CATALYTIC EFFECT OF GSSG ON REDUCTION OF CYTOCHROME C BY GSH - POSSIBLE MODEL FOR FACILITATION OF ELECTRON TRANSFER AND ENERGY CONSERVATION BY SULFONIUM ION FORMATION*

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SUMMARY

The reduction of cytochrome c by GSH can occur by two reactions. One is metal catalyzed, prevented by EDTA, and is greatly inhibited by the ionic strength of isotonic solutions. The other is GSSG catalyzed and is completely insensitive to ionic strength. It is suggested that a complex between GSSG and GSH, possibly involving a ring of the three sulfur atoms, facilitates the electron donating activity of GS^- and stabilizes the first and second oxidation products, GS· and GS^+. The stabilized sulfonium ion might represent a type reaction involved in energy conservation in oxidative phosphorylation.

When the reduction of purified cytochrome c by GSH was studied in 0.175 M KCl-0.025 M Tris, pH 7.4, it was found that the rate varied a great deal from one sample of GSH to another, and that the rate was not proportional to either the GSH concentration or to the square of the GSH concentration in a predictable way. This suggested that the rate was being determined by some contaminant, such as metal ions or the GSSG that is present in all samples of GSH in varying amounts.

Direct studies with added GSSG are presented in Fig. 1.

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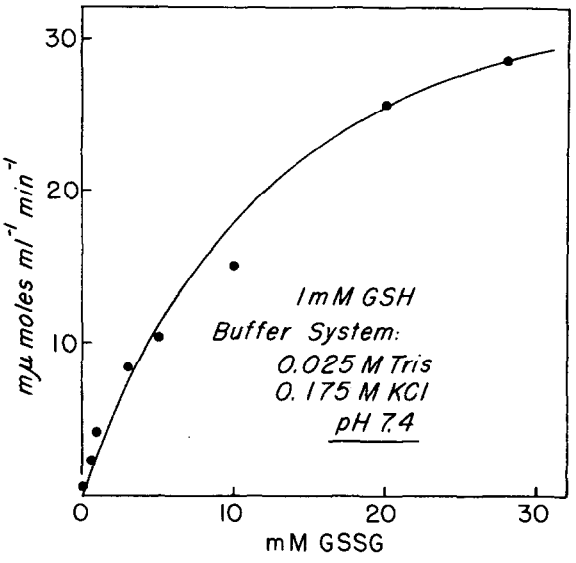

Fig. 1. Effect of added GSSG on the rate of reduction of cytochrome c by 1 mM GSH. Initial rates from the increase in absorption at 550 nm. Buffer medium, 0.175 M KCl plus 0.025 M Tris·HCl pH 7.45; cytochrome c 10 μM; Temp. 25°.

Addition of GSSG progressively increased the rate of reduction of cytochrome c by 1 mM GSH many fold. The effects of EDTA on the reaction with GSH and with GSH + GSSG are presented in Fig. 2. The rates are not comparable with those in Fig. 1 because the experiment was carried out at pH 8.0 instead of 7.4 and without KCl, making the rate with GSH alone much higher (See discussion below on the effect of high ionic strength). The presence of 0.2 mM GSSG significantly increases the rate of reduction of cytochrome c by 0.5 mM GSH. In addition 1 mM EDTA almost completely eliminates the reduction by GSH alone, while it only partially inhibits the reaction when GSSG is added. Other experiments indicated that less inhibition by EDTA occurred with higher concentrations of GSH, when there would be higher concentrations of GSSG present. These data suggested that there might be two reactions between

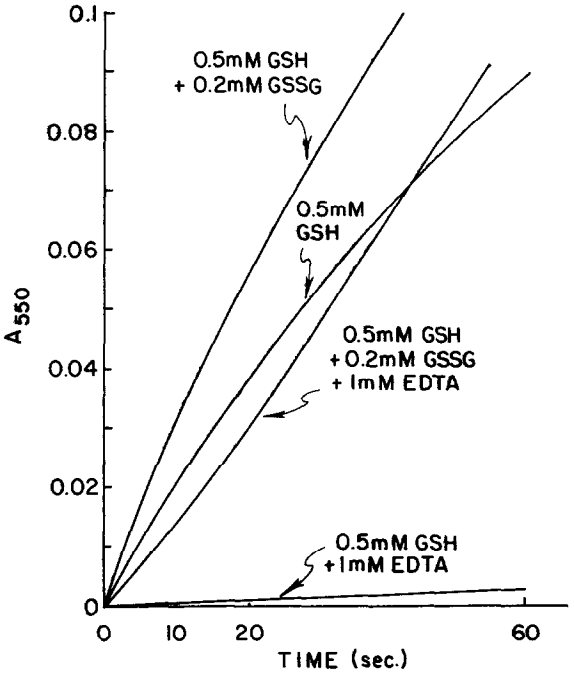

Fig. 2. Effect of EDTA on the rate of reduction of cytochrome c by GSH and by GSH plus GSSG. Medium, 10 mM Tris·HCl pH 8.0 (no KCl); cytochrome c 10 μM; Temp. 25°.

GSH and cytochrome c, one catalyzed by divalent metal ions and the other by GSSG (1, 2).

The stimulating effect of GSSG occurred even under conditions such that the metal catalyzed reaction is largely suppressed (high ionic strength or the presence of EDTA). However, to make certain that the increase in rates with added GSSG was not due to metal contaminants, additional experiments were done with several metal complexing agents. As is shown in Table I, the rate of reduction of cytochrome c by 1 mM GSH in the presence of 5 mM GSSG is not inhibited by the metal complexing agents EDTA, 8-hydroxyquinoline, or o-phenanthroline at 1 mM. Thus, contaminating metals do not seem to be involved in the GSSG effect.

If GSSG is acting as a catalyst, then the reduction of cyto-

TABLE 1

Effect of Metal Ion Chelators on the Rate of Reduction of 10 μM Cytochrome c by 1 mM GSH + 5 mM GSSG

Buffer Medium: 0.025 M Tris + 0.175 M KCl pH 7.45

Addition	Initial rate (mμmoles/ml/min.)
GSH + GSSG (control)	18.2
+ 1 mM 8-hydroxy-quinoline	22.4
+ 1 mM orthophen-anthroline	24.0
+ 1 mM EDTA	20.6

chrome c by GSH ought to be autocatalytic because GSSG is being formed. Because GSSG does not stimulate in trace amounts at pH 7.4, the autocatalytic effect was difficult to demonstrate starting with GSH alone in the presence of EDTA. However, as is illustrated in Fig. 2, an acceleration of rate with time can be shown when 0.2 mM GSSG is added at the beginning. In the experiment in Fig. 2 the metal catalyzed reaction was suppressed by EDTA. Other experiments indicate, as would be expected from the decrease in rate with very low concentrations of GSSG under some conditions, that a GSSG-metal complex is not the active catalyst and therefore is not responsible for the catalysis by GSSG.

Once it was clear that GSSG *per se* and not a metal contaminant or GSSG-metal complex catalyzed the reduction of cytochrome c by GSH, we investigated the reaction in several other respects. The original studies were done in 0.175 M KCl-0.025 M Tris pH 7.4 as part of a study of the action of GSSG + GSH mixtures in producing lipid peroxidation in mitochondria in this medium. However, the catalytic effect of GSSG is clearly seen at pH 8.0, which is optimal for GSH reduction of cytochrome c, as well as at pH 7.4, which is considerably removed from that optimum. An important

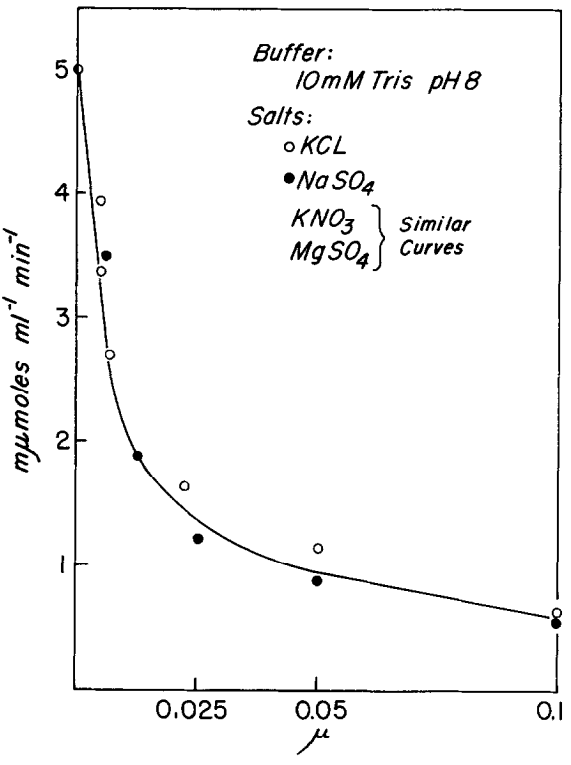

Fig. 3. Effect of increasing ionic strength on the rate of reduction of cytochrome c by GSH. All solutions contained 10 mM Tris·HCl pH 8.0 as buffer. Cytochrome c 10 μM, GSH 0.2 mM, Temp. 25°. Initial rates.

finding was that high ionic strength greatly inhibited the metal catalyzed reaction with GSH alone. Fig. 3 presents experiments in which the ionic strength was raised by four different salts. The GSSG catalyzed reaction, on the other hand, is completely insensitive to changes in ionic strength. Thus, the fact that most of the initial studies were carried out in 0.175 M KCl-0.025 M Tris was instrumental in permitting us to study the GSSG catalyzed reaction. The difference in sensitivity to ionic strength emphasizes the differences in mechanism, or in the rate limiting step, between the metal catalyzed and the GSSG catalyzed reactions.

The data in Table II indicate that the GSSG catalyzed reac-

TABLE 2

Reduction of Cytochrome c by GSH and by GSH plus GSSG under Anaerobic as Compared to Aerobic Conditions

Buffer medium: 0.025 M Tris + 0.175 M KCl, pH 7.45; cytochrome c 10 μM; Temp., 25°.

Additions	Rate of Reduction (mμmoles/ml/min.)	
	Aerobic	Anaerobic
1 mM GSH	0.52	0.42
10 mM GSH	4.06	3.42
1 mM GSH + 5 mM GSSG	17.6	40
500 μM Cysteine	3.37	2.33

Rates are initial rates. The anaerobic rate with GSH + GSSG may be considerably greater than 40, which is based on tracings obtained following the slightly greater delay after mixing in the anaerobic cuvette.

tion for reduction of cytochrome c by GSH is much faster under anaerobic conditions than under aerobic conditions. Since earlier experiments by Schneider et al. (3) have indicated that oxidation of GSH in a GSH + GSSG mixture by dissolved O_2 could be only partially inhibited by 10 mM EDTA, it is very likely that GSSG also catalyzes a reaction with O_2. We propose that the slower rate of reduction of cytochrome c under aerobic conditions is due to competition between O_2 and cytochrome c for an activated form of GSH which exists as the result of interaction with GSSG. The data in Table II do not necessarily answer the question about the possible role of O_2 in the metal catalyzed reaction. The experiment was conducted under conditions (high ionic strength) under which the metal ion catalyzed reaction would be greatly inhibited.

The catalysis by GSSG is not specific for GSH, nor is acceleration of thiol oxidation specific for GSSG. Several thiols and disulfide compounds exhibit similar properties. There is ample evidence to indicate that GSSG catalysis is due to the disulfide

TABLE 3

Rate of Reduction of 10 μM Cytochrome c by Various Thiols and Thiols + GSSG

Buffer solution: 0.025 M Tris + 0.175 M KCl pH 7.45, 25°.

Conc. Thiol.	Rate mμmoles/ml/min.	GSSG added	Rate with GSSG mμmoles/ml/min.	Rate with GSSG / Rate without GSSG
1 mM 2-Mercaptoethanol	0.83	5 mM	42.8	51.6
1 mM 3-Mercaptopropionic Acid	0.31	5 mM	3.94	12.7
10 μM 2,3-Dimercaptopropanol	7.05	5 mM	5.8	0.82
500 μM Cysteine	1.50	5 mM	44.3	29.5
500 μM Cysteine + 125 μM Cystine	19	--	--	--

The rates represent the initial rates.

group and not other parts of the GSSG molecule (Table III).

Fig. 4 indicates the probable reactions between GSH and cytochrome c. The GSH undoubtedly reacts as the mercaptide ion, GS^-. On the basis of the experiments discussed here, we postulate an interaction between GSSG and GS^- to give an intermediate

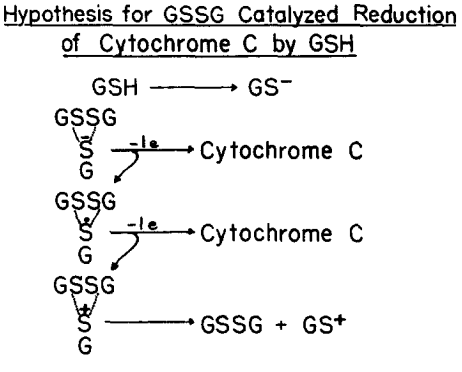

Fig. 4. Possible reactions in the reduction of cytochrome c by GSH in the presence of GSSG.

which donates its electrons more rapidly to cytochrome c. Removal of one electron from the mercaptide ion would yield the mercaptyl or thiyl radical (GS•). This may be stabilized by the interaction with GSSG and react with cytochrome c instead of combining with another GS• to form GSSG. The product from removal of 2 electrons from GSH or GS^- in a complex with GSSG would be the equivalent of a sulfonium ion (GS^+). As has been suggested, this may have implications as a primary form in energy conservation (4-7).

The disulfide catalysis may be significant in facilitating rapid electron transfer into and out of cytochromes and in energy conservation via a sulfonium ion formation. In mitochondrial systems the thiol and the disulfide may not be supplied by small molecules like glutathione, but by appropriate groups in proteins. The lipophilic character of the postulated complex involving three sulfur atoms as well as the complete insensitivity of the reaction to ionic strength may be of considerable significance if such a reaction functions in mitochondrial electron transport and energy conservation.

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