Reversible Activation of Rat Liver Microsomal Glutathione *S*-Transferase Activity by 5,5′-Dithiobis(2-nitrobenzoic acid) and 2,2′-Dipyridyl Disulfide*

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Rat liver microsomes have been shown to have glutathione *S*-transferase activity with 1-chloro-2,4-dinitrobenzene (CDNB) as the second substrate.[1,2] Recently, we have demonstrated that this activity can be stimulated 8- and 4-fold by the sulfhydryl reagents *N*-ethylmaleimide and iodoacetamide, respectively.[2] The product of this essentially irreversible chemical modification is presumably a stable thioether in both cases.

In order to test the possibility that reagents which form disulfide bonds with protein sulfhydryl groups can also activate this glutathione *S*-transferase reversibly, we have treated microsomes with 5,5′-dithiobis(2-nitrobenzoic acid) (DTNB) and with 2,2′-dipyridyl disulfide (DTP).[3,4] Since disulfides formed from these reagents are easily reduced by dithioerythritol, treatment with this substance was used in an attempt to reverse the activation observed and to further confirm the nature of the modification achieved.

Experimental. Microsomes were prepared as described previously from the livers of 180–200 g male Spraque-Dawley rats that had been starved overnight.[5] Microsomes were subsequently subjected to an additional wash with 0.15 M Tris-Cl, ph 8, in order to assure the removal of adsorbed cytosolic proteins. The microsomes were incubated at room temperature in 90 mM sodium phosphate, pH 7.5, at a final concentration of 1 – 1.3 mg protein/ml and with the concentrations of reagents indicated in Table 1 and Fig. 1. At the times indicated, aliquots were assayed for glutathione *S*-transferase activity towards CDNB according to Ref. 6, except that the concentration of glutathione was 5 mM instead of 1 mM. Protein was determined using the procedure of Lowry *et al.*[7]

As can be seen from Table 1, treatment of microsomes with DTNB or DTP activates the glutathione *S*-transferase activity about 4-fold. This finding suggests that formation of a disulfide bond, presumably involving the same sulfhydryl group involved in formation of a thioether bond with *N*-ethylmaleimide and iodoacetamide, can also activate the enzyme.

Table 1. Effect of DTNB and DTP treatment on glutathione *S*-transferase activity in rat liver microsomes.

Conc./ mM	Activity to CDNB nmol/ mg min [a]	Treated/ control
DTNB; incubation time 1 min		
0	98.7(59)	
0.01	234(12)	2.4
0.05	345(18)	3.5
0.1	388(13)	3.9
1	441(45)	4.5
DTP; incubation time 2 min		
0	114(1)	
0.01	188(10)	1.7
0.05	342(30)	3.0
0.1	408(41)	3.6
1	444(39)	3.9

[a] Values are the mean ± the standard error of the mean for three rats.

However, interpretation of this experiment is complicated by the possibility that the halves of the DTNB and DTP molecules which form disulfide bonds with microsomal sulfhydryl groups might be replaced by the large amount of glutathione present in the assay medium itself. Indeed, Fig. 1 shows that if DTNB-activated microsomes are preincubated with 5 mM glutathione before assay, an even greater glutathione *S*-transferase activity is observed and this elevated activity persists for several minutes.

These results suggest three conclusions: (1) The halves of the DTNB and DTP molecules which form disulfide bonds with microsomal sulfhydryl groups can be replaced by glutathione. (2) The formation of a disulfide bond between glutathione and microsomal sulfhydryl groups results in activation of the glutathione *S*-transferase activity. Indeed, it is not at this point possible to decide whether DTNB and DTP themselves activate the microsomal enzyme or simply facilitate the formation of a disulfide bond involving glutathione and thereby lead to activation. (3) The disulfide bond formed between glutathione and microsomal sulfhydryl groups which results in activation of the transferase activity appears to be relatively stable. For instance, this disulfide bond is not readily reduced by a large excess of glutathione.

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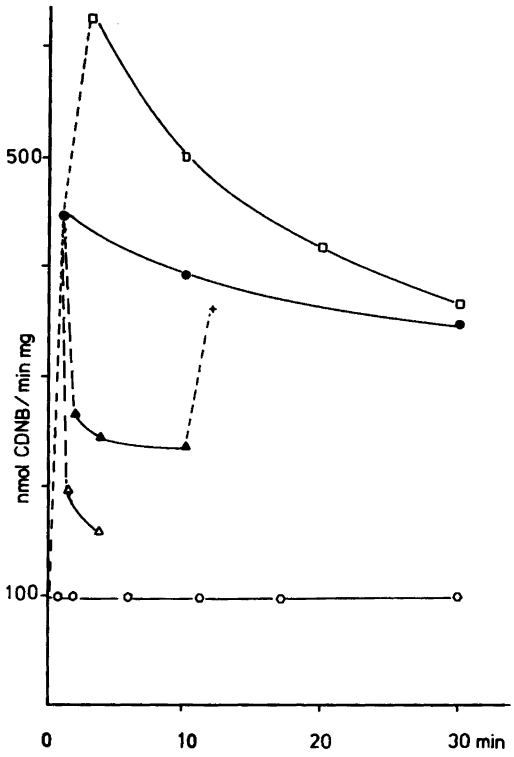

Fig. 1. Glutathione *S*-transferase activity in microsomes activated with 0.1 mM DTNB (●) subsequently treated with 5 mM GSH (□) or 0.3 mM (▲) and 5 mM dithioerythritol (△). DTNB-dithioerythritol treated microsomes could be activated again with 3 mM DTNB (+). Control microsomes incubated without DTNB or with 5 mM dithioerythritol only (○).

The reversibility of the activation of microsomal glutathione *S*-transferase activity achieved by DTNB or DTP is also illustrated in Fig. 1. Addition of large amounts of dithioerythritol almost completely reverses the activation. The high activity can be restored by again adding excess DTNB (3 mM). Microsomes which are subjected to treatment with 5 mM dithioerythritol alone do not exhibit any change in their glutathione *S*-transferase activity. Not only does this observation serve as a control to to the experiment shown in the figure, it also suggests that glutathione *S*-transferase activity in our isolated rat liver microsomes has not already been partially activated *in vivo* by the formation of disulfide bonds.

The study presented here demonstrates that the glutathione *S*-transferase activity of rat liver microsomes can be activated by reagents which form disulfide bonds with microsomal sulfhydryl groups. The mechanism of this activation is still far from clear. A very basic question is whether the microsomal sulfhydryl groups involved are localized on the protein which catalyzes the glutathione *S*-transferase activity or whether the effect is an indirect one. This question can best be answered after isolation of the protein involved and we are presently carrying out this isolation.

Also unclear is the physiological significance of the activation of microsomal glutathione *S*-transferase activity. Of special interest in this respect are the present findings that formation of a disulfide bond involving glutathione results in activation and that the activation achieved through formation of a disulfide bond is reversible. It is possible that addition to and removal of glutathione or some other thiol from sulfhydryl groups on the endoplasmic reticulum may play a role in the regulation of glutathione *S*-transferase activity *in vivo*.

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