REVERSIBLE INACTIVATION BY ELEMENTAL SULFUR AND MERCURIALS OF RAT LIVER SERINE DEHYDRATASE AND CERTAIN SULFHYDRYL ENZYMES

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SUMMARY

Rat liver serine dehydratase can be readily inactivated by elemental sulfur. Dithioerythritol can prevent and reverse this inactivation. The inactivation of serine dehydratase by sulfur is very similar in a number of aspects to that obtainable with p-chloromercuriphenylsulfonate. K^+ ions at physiological concentrations effectively protect the enzyme against inactivation by both sulfur and the mercurial. Alcohol dehydrogenase and 3-hydroxybutyrate dehydrogenase are also sensitive to elemental sulfur in the absence of dithioerythritol.

L-Serine dehydratase has been reported to be specifically inhibited by elemental sulfur (1), and this inhibition has been considered as a physiological device for the control of methionine metabolism (2). We have investigated this question and found that the apparent inhibition of serine dehydratase by sulfur involves an inactivation which can be prevented and reversed by thiols and it is paralleled by that obtainable with p-chloromercuriphenylsulfonate. Alcohol dehydrogenase and 3-hydroxybutyrate dehydrogenase, both sensitive to sulfhydryl reagents, can also be inactivated by elemental sulfur, and this inactivation is also counteracted by thiols. These observations suggest, for the first time to our knowledge, that elemental sulfur can behave as a sulfhydryl poison for a variety of enzymes.

MATERIALS AND METHODS

Serine dehydratase and homoserine dehydratase were assayed

in extracts of livers from rats fed a high protein diet as previously described (3). Homoserine dehydratase assay was similar to that of serine dehydratase, but in this case the reaction was started by addition of DL-homoserine for 25 mM final concentration. Yeast alcohol dehydrogenase (Sigma) and 3-hydroxybutyrate dehydrogenase from Rodopseudomonas spheroides (Boehringer) were assayed with 0.5 mM DPN^+, 0.05 M Tris-HCl pH 7.5 or 8.5, and 0.1 M ethanol or 20 mM DL-hydroxybutyrate respectively. Elemental sulfur was added as a coloidal suspension prepared according to Kato et al. (1) unless indicated otherwise. Assays were carried out at room temperature (ca. 23° C) in 1 cm light path cuvettes, and changes in optical density were recorded in a Cary 15 spectrophotometer.

TABLE I

Inhibition of serine dehydratase and homoserine dehydratase by preincubation with elemental sulfur and its counteraction by dithioerythritol

Preincubations were carried out for 10 minutes at 37° C with the entire assay mixture minus substrates, auxiliary enzymes and pyridin nucleotide, in presence of sulfur and DTE as indicated below. The final concentration of ethanol (added as initial solvent of the elemental sulfur) did not affect the enzymes.

Enzyme	Preincubation			Activity
	Buffer 0.05 M*	Sulfur 1.6 µg/ml	DTE 1 mM	
Serine dehydratase	Na borate, pH 8.3	-	-	100
	"	+	-	4
	"	-	+	105
	"	+	+	110
	Tris-HCl, pH 8.5	-	+	100
	"	+	-	5
	"	+	+	105
	K phosphate, pH 8.5	-	+	100
	"	+	-	25
	"	+	+	95
Homoserine dehydratase	Na borate, pH 8.3	-	-	100
	"	+	-	42
	"	+	+	93

* respect to the anion.

RESULTS AND DISCUSSION

As shown in Table I, preincubation with elemental sulfur causes a strong inactivation of serine dehydratase, assayed in three different buffers, except when dithioerythritol (DTE) was present in excess. Reduced glutathione can replace DTE as a protective agent. Fig. 1 shows the time course of the inactivation of serine dehydratase by elemental sulfur and its reversal after addition of DTE. We have observed that both DTE and reduced glutathione can react directly with elemental sulfur since they clear sulfur suspensions, although glutathione acts much more slowly than DTE. Mercaptoethanol is nearly as effective as DTE.

Kato et al. (1) reported that, in addition to serine dehydratase, homoserine dehydratase could also be inhibited by sulfur, although only in the presence of an alkaline sodium borate buffer. We have confirmed this observation and found that this inhibition can be prevented by DTE.

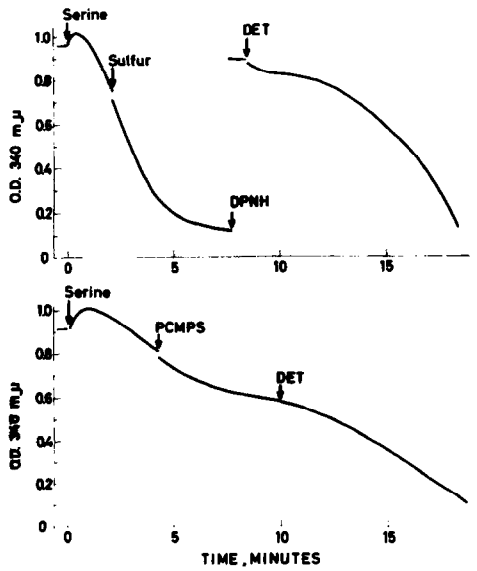

Fig. 1. Time course of the inactivation of serine dehydratase by sulfur and p-chloromercuriphenylsulfonate and its reversion by dithioerythritol. The assays were carried out in 0.1 M sodium borate, pH 8.3. The amount of reactants added, as indicated by the arrows, were for the following final concentrations: 0.1 M for serine; 1.6 μg/ml for sulfur; 0.01 mM for pCMPS; and 1 mM for DTE.

TABLE II

Inactivation of alcohol dehydrogenase and 3-hydroxybutyrate dehydrogenase by preincubation with elemental sulfur

Preincubations were carried out with the entire assay mixture minus substrate and coenzyme, in presence of sulfur and DTE as indicated below. For alcohol dehydrogenase, elemental sulfur was added as an alcohol-free, colloidal aqueous suspension (6). Each of these preincubations were carried out at 37° C for 10 minutes. In the case of 3-hydroxybutyrate dehydrogenase, preincubations were carried out at room temperature for 30 minutes.

Enzyme	1st preincubation			2nd preincubation	
	Sulfur 1.6 µg/ml	DTE 1 mM	Activity	DTE 1 mM	Activity
Alcohol dehydrogenase	-	-	100		
	+	+	95		
	+	-	40		
	+	-		-	42
	+	-		+	85
	-	-		+	103
3-Hydroxybutyrate dehydrogenase	-	-	100		
	+	+	96		
	+	-	55		
	+	-		-	27
	+	-		+	45
	-	-		+	101

Two -SH enzymes, alcohol dehydrogenase (4) and 3-hydroxybutyrate dehydrogenase (5) can also be inactivated by preincubation with elemental sulfur as shown in Table II. This Table also shows that DTE can prevent and largely reverse the sulfur inactivation of alcohol dehydrogenase. In similar conditions, 3-hydroxybutyrate dehydrogenase partially inactivated by preincubation with sulfur was not reactivated by a subsequent incubation with DTE, although the latter did prevent any further decrease in activity.

The requirements for the inhibitory effect of elemental sulfur (preincubation and absence of thiols), together with the well known sulfhydryl reactivity of alcohol dehydrogenase and 3-hydroxybutyrate

dehydrogenase would suggest that the inactivation of serine dehydratase by elemental sulfur may depend on a blockage of -SH groups (7) of the enzyme. The fact that p-chloromercuriphenylsulfonate (pCMPS) inactivates serine dehydratase similarly to sulfur is consistent with this interpretation. Fig. 1 shows that the time course of the inactivation by pCMPS and of its reversal by DTE are very similar to those observed with sulfur. The parallelism between the inactivation of serine dehydratase by elemental sulfur and pCMPS is further emphasized by the data in Table III. 0.1 M K^+ ions strongly protected against both inactivating agents. With threonine as substrate, at pH 8.3, the enzyme is considerably less sensitive to inactivation by these agents. At neutral pH, threonine did not protect, but K^+ ions still protected in a similar degree from inactivation by either sulfur or pCMPS (Fig. 2). Fig. 2 shows the effect of increasing K^+ concentrations on the inactivation of serine dehydratase by sulfur, at pH 7; half protection was achieved by a 0.01

TABLE III

Inactivation of serine dehydratase by elemental sulfur and p-chloromercuriphenylsulfonate and protection by threonine and K^+ ions

The assays were carried out in 0.1 M sodium borate, pH 8.3, with 0.1 M serine or threonine and with or without K^+ as indicated below. The reaction was allowed to proceed at room temperature for 3 to 5 minutes (v_o) before the addition of the inactivator. v_i stands for the velocity measured at 10 minutes after the addition of the inactivator.

Substrate	KCl 0.1 M	v_o*	Inactivator**	v_i*	Inhibition per cent
Serine	-	470	Sulfur	15	97
Serine	-	450	pCMPS	7	98
Serine	+	270	pCMPS	240	10
Serine	+	240	Sulfur	210	12
Threonine	-	120	Sulfur	70	42
Threonine	-	150	pCMPS	85	43

* v_o and v_i are expressed as the decrease in optical density per minute x 10^3.

** Sulfur and pCMPS, when indicated, were added for final concentrations of 1.6 µg/ml and 0.01 mM respectively.

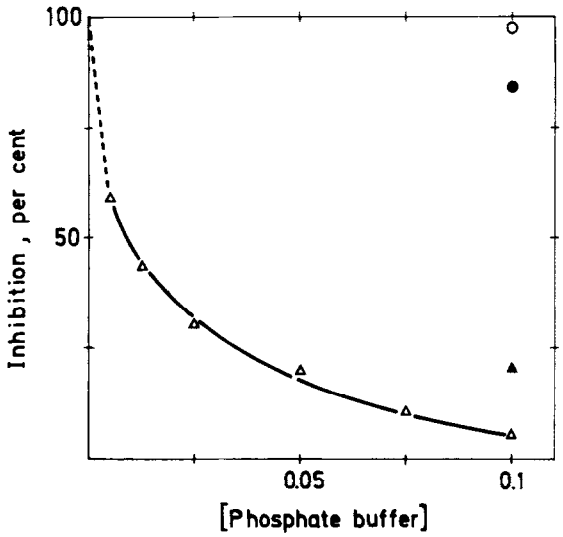

Fig. 2. Protection by K^+ ions against the inactivation of serine dehydratase by sulfur and p-chloromercuriphenylsulfonate. The assays were carried out in phosphate buffer, pH 7, with either sodium (circles) or potassium (triangles) as cation. The reaction was started with 0.1 M serine and was allowed to proceed for 3 to 5 minutes (v_o) before the addition of the inactivator. The velocity measured at 10 minutes after addition of the inactivator (v_i) was used to estimate the degree of inhibition $[(v_o - v_i) \times 100/v_o]$. Open symbols stand for sulfur and solid symbols for pCMPS.

M concentration, and almost complete protection took place in the physiological range of K^+ ions. This protective effect of K^+ ions can account for the much lower apparent affinity of sulfur for serine dehydratase when assayed with 0.1 M potassium phosphate instead of 0.1 M sodium borate buffer (1).

The marked effect of K^+ ions on the sensitivity of rat liver serine dehydratase to inactivation by elemental sulfur and pCMPS, in addition to the previously reported (3) effect on the apparent affinity of the enzyme protein for pyridoxal phosphate indicates that the native enzyme is markedly dependent on an environment with K^+ ions in the physiological concentration range. For this reason it is suggested that despite the fact that the activity of the enzyme can be somewhat greater in the absence of K^+ ions (because of the greater affinity for the sub-

strate*) any study aiming to a better knowledge of properties of potential physiological significance of the enzyme should in principle be carried out in the presence of K^+ ions.

We believe that the results above described clearly indicate that the previously reported "non competitive inhibition" of serine dehydratase by elemental sulfur is a reversible inactivation involving -SH groups of the enzyme. The apparent lack of specificity of this inactivation, together with the protective role of K^+ ions seems to exclude any physiological significance of elemental sulfur for the regulation of serine dehydratase activity.

In contrast with the parallelism in the inactivation of serine dehydratase by elemental sulfur and pCMPS, alcohol dehydrogenase is much more sensitive, both in time and extent, to inactivation by pCMPS than by sulfur. While the parallelisms suggest involvement of -SH groups in the marked sensitivity of serine dehydratase to elemental sulfur, the quantitative differences between the latter and the mercurial in the case of alcohol dehydrogenase are consistent with the hypothesis that sensitivity to elemental sulfur might be markedly related to the occurrence of neighbouring -SH groups that could give disulfide bridges (8) or larger bridges involving more than two sulfur atoms.

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