the presence of ATP, magnesium ions, and bicarbonate (15). Whatever the nature of the group oxidized in the presence of dithiothreitol, it appears to be involved in catalysis rather than in the noncovalent binding of glutamine to the enzyme. This conclusion is consistent with the data which indicate that both glutamine and chloroketone can bind to the dithiothreitol-inhibited enzyme (Table I). An alternative hypothesis which must be considered is that the group (or groups) oxidized does not participate directly in catalysis but that the oxidation caused by dithiothreitol or hydrogen peroxide triggers a conformational change which renders the catalytic site inactive. The fact that glutamine protects against inhibition would then imply that there must also be a glutamine-induced conformational change.

It is of interest that the conditions employed here in the reaction with hydrogen peroxide (0.2 mM; 37°C; 30 min at pH 7.8) are much milder than those previously employed for the modification of enzymes and polypeptides (22). This may indicate that the oxidative reaction with carbamyl phosphate synthetase may be highly selective for a critical residue at the glutamine binding site. It is notable also that the oxidation reaction is not readily reversible by incubation with a high concentration of dithiothreitol. However, in those cases in which reversibility by thiols has been observed (21, 22), more stringent conditions for reduction have generally been employed. It is tempting to speculate that other glutamine amidotransferases may be susceptible to oxidation of the type found here for carbamyl phosphate synthetase. Indeed, the present work raises the question as to whether use of dithiothreitol (or other thiols) may produce inhibition of other enzymes by generating hydrogen peroxide and suggests that indiscriminate use of such thiols for enzyme stabilization may be inadvisable.

As noted previously (13) earlier observations on glutamine amidotransferases in which unexpected variability was found in the relative rates of reaction with ammonia and glutamine may have been due to effects, such as those of dithiothreitol described here, on the glutamine binding site.

*Note Added in Proof.—While this paper was in press, it was...
reported (23) that prevention of a reaction by catalase is not necessarily evidence for the formation of hydrogen peroxide since certain catalase preparations are contaminated with superoxide dismutase. Indeed we have confirmed this observation in studies on a number of commercial catalase preparations, including the Worthington sterile catalase preparation used in the studies reported here. However, when we separated the superoxide dismutase from catalase by Sephadex G-100 chromatography, this purified catalase also completely protected against inhibition of glutamine-dependent carbamyl phosphate synthetase by dithiothreitol. On the other hand, purified superoxide dismutase (obtained from Truett Laboratories, Dallas, Texas) was found to have no effect on the inhibition by dithiothreitol. These findings show that it is hydrogen peroxide rather than superoxide ion which is produced in the oxidation of dithiothreitol. (The authors wish to acknowledge the valuable suggestions and advice on this problem given to us by Dr. I. Fridovich.)

Acknowledgments—The authors are indebted to Dr. Suresh S. Tate of this department for his valuable advice, especially for his suggestion that inhibition of the enzyme by dithiothreitol might be associated with formation of hydrogen peroxide. We thank Mrs. Kim Li for her skillful technical assistance.

REFERENCES
1. Trotta, P. P., Haschemeyer, R. H., and Meister, A. (1971) Fed. Proc. 30, 1058
2. Trotta, P. P., Burt, M. E., Haschemeyer, R. H., and Meister, A. (1971) Proc. Nat. Acad. Sci. U. S. A. 68, 2599-2603
3. Khedouri, E., Anderson, P. M., and Meister, A. (1966) Biochemistry 5, 3552-3557
4. Wellner, V. P., Anderson, P. M., and Meister, A. (1973) Biochemistry 12, 2061-2066
5. Trotta, P. P., Wellner, V. P., Pinkus, L. M., and Meister, A. (1973) Proc. Nat. Acad. Sci. U. S. A. 70, 2717-2721
6. Pinkus, L., and Meister, A. (1972) J. Biol. Chem. 247, 6119-6127
7. Anderson, P. M., Wellner, V. P., Rosenthal, G. A., and Meister, A. (1970) Methods Enzymol. 17A, 235-243
8. Trotta, P. P., Pinkus, L. M., Haschemeyer, R. H., and Meister, A. (1974) J. Biol. Chem. 249, 492-499
9. Bernt, E., and Bergmeyer, H. V. (1963) in Methods of Enzymatic Analysis (Bergmeyer, H. V., ed) pp. 384-388, Academic Press, New York
10. Anderson, P. M., and Meister, A. (1966) Biochemistry 5, 3157-3163
11. Ellman, G. L. (1959) Arch. Biochem. Biophys. 82, 70-77
12. Schroeder, D., Allison, J., and Buchanan, J. (1969) J. Biol. Chem. 244, 5856-5865
13. Trotta, P. P., Pinkus, L. M., Wellner, V. P., Estis, L., Haschemeyer, R. H., and Meister, A. (1973) in The Enzymes of Glutamine Metabolism (Prujinis, N., and Stadtman, E. R., eds) American Chemical Society Symposium, pp. 451-462, Academic Press, New York
14. Weber, K., and Osborn, M. (1960) J. Biol. Chem. 244, 4406-4412
15. Foley, R., Poon, J., and Anderson, P. M. (1971) Biochemistry 10, 4962-4969
16. Anderson, P. M., and Carlson, J. D. (1973) Abstracts of the American Chemical Society Meeting, Chicago, Illinois, August 27-31, Abstr. 5
17. Anderson, P. M., Carlson, J. D., Rosenthal, G. A., and Meister, A. (1973) Biochem. Biophys. Res. Commun. 55, 246-252
18. Jockly, P. C. (1972) Biochemistry of the SH Group, pp. 94-115, Academic Press, New York
19. Lichtenberg, L. A., and Wellner, D. (1968) Anal. Biochem. 20, 317-319
20. Cavallini, D., De Marco, C., and Dupre, S. (1968) Arch. Biochem. Biophys. 124, 18-26
21. Means, G. E., and Fenni, R. E. (1971) Chemical Modification of Proteins, pp. 162-165, Holden-Day, Inc., San Francisco
22. Neumann, N. P. (1967) Methods Enzymol. 11, 485-487
23. Halliwell, B. (1973) Biochem. J. 135, 379-381
Inhibition by Dithiothreitol of the Utilization of Glutamine by Carbamyl Phosphate Synthetase: EVIDENCE FOR FORMATION OF HYDROGEN PEROXIDE
Paul P. Trotta, Lawrence M. Pinkus and Alton Meister

J. Biol. Chem. 1974, 249:1915-1921.

Access the most updated version of this article at http://www.jbc.org/content/249/6/1915

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 0 references, 0 of which can be accessed free at http://www.jbc.org/content/249/6/1915.full.html#ref-list-1