INHIBITION OF BRAIN GLUTATHIONE REDUCTASE BY SODIUM ARSENITE

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SUMMARY

The aim of this study is to investigate the inhibition characteristics of brain glutathione reductase (GSSGR) under reducing conditions. Sheep brain glutathione reductase (GSSGR, NAD(P)H: oxidized glutathione oxidoreductase, EC 1.6.4.2) was purified about 11000 fold with a method employing ammonium sulphate fractionation, heat denaturation, 2',5'-ADP Sepharose 4B and Sepharose C16B chromatography steps. NADPH is an inhibitor of brain GSSGR. Sodium arsenite, which is a specific inhibitor of enzymes having two nascent -SH groups, had little inhibitory effect on GSSGR when incubated with the enzyme alone. When the enzyme was incubated with both NADPH and arsenite, inhibition was intensified. 2-Mercaptoethanol and reduced glutathione enhanced the inhibition further; whereas oxidized glutathione protected the enzyme from inhibition. The reaction mechanism of the enzyme is discussed in the light of these findings.

Key Words: Glutathione reductase, sheep brain, inhibition, NADPH, sodium arsenite, thiol reagents.

INTRODUCTION

Glutathione reductase [NAD(P)H: Oxidized Glutathione Oxidoreductase, EC 1.6.4.2], (GSSGR), catalyzes the reduction of oxidized glutathione (GSSG) to reduced glutathione (GSH) by the help of nicotinamide adenine dinucleotide phosphate (NADPH) as the cofactor. The enzyme is responsible for maintaining glutathione in the reduced state. By coupling with the glutathione peroxidase reaction, GSSGR prevents cellular damage by oxidative stress (1).

The enzyme was purified from sheep brain and some of its molecular and kinetic properties were investigated. Brain GSSGR is a homodimer with molecular weight of 116,000, and contains one molecule of FAD per subunit (2). The enzyme follows a hybrid Ping Pong-ordered kinetics with double substrate inhibition (3).

The aim of this study is to investigate the inhibition characteristics of brain GSSGR under reducing conditions.

MATERIALS AND METHODS

Fresh sheep brains were obtained from an Ankara slaughterhouse. All the other reagents were analytical grade.

Enzyme was purified about 11000 fold with some modifications of the previously described method (2). The method included ammonium sulphate fractionation (35-55%), heat denaturation (1 h at 65°C), 2', 5'-ADP Sepharose 4B chromatography from which enzyme was eluted with 0-2 M NaCl gradient, and Sepharose C16B chromatography steps. Specific activity of the eluate was about 190 IU/mg.

GSSGR assays were carried out spectrophotometrically with LKB Ultraspec Plus, following the decrease of absorbance at 340 nm due to the oxidation of NADPH, at 37°C. The assay system contained 100 mM sodium phosphate buffer, pH 7.5, 1 mM GSSG and 0.12 mM NADPH. A unit of activity is defined as the amount of enzyme that catalyzes the oxidation of 1 µmol of NADPH per minute under the assay conditions.

For inhibition and protection assays, aliquots of enzyme solution which contain 50 ng of enzyme were incubated with the reagents indicated. For determination of the control activities enzyme was incubated with distilled water at identical conditions. The assays were repeated at least twice.

RESULTS

Figure 1 shows the GSSGR activity versus time with 2.86 mM sodium arsenite in the presence and absence of 7.9 µM NADPH. The inhibition of GSSGR by sodium arsenite in the presence of NADPH increases with time.

Figure 2 shows that, when increasing concentrations of sodium arsenite (up to 3. OmM) was incubated with the enzyme alone, little or no inhibition was observed. On the other hand when the incubation was in the presence of 7.9 µM NADPH, enzyme was inhibited. In this case the enzyme showed approximately 60% of its original activity at zero

concentration of sodium arsenite due to the inhibition of the enzyme by NADPH.

Figure 4 shows the bar diagrams of the enzyme inhibition-reactivation assays. When the enzyme was incubated with 2.86 mM sodium arsenite little or no activity loss was observed (I a). NADPH is the inhibitor of the brain GSSGR (3). When the enzyme was incubated with 7.9 µM NADPH for the same period of time, activity was found to be about 65% of the control (I b). When the enzyme was incubated with both of the reactants (I c) the inhibition was intensified. It is seen from the figure that GSH or 2-mercaptoethanol (2-ME) also help in the inhibition of the enzyme by sodium arsenite. When the enzyme was incubated with 2.86 mM sodium arsenite and 2.86 mM GSH (II a) or 1.43 mM 2-ME (III a) for 10 minutes inhibition was observed. These two thiol reagents potentiated the inhibition of 7.9 µM NADPH also (II b and III b). When the enzyme was incubated with 2.86 mM sodium arsenite and 7.9 µM NADPH, addition of 2.86 mM GSH (II c) or 1.43 mM 2-ME (III c) to the medium enhanced the inhibition further. On the other hand, addition of 1.25 mM GSSG to the medium protected the enzyme against NADPH (IV b) and sodium arsenite inhibitions (IV c) to some extent.

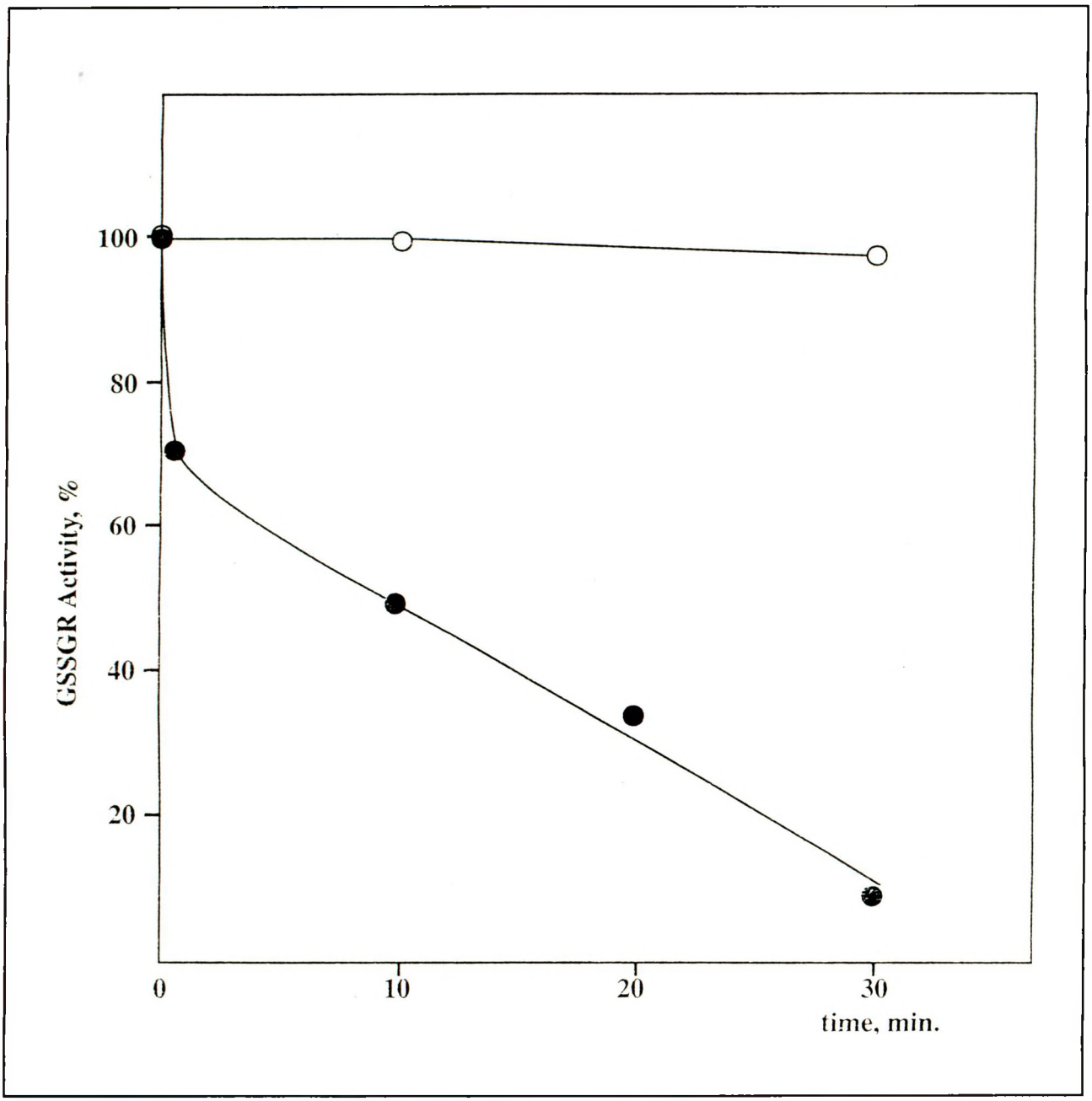

Fig 1. % GSSGR activity vs time when the enzyme was incubated at 25°C: o- alone; and ● - in the presence of 7.9 µm of NADPH and 2.86 mM sodium arsenite.

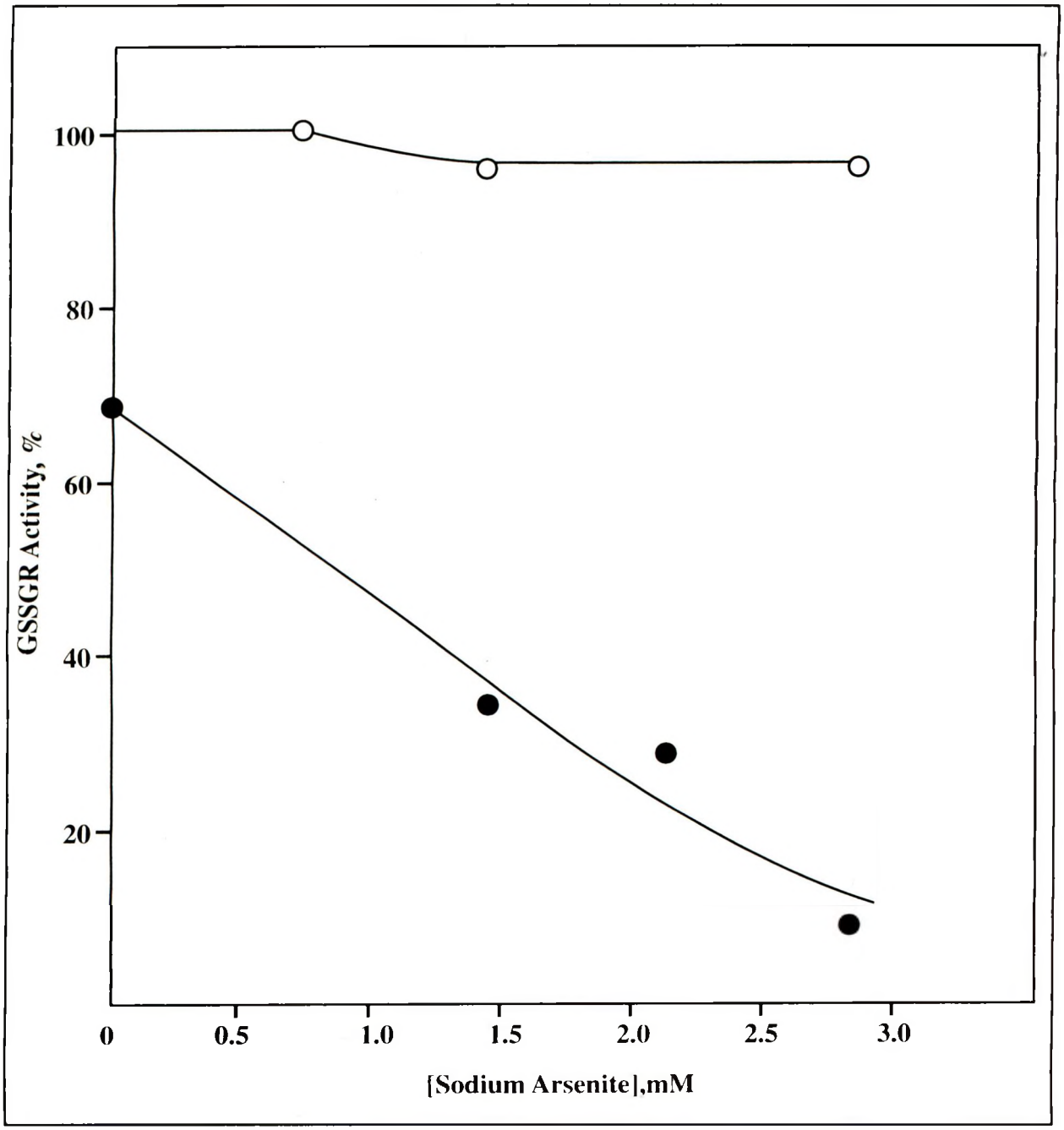

Fig 2. % GSSGR activity vs increasing concentrations of sodium arsenite when the enzyme was incubated for 30 min. at 25°C: o-in the absence, and ●- in the presence of 7.9 μm NADPH.

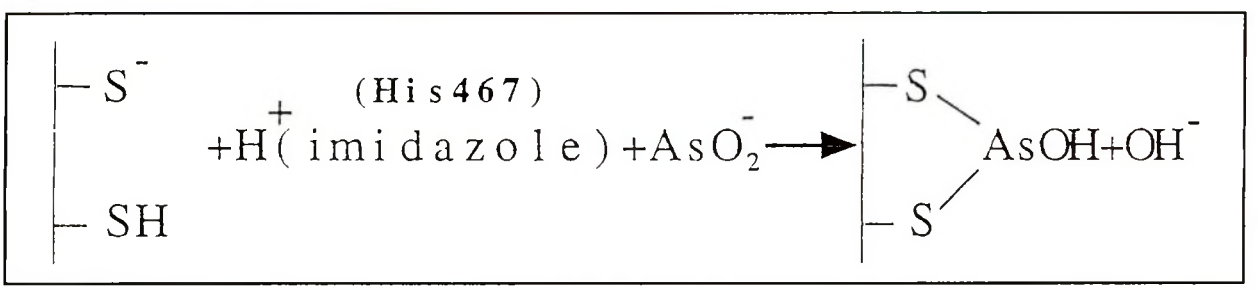

Fig 3. Reaction of sodium arsenite with GSSGR

DISCUSSION

It is known that arsenicals inhibit only the reduced form of the enzyme (4,5). It is shown in figure 2 that increasing concentrations of sodium arsenite alone did not affect the enzyme activity. On the other hand, in the presence of NADPH time dependent inhibition was observed (Fig. 1).

When NADPH is bound to the enzyme, according to the reaction mechanism proposed for human erythrocyte enzyme, disulphide bond between cysteine 58 and cysteine 63 at the active site is broken down, and an -SH group and a thiolate ion are formed and histidine 467 is protonated. This form is called as the EH_2 form of the enzyme (6). Arsenite probably forms a complex with the EH_2 form of the enzyme and block this mechasim. The reaction can be given with the equation defined for lipoamide dehydrogenase (Fig.3), (5).

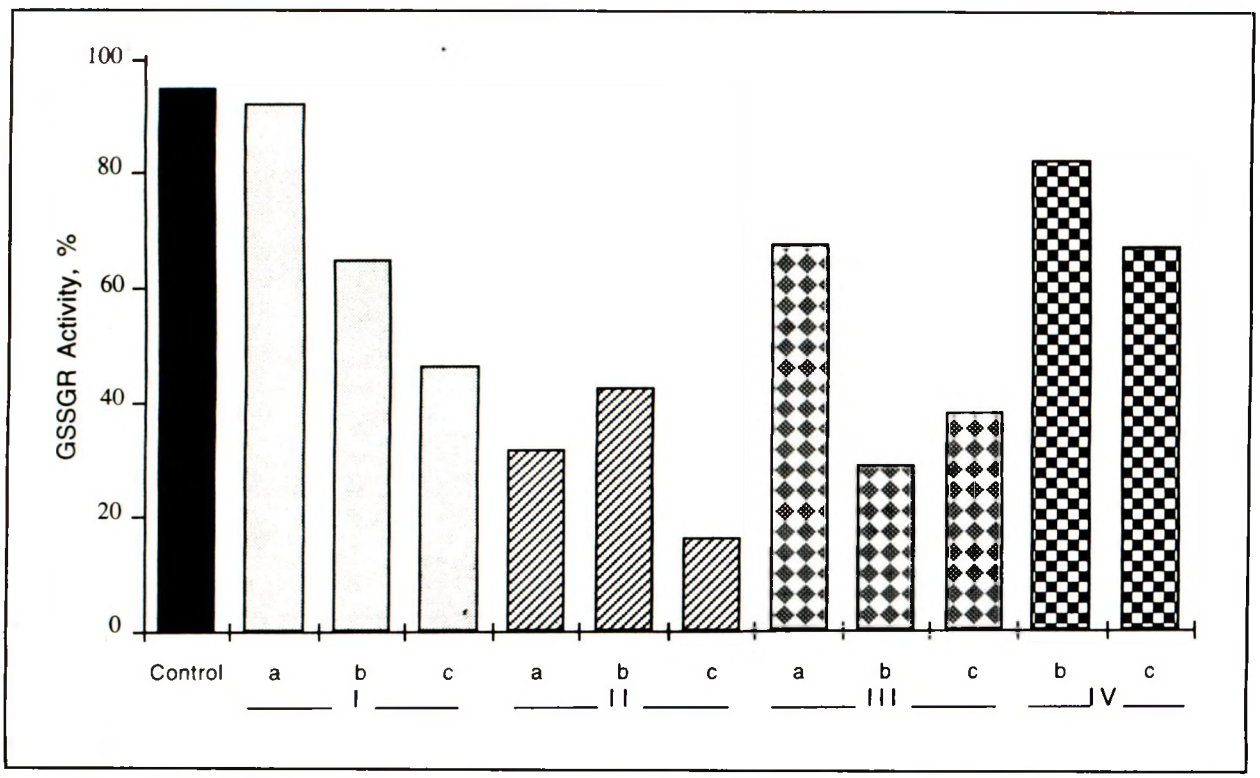

Fig 4. Effect of GSH, 2-ME and GSSG on the activity of GSSGR. The enzyme was incubated for 10 min. at 25°C with (a) 2.86 mM sodium arsenite, (b) 7.9 μM NADPH or (c) both in the presence of (II) 2.86 mM GSH, (III) 1.43 mM 2-ME, (IV) 1.25 mM GSSG, or (I) without any addition.

GSH or 2-ME leads to the inhibition of the enzyme by sodium arsenite enhancing the formation of EH_2 form, but in this case EH_2 form has two - SH groups, and arsenite is bound to these nascent -SH groups. GSSG protects the enzyme against NADPH and arsenite inhibition to some extent. It is thought that this protection effect of GSSG is due to its interaction with the -SH group produced after NADPH binding. When GSSG is bound to this -SH group as a mixed disulphide, the reaction given in figure 4 cannot proceed. Our findings show that the reaction mechanism of sheep brain enzyme is similar to the reaction mechanism proposed for human erythrocyte enzyme. However, these findings are somewhat contrary to the findings of Pinto et al. (4), who had found that both reduced and oxidized forms of glutathione and 2-ME restored the activity of the yeast GSSGR.

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