2,2'-Dithiodipyridine Activates Aldehyde Dehydrogenase and Protects the Enzyme against Inactivation by Disulfiram

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Small concentrations of 2,2'-dithiodipyridine cause a rapid activation of sheep liver cytoplasmic aldehyde dehydrogenase in the presence of NAD⁺. Enzyme pre-modified by 2,2'-dithiodipyridine is largely protected against the potent inactivatory effect of disulfiram. 2,2'-Dithiobis-(5-nitropyridine) inactivates the enzyme. The implications of these results are discussed with reference to various possible classes of thiol group in aldehyde dehydrogenase.

An important property of mammalian hepatic aldehyde dehydrogenase is its susceptibility to inactivation by thiol-modifying reagents. These include disulfiram (tetraethylthiuram disulphide, Antabuse) (Kitson, 1975, 1977, 1978), a number of other thiuram disulphides (Kitson, 1976), 5,5'-dithiobis-(2-nitrobenzoate) and iodoacetamide (Hart & Dickinson, 1977), and *p*-chloromercuribenzoate (Deitrich, 1967; Kraemer & Deitrich, 1968). On the basis of this and other properties, a mechanism has been put forward for the action of aldehyde dehydrogenase involving acyl-enzyme formation between the acyl moiety of the aldehyde substrate and a catalytically essential enzymic thiol group (Feldman & Weiner, 1972; Hart & Dickinson, 1978*a,b*). The thiol-modifying reagent 2,2'-dithiodipyridine, however, has been reported to be an activator of sheep liver cytoplasmic aldehyde dehydrogenase (Kitson, 1978). The purpose of the present work was further to investigate this phenomenon, in particular by comparing it with the better-known inactivation of aldehyde dehydrogenase by disulfiram.

Experimental

Materials

The following compounds were obtained from the sources indicated: disulfiram, 2,2'-dithiodipyridine, 4,4'-dithiodipyridine and 2,2'-dithiobis-(5-nitropyridine). Sigma Chemical Co. (St. Louis, MO, U.S.A.); acetaldehyde and 2-mercaptoethanol, Fluka A.G. (Buchs, Switzerland); NAD⁺, BDH Chemicals (Poole, Dorset, U.K.).

Methods

Cytoplasmic aldehyde dehydrogenase from sheep liver was prepared as previously described (Kitson, 1978). Before use the enzyme solutions were

thoroughly dialysed to remove 2-mercaptoethanol. Enzyme activity was assayed as described previously (Kitson, 1975) at pH 7.3. The effect of the modifiers studied on the enzyme activity was determined by incorporating the modifier in solution in 0.1 ml of ethanol directly in the assay system. Protein concentrations were determined by the method of Bradford (1976). Enzyme concentration in the assay system was approx. 0.5 μ M (based on mol.wt. 212 000; Crow, 1975).

Results and Discussion

The first part of Table 1 records the effect of 2-mercaptoethanol on the interaction between 2,2'-dithiodipyridine and cytoplasmic sheep liver aldehyde dehydrogenase. First it is confirmed that very low concentrations of the modifier cause an appreciable increase in enzyme activity; this process is complete within the time of mixing (Expts. A–D). The subsequent addition of a moderate excess of 2-mercaptoethanol does not abolish this activation (Expt. E), although pre-mixing of the thiol and the modifier does result in a rate very close to the control (as would be expected from the destruction of 2,2'-dithiodipyridine by 2-mercaptoethanol; Expt. F). When a massive excess of 2-mercaptoethanol (0.4 M) is used, the activatory process can be reversed (Expt. H). These results are very similar to those found with disulfiram (Kitson, 1978) (except there, of course, an inactivation occurred), and suggest that the effect of 2,2'-dithiodipyridine is caused by a covalent interaction between this compound and the enzyme, involving a disulphide interchange.

Table 1 shows that when the enzyme is treated with 2,2'-dithiodipyridine, and then excess 2-mercaptoethanol is added (which largely removes unchanged modifier) before NAD⁺ and acetaldehyde are added

Table 1. *Effect of 2,2'-dithiodipyridine and disulfiram on cytoplasmic aldehyde dehydrogenase*

The activity of the enzyme at various concentrations of modifiers (added in the order shown) was assayed as described under 'Methods'. The initial rate of the enzyme reaction in the presence of modifier is expressed as a percentage of the rate in its absence. Numbers in parentheses refer to the numbers of results that were averaged to give the data shown. Abbreviation: 2,2'-DTDP, 2,2'-dithiodipyridine.

Expt.	2,2'-Dithiodipyridine (μM)	2-Mercaptoethanol (mM)	Order of mixing	Initial activity (% of control rate)
A	1	0	2,2'-DTDP, buffer, enzyme, substrates	116 (4)
B	2	0	As Expt. A	153 (7)
C	5	0	As Expt. A	154 (4)
D	2	0	Buffer, enzyme, substrates, 2,2'-DTDP	157 (2)
E	2	0.1	2,2'-DTDP, buffer, enzyme, substrates; thiol added 2 min later	155 (3)
F	2	0.1	2,2'-DTDP, buffer, thiol, substrates; enzyme added 2 min later	110 (5)
G	2	0.1	2,2'-DTDP, buffer, enzyme; thiol added 2 min later; substrates added after further 2 min	96 (3)
H	2	400	2,2'-DTDP, buffer, enzyme, substrates; thiol added 2 min later	90 (1)
	2,2'-Dithiodipyridine (μM)	Disulfiram (μM)		
I	0	1	Disulfiram, buffer, enzyme, substrates	13 (2)
J	1	1	2,2'-DTDP, buffer, enzyme, substrates; disulfiram added 2 min later	75 (2)
K	2	1	As Expt. J	113 (2)
L	5	1	As Expt. J	129 (2)
M	1	1	2,2'-DTDP, disulfiram and buffer mixed; then enzyme and substrates	22 (1)
N	2	1	As Expt. M	27 (2)
O	5	1	As Expt. M	43 (2)

Fig. 1. *Effect of 2,2'-dithiobis(5-nitropyridine) on cytoplasmic aldehyde dehydrogenase*

Enzyme activity was measured as described under 'Methods' at pH 7.3 and is plotted against the concentration of modifier added to the assay mixture. ●, 2,2'-Dithiobis(5-nitropyridine); ○, disulfiram.

to initiate the enzyme reaction, no activation results (Expt. G). It thus appears that 2,2'-dithiodipyridine reacts rapidly with the enzyme only in the presence of NAD^+ . [The enzyme exhibits an obligatory sequential order of binding with NAD^+ as leading substrate (MacGibbon *et al.*, 1977).]

In contrast with the above result, disulfiram reacts rapidly with cytoplasmic aldehyde dehydrogenase regardless of whether NAD^+ is present or not (Kitson, 1978).

The second half of Table 1 shows what happens when a sample of aldehyde dehydrogenase is treated with both 2,2'-dithiodipyridine and disulfiram. First it is evident that pre-modification of the enzyme with 2,2'-dithiodipyridine largely prevents the substantial inactivation of the enzyme that a small concentration of disulfiram would otherwise cause (Expts. I-L). However, when aldehyde dehydrogenase is presented with a mixture of the two modifiers, an extensive loss of activity results, showing that the enzyme has a greater affinity for disulfiram than for 2,2'-dithiodipyridine (Expts. M-O).

Qualitatively similar results were obtained with 4,4'-dithiodipyridine, but the activation, and protection against disulfiram inactivation, were

quantitatively less impressive than with the 2,2'-isomer. In contrast, 2,2'-dithiobis-(5-nitropyridine) is an inactivator of cytoplasmic aldehyde dehydrogenase and gives a 'titration' profile similar to that obtained with disulfiram (Kitson, 1978), although disulfiram is the more effective inactivator (see Fig. 1).

There are two explanations for the results reported here, which differ depending on whether it is assumed that disulfiram and 2,2'-dithiodipyridine react with the same or different enzymic thiol groups, and in both cases the implications are intriguing. On the one hand, aldehyde dehydrogenase may indeed have one or more catalytically essential thiol groups which are modified (with consequent loss of activity) by disulfiram and the other thiol reagents listed in the introduction. If this is the case, the enzyme must also possess different thiol groups which are uniquely reactive to 2,2'-dithiodipyridine, and this latter reaction must result in modified enzyme which is not only better at catalysing the oxidation of acetaldehyde, but is very effectively protected against the otherwise potent inactivatory effect of disulfiram. These effects would presumably be mediated by steric factors or subtle three-dimensional changes in the enzyme when modified by 2,2'-dithiodipyridine. It is interesting to note that, on this basis, two reagents of such close structural similarity as 2,2'-dithiodipyridine and 2,2'-dithiobis-(5-nitropyridine) apparently react with different thiol groups.

On the other hand, it may be envisaged that among its approx. 36 cysteine residues (MacGibbon, 1976) cytoplasmic aldehyde dehydrogenase possesses only one class of thiol group that is particularly reactive to thiol-modifying reagents. Of course, since with 2,2'-dithiodipyridine modification results in increased enzyme activity, such thiol groups could not be involved directly in the enzyme's catalytic mechanism. Again, steric factors or changes in tertiary structure of the modified enzyme could give rise to altered binding of substrates to enzyme, or altered catalytic efficiency of the enzyme, such that with most reagents there is a decrease in the enzyme activity, but with 2,2'-dithiodipyridine a more active enzyme molecule happens to result. As before, it seems rather surprising that 2,2'-dithiodipyridine and 2,2'-dithiobis-(5-nitropyridine) have such different effects.

[The idea that the thiol groups with which disulfiram reacts may not be essential for catalytic activity

has been suggested as one possible explanation for the insensitive residual activity (approx. 10%) of disulfiram-treated aldehyde dehydrogenase (Kitson, 1978; Hempel & Pietruszko, 1979). However, it has recently been shown that this disulfiram-insensitive activity is probably due to contamination with mitochondrial aldehyde dehydrogenase (Dickinson & Berrieman, 1979).]

Resolution of the question as to whether disulfiram and 2,2'-dithiodipyridine react with the same thiol groups in aldehyde dehydrogenase may have to await the characterization of the peptides around the cysteine residues involved; for disulfiram this work has started (Hempel & Pietruszko, 1979). In the meantime, the fact that the various thiol-modifying reagents referred to here have such widely differing effects on the enzyme remains an interesting phenomenon, and future work on this topic may well lead to a greater understanding of the properties of aldehyde dehydrogenase.

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