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DI-γ-GLUTAMYLCYSTINE AS A SUBSTRATE FOR GLUTATHIONE REDUCTASE*

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SUMMARY

Di-γ-glutamyl cystine will substitute for oxidized glutathione as a substrate in the reaction catalyzed by glutathione reductase. Using this substrate the apparent K_m is much higher and the v_{max} decreased.

EXPERIMENTAL

Glutathione reductase (reduced NAD(P):oxidized glutathione oxidoreductase, EC 1.6.4.1) catalyzes the reduction of glutathione and is present in most biologically active materials. The enzyme has been purified from *Escherichia coli*, yeast, peas, rat liver, and human erythrocytes. Initially, glutathione reductase was regarded as highly specific because the enzyme for wheat germ failed to reduce L-cystine, DL-homocystine, di-γ-glutamylcystine, oxidized asparathione, and oxidized coenzyme A and was inactive with NADH[1].

Subsequently the enzyme from yeast, liver, and human erythrocytes was shown to utilize NADH[2,3], and the erythrocyte enzyme could catalyze the reduction of cystine and homocystine[3]. While determining the concentration of oxidized glutathione in di-γ-glutamylcystine (GCCG), we found that GCCG could serve as a substrate for yeast glutathione reductase.

Glutathione reductase (yeast), NADPH, GSSG, and carboxypeptidase A were obtained from the Sigma Chemical Company. Di-γ-glutamylcystine was prepared from commercial oxidized glutathione as described by STRUMEYER[4].

Glutathione reductase was measured spectrophotometrically in a Gilford Model 2400 Absorbance Recorder by following the disappearance of NADPH at 340 nm. The assay system (1 ml) contained Tris–HCl (50 μmoles, pH 8.0), EDTA (20 μmoles), NADPH (0.1 μmole), either GCCG or GSSG, and glutathione reductase (0.6 enzyme unit when GCCG was used as a substrate or 0.006 for GSSG).

Fig. 1 illustrates the oxidizing of NADPH by adding glutathione reductase to a solution of GCCG. Residual GSSG caused the initial rapid decrease in absorbance

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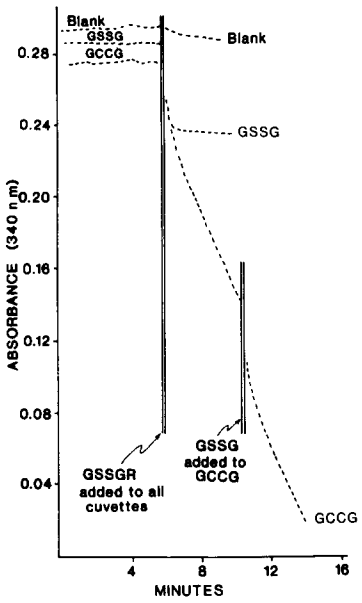

Fig. 1. Oxidation of NADPH with glutathione reductase (GSSGR.) One cuvette did not contain either GCCG or GSSG, one cuvette contained GSSG, and Cuvette C contained GCCG (2 mM). After 6 min incubation glutathione reductase was added to all cuvettes. 5 min later GSSG was added to the cuvette containing GCCG. Beginning absorbances were adjusted to the appropiate positions by using offset controls.

in Cuvette C; thereafter, the reaction failed to stop but continued at a slower rate as GCCG was reduced. By extrapolation, the reaction indicated that GCCG, when prepared by carboxypeptidase, contained approximately 0.24% GSSG. Adding GSSG to the GCCG reaction again elicited a rapid drop in absorbance.

There is a possibility that the observed change in absorbance is not a direct reaction between GCCG and NADPH. Since the GCCG is still contaminated with GSSG, the following reactions might be expected[5]:

$$RSSR + GSH \rightarrow RSSG + RSH$$

$$RSSG + GSH \rightarrow GSSG + RSH$$

$$GSSG + NADPH + H^+ \rightarrow 2\,GSH + NADP^+$$

If NADPH oxidation occurred in this fashion, GSH should accelerate the observed reaction. However, addition of 2 mM GSH (freshly electrolytically reduced) caused a 20% reduction in NADPH oxidation.

The apparent Michaelis–Menten constant was much higher (37-fold) for GCCG than for the more natural substrate, GSSG, and concomitantly maximum velocity decreased (1/5). The observed results were:

	K_m (μM)	v_{max}
GCCG	11600 ± 700	64.3
GSSG	34.4 ± 4.4	307.0

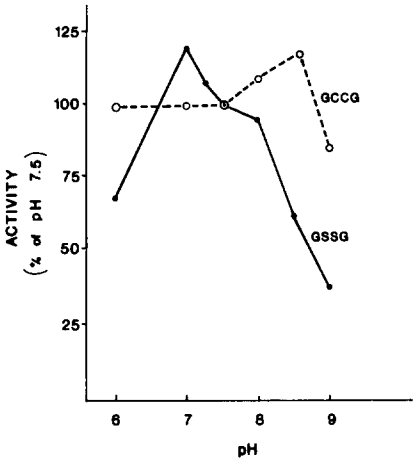

Fig. 2. Effect of pH on glutathione reductase activity, using GSSG (0.1 μM) and GCCG (20 mM) as substrates.

Apparently GCCG was a better substrate than cystine or homocystine, whose reaction rates with the erythrocyte enzyme were, respectively, only 0.5% and 0.1% of the GSSG reduction rate[3].

Glutathione reductase had a lower pH optimum with GCCG than with GSSG (Fig. 2).

Although glutathione reductase reacted relatively feebly with GCCG, it provides a reaction to keep γ-glutamylcystine reduced and thus available for glutathione synthesis.

ACKNOWLEDGMENTS

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