INHIBITION OF GLUTATHIONE PEROXIDASE BY COENZYME A*

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Summary: Glutathione peroxidase has been found to be extremely sensitive to inhibition by coenzyme A. Blocking the SH group of coenzyme A reduces the inhibitory effectiveness about 6-fold. It is thus possible that GSH peroxidase activity is regulated in vivo by the CoA/acyl CoA ratio. Dephospho-CoA was about 11-fold less effective than CoA, and pantetheine some 100-fold less effective. The kinetics of CoA inhibition were similar to those of 5'-ATP inhibition. It seems that CoA inhibits mainly because of its nucleotide properties.

GSH peroxidase (hydrogen peroxide: GSH oxidoreductase, EC 1.11.1.9) is probably the main agent responsible for the decomposition of hydrogen peroxide and lipid peroxides in the liver and erythrocyte cell (1). In a recent report from this laboratory (2) it was shown that the enzyme possesses allosteric properties and is susceptible to allosteric inhibition by a wide range of nucleotides, particularly NADPH, $NADP^+$ and 5'-ATP.

Whilst investigating the effects of further nucleotides and their derivatives it was discovered that CoA was a much more potent inhibitor of GSH peroxidase than the other nucleotides. Whereas many nucleotides have been implicated in the allosteric

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regulation of enzymes, no such role has yet been suggested for CoA. Because of the possibility that CoA could act as an allosteric modifier, the inhibition was studied further.

Methods and Materials

Pig erythrocyte GSH peroxidase was purified as previously described (2). The enzyme was assayed by the method of Paglia and Valentine (3) as modified by Little et al (2). Pantetheine was prepared by reduction of pantethine with sodium borohydride. Excess borohydride was destroyed by acidification after reduction. N-ethylmaleimide (NEM) blocking of CoA and dephospho-CoA was carried out by incubating the thiol with 1 equivalent of NEM at pH 7 and 23° for 20 min.

CoA, acetyl CoA, pantethine, GSH, NEM, GSSG reductase and all nucleotides were obtained from Sigma. Dephospho-CoA was supplied by P-L Biochemicals, Wisc., and cumeme hydroperoxide (ROOH) by Matheson, Coleman, and Bell. All other reagents used were of analytical reagent grade of purity.

Results and Discussion

Effect of CoA and derivatives on enzyme activity

The sensitivity of GSH peroxidase activity to inhibition by CoA and several compounds related to CoA is shown in Table I. Under the reaction conditions, only 25 μM CoA was required for 30% inhibition. Blocking the SH-group of CoA either by acetylation or treatment with NEM reduces the inhibitory effectiveness 6-8-fold. It thus appears that a free SH-group greatly enhances the inhibition.

GSH peroxidase is inhibited by a wide range of nucleotides and so the effect of changes in the nucleotide moiety on

TABLE I

Inhibition of GSH Peroxidase by CoA Derivatives

Inhibitor	Concentration required for 50% inhibition	Concentration required for 30% inhibition
	mM	mM
Coenzyme A	0.06	0.025
S-Acetyl Coenzyme A	0.37	0.17
NEM-blocked Coenzyme A	0.46	0.20
Dephospho Coenzyme A	0.8	0.27
Pantetheine	5.5	2.2
Pathothenate	No effect at 5 mM.	

the inhibitory powers of CoA was examined. Removal of the 3'-phosphate as in dephospho-CoA decreases inhibitory effectiveness some 13-fold, whereas total removal of the nucleotide moiety including all phosphate groups to give pantetheine yields a weak inhibitor about 100-fold less potent than CoA. It would thus appear that the nucleotide residue is the most important part of

TABLE II

Effect of 5'-adenosine compounds on GSH Peroxidase activity

Inhibitor	Conc. required for 30% inhibition (mM)
5'-AMP	7.5
5'-ADP	1.5
5'-ATP	0.7
5'-Adenosine tetra-phosphate	0.5
NEM-blocked dephospho-CoA	0.4
Dephospho-CoA	0.27

CoA for the inhibition. Dephospho-CoA may be regarded as a derivative of the 5'adenosine phosphates. It was therefore of interest to compare the inhibitory effectiveness of dephospho-CoA with the other 5'adenosine phosphates (Table II). It was noted previously that as the 5'phosphate chain increased, so did the inhibition (2). It is apparent that dephospho CoA fits into the pattern set by the four 5'adenosine phosphates. Previously it was noted that 3'5'-AMP was significantly better inhibitor than 5'AMP and that 3'AMP was better still (2). On this basis it is possible to rationalize the fact that CoA with phosphate residues at both the 3' and 5' positions on the adenosine ring is a much better inhibitor than dephospho-CoA.

It thus seems reasonable to suggest that the nucleotide portion of CoA is mainly responsible for the inhibition. However, the SH-groups also appear to play an important, but as yet unclear role. One possibility might be that whereas the nucleotide moiety of CoA acts at the allosteric site, the SH group end of the molecule interacts at the catalytic site. CoA itself was found to be a poor substrate for GSH peroxidase, being oxidized at <1% of the rate of GSH oxidation. However, some form of direct CoA interaction at the catalytic site cannot be ruled out.

Kinetics of CoA inhibition

Further evidence for the involvement of the nucleotide residue of CoA in the inhibition was obtained from an investigation of the kinetics of inhibition and the kinetic interactions between inhibitor and the two substrates. Fig. 1 shows that increased GSH levels markedly decrease the inhibition. The double reciprocal Webb Plot (4) (Fig. 1 inset) shows that the CoA inhibition is competitive with respect to GSH and suggests total exclusion of

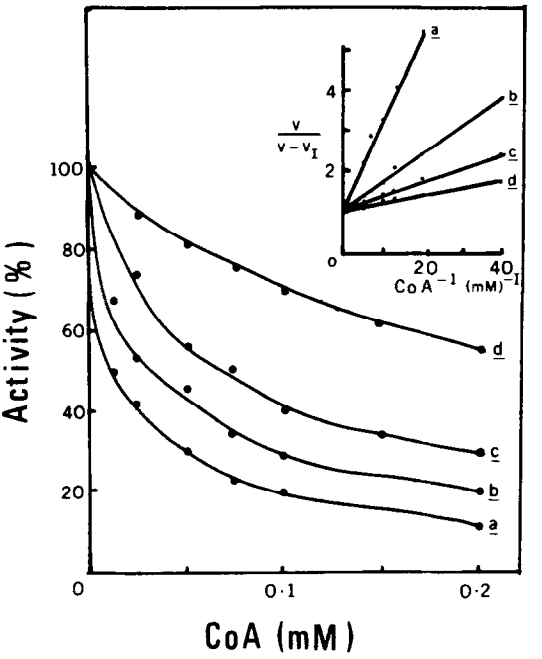

Fig. 1 Effect of [GSH] on inhibition by CoA. Assays were carried out at 0.125 mM ROOH. The levels of GSH used in the assay were (a) 0.125 mM, (b) 0.25 mM, (c) 0.5 mM, (d) 1 mM.

GSH from the active site rather than a simple reduction in the enzyme affinity for substrate. However, the interaction between the hydroperoxide substrate and CoA is cooperative rather that competitive since the inhibition is enhanced by increasing hydroperoxide levels (Fig. 2).

The Webb Plot (Fig. 2 inset) shows the interaction to be of a mixed type. It therefore seems that the inhibition is of an uncompetitive or coupling nature.

It is of significance to note that the kinetics of GSH peroxidase inhibition by 5'ATP were identical to the above. However, the interaction between GSH and CoA is much stronger than between GSH and 5'ATP. The K_i for the latter interaction was 2.9 mM, whereas with CoA the K_i was exceedingly small, being <5 μM.

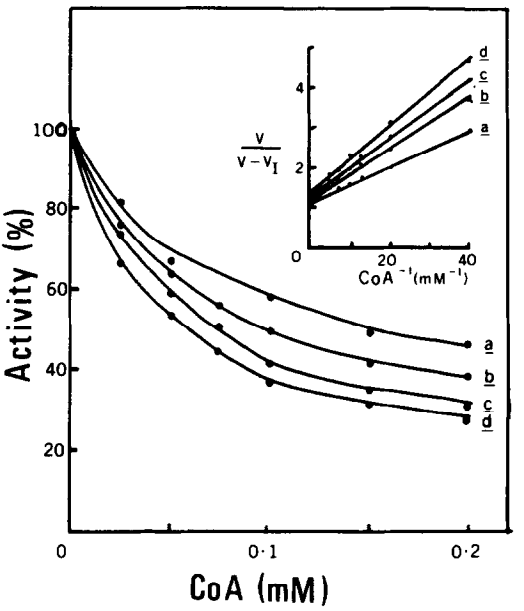

Fig. 2 Effect of [ROOH] on inhibition by CoA. Assays were carried out at 0.5 mM GSH. The levels of ROOH used in the assay were (a) 0.03 mM, (b) 0.07 mM, (c) 0.125 mM, (d) 0.5 mM.

In view of the very low levels of CoA in the erythrocyte (about 3 μM), it seems unlikely that CoA inhibition is of physiological relevance here. However, in the liver, where CoA levels are about 0.05-1 mM in the cytosol and 0.75-1.89 in the mitochondria (5), the inhibition may be of physiological relevance. In addition, the markedly low effectiveness of CoA after SH blocking may suggest that GSH peroxidase activity could be regulated *in vivo* in certain tissues by the free CoA/acyl CoA ratio. Thus, the conversion of CoA to acyl CoA as a result of glycolysis or β-oxidation of fatty acids would result in a relative activation of GSH peroxidase in the cytosol or mitochondrial matrix.

As GSH peroxidase is so sensitive to CoA inhibition it could well be instructive to examine the effects of CoA on other allosteric enzymes with adenosine nucleotides as effectors.

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