INHIBITION OF PROTEIN SYNTHESIS BY GLUTATHIONE DISULFIDE IN THE PRESENCE OF GLUTATHIONE[1]

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SUMMARY: As little as 5×10^{-5} M glutathione disulfide (GSSG) in the presence of 80×10^{-5} M glutathione (GSH) in a rabbit reticulocyte lysate caused a profound inhibition of initiation of protein synthesis. A potential physiological regulatory role for GSSG in protein synthesis is thus revealed.

In previous work we found that protein synthesis in the intact rabbit reticulocyte was halted instantaneously upon the addition of the thiol-oxidizing agent, diamide (1), along with the immediate complete oxidation of glutathione (GSH) to glutathione disulfide (GSSG) (2, 3). Both translation and initiation were affected, the former recovering after partial regeneration of GSH, whereas recovery of initiation was delayed until regeneration was complete (3).

The fact that elongation (and release) is inhibited with complete oxidation of GSH but recovers along with partial regeneration of GSH suggests that these stages require some GSH (or equivalent reactive groups), but are not

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inhibited by the presence of a significant concentration of GSSG. The resumption of efficient initiation only with the full regeneration of GSH could be due to dependence of initiation on the full complement of cellular GSH or to inhibition of initiation by the presence of relatively small amounts of GSSG.

We report here that initiation of protein synthesis is inhibited by GSSG in small concentrations in the absence of any other oxidant and in the presence of GSH. These results indicate one possible physiological role of GSSG, heretofore unexplored, in cellular function.

MATERIALS AND METHODS

Reticulocyte levels of rabbit blood were raised to 20-40% by repeated bleeding and one injection of imferon. Lysate of reticulocyte enriched blood was prepared by the addition of one volume of water to one volume of centrifuged washed cells and removal of the stroma by centrifugation at 25,000 x g for 30 minutes. Lysates were kept frozen in liquid nitrogen until use.

Initial incubation mixture, made according to Maxwell and Rabinovitz (4) with slight modifications, consisted of: lysate, with final hemoglobin concentration of 0.9-1.1 mM; Heme, 0.033 mM; Tris buffer, 10 mM, pH 7.8; KCl, 75 mM; $MgCl_2$, 2 mM; ATP/GTP, 1 mM/0.2 mM (stock solution adjusted to pH 7.0 with NaOH); creatine phosphate, 6 mM; creatine phosphate kinase (Boehringer) 18 U/ml; amino acid mixture, 10% of that described by Borsook et al (5), without alanine; ^{14}C-alanine (specific activity 123 $\mu c/\mu M$), 3 μc/ml.

Ten volumes of this incubation mixture were mixed with one volume of GSSG solution made in Tris buffer 0.1 M, pH 7.8, or with Tris buffer alone, at 4° C. Incubation was then carried out at 33° C. Aliquots were removed at time intervals, the protein precipitated and counted (3). Ribosomal analysis was done as previously described (3).

RESULTS

Addition of GSSG to lysed reticulocytes (the final preparation having

a GSH content of 7-10 x 10^{-4} M) does not stop protein synthesis instantaneously, but leads to complete inhibition following an initial period during which the rate of protein synthesis is equal to that of control sample. With GSSG additions at the level of 1-2 x 10^{-4} M, incorporation of ^{14}C-amino acid proceeds at the control rate for 6-8 minutes, slows down over 2-4 minutes, then stops completely. Higher concentrations of GSSG do not eliminate the initial period. Lower concentrations (5-7 x 10^{-5} M) of GSSG lengthen the initial period and also cause a marked decrease in the rate of protein synthesis. The time course of incorporation of label into protein in the presence of different initial concentrations of GSSG is illustrated in Fig. 1.

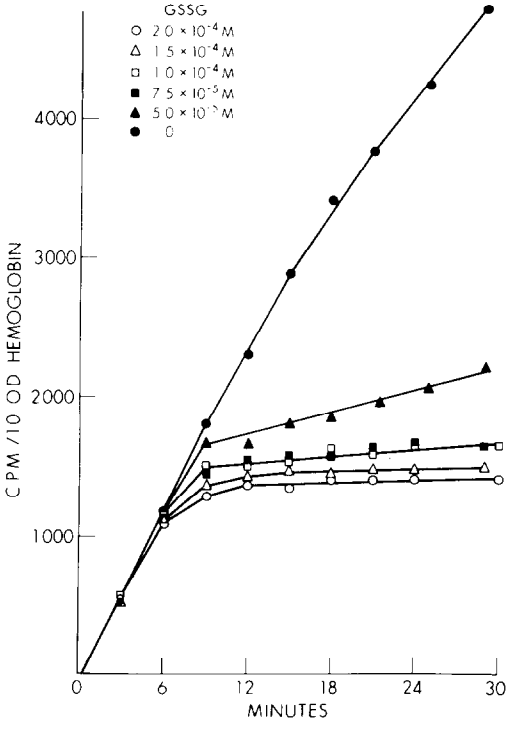

Fig. 1: Effect of added GSSG on the incorporation of ^{14}C-alanine into protein in lysed rabbit reticulocytes. In the experiment shown here, the GSH concentration was 0.84 mM. GSSG was added (final concentrations of 0.05-0.2 mM) without any being reduced by this preparation. In some lysates a partial reduction of the added GSSG occurred during the initial critical period of 2-4 minutes of incubation. In these cases, slightly higher amounts of GSSG were added.

The inhibition of protein synthesis by GSSG can be reversed to varying extents by the addition of glucose and NADP to the lysate, additions which lead to NADPH and then reduction of GSSG to GSH via glutathione disulfide reductase. These additions are completely effective in preventing GSSG inhibition if made at the beginning of the incubation. During incubation partial recovery can be observed if additions are made before the rate of protein synthesis falls (Fig. 2). Once the inhibition of synthesis has been expressed, additions of glucose and NADP do not reactivate the synthesis of protein, but do lead to the reduction of GSSG to GSH, recovery being between 70-100%. Parallel observations are made if dithiothreitol (DTT) is added in place of glucose and NADP.

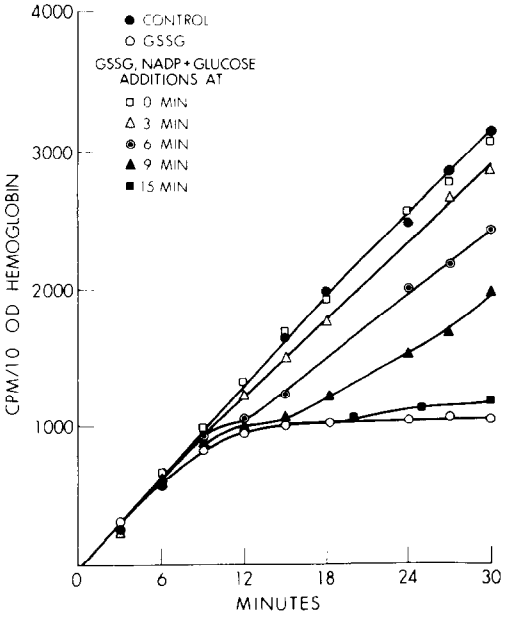

Fig. 2: Reduction of GSSG and reversal of inhibition of protein synthesis. GSSG was added to a concentration of 0.2 mM at 0 time. Glucose and NADP were added (final concentrations of 5 mM and 0.5 mM, respectively) at times: 0, 3, 6, 9, 15 minutes of incubation.

The ribosomal profile is the same for the GSSG-treated and control lysates during the initial period when both exhibit the same rate of protein synthesis. The inhibition of protein synthesis is accompanied by a conver-

sion of polysomes to monosomes, with essentially complete loss of labeled protein from the ribosomal fraction. Addition of cycloheximide prevents both the breakdown of polysomes and the loss of labeled protein from the ribosomes. The ribosomal patterns at different stages are illustrated in Fig. 3.

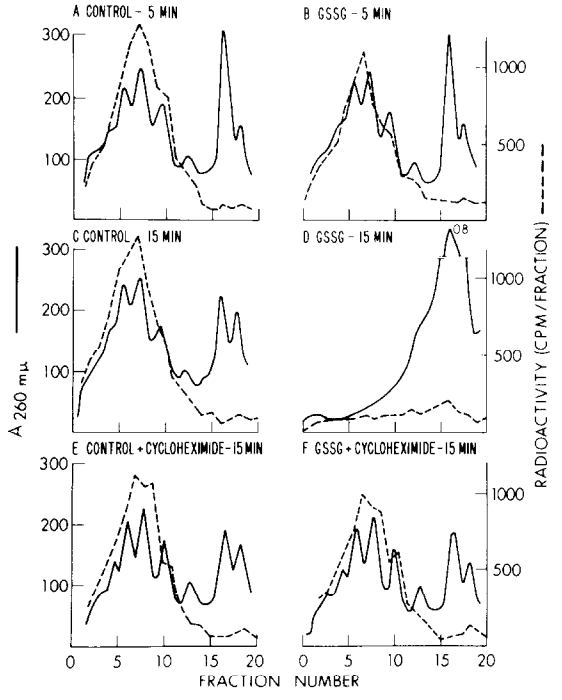

Fig. 3: Ribosomal patterns and ^{14}C labeling in control and GSSG-lysates, in the presence and absence of cycloheximide. GSSG was added (final concentration of 0.2 mM) at 0 time (B, D, F). Cycloheximide was added (final concentration of 0.1 mM) at 5 minutes of incubation (samples E, F).

A. Control at 5 minutes of incubation
B. GSSG-lysate at 5 minutes of incubation
C. Control at 15 minutes of incubation
D. GSSG-lysate at 15 minutes of incubation
E. Control and cycloheximide at 15 minutes of incubation
F. GSSG-lysate + cycloheximide at 15 minutes of incubation

CONCLUSIONS

There are a number of conclusions which follow directly from these results. First, it is clear that glutathione disulfide, in amounts which might well be attained within the cell under some conditions (6), has a pro-

found effect on protein synthesis. Second, the initial period of normal rate after GSSG addition to a system indicates that the sites affected by the GSSG are present in a pool which must be consumed before protein synthesis stops. Third, the recovery of GSSG as GSH is high, suggesting that the concentration of affected sites is low, perhaps of the same (or somewhat higher) order of magnitude as the ribosomal concentration.

That GSSG might have an effect on elongation reactions is indicated from studies on transfer factors (7) and with liver endoplasmic reticulum (8). In the present studies GSSG is demonstrated to exert a marked, preferential inhibition on initiation of protein synthesis when present in small concentrations, even in the presence of GSH. The significance of these results, in relation to a physiological regulatory role of GSSG in normal and genetically deficient systems (i.e., glucose-6-phosphate dehydrogenase deficiency (9), GSSG-reductase deficiency (10)) will be explored elsewhere. The sites affected by the GSSG are currently under study.

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