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High concentrations of aldehydes slow the reaction of cytoplasmic aldehyde dehydrogenase with thiol-group modifiers

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High concentrations of aldehydes slow the inactivation of cytoplasmic aldehyde dehydrogenase by disulfiram and also slow the reaction of the enzyme with 2,2′-dithiodipyridine. It is concluded that a low-affinity aldehyde-binding site is probably the site at which thiol-group modifiers react with aldehyde dehydrogenase, as well as being the active site for hydrolysis of 4-nitrophenyl acetate.

The cytoplasmic aldehyde dehydrogenase of sheep liver catalyses the hydrolysis of 4-nitrophenyl acetate, as well as the oxidation of aldehydes by NAD^+. The relationship between the kinetic properties of the dehydrogenase and esterase activities has been extensively studied (MacGibbon *et al.*, 1978; Blackwell *et al.*, 1983). The effect of thiol-modifying reagents such as disulfiram (tetraethylthiuram disulphide) on the two activities of the enzyme has also been examined (Kitson, 1982*a*). Blackwell *et al.* (1983) argue persuasively that the esterase and dehydrogenase active sites are different. They envisage two binding domains, P1 and P2. Of these, P1 is the normal active site for the dehydrogenase activity and is occupied by propionaldehyde only in the presence of NAD^+. At high propionaldehyde concentration (of the order of 20mM) P2 is also occupied, and this has the effect of stimulating the dehydrogenase action at P1. (No oxidation of aldehyde is thought to take place at P2.) On the other hand, 4-nitrophenyl acetate binds and is hydrolysed only at P2; the hydrolysis is competitively inhibited by a high concentration of propionaldehyde.

There is also a significant difference in the effects of disulfiram on the two activities. Adding disulfiram to an on-going dehydrogenase assay under normal conditions (pH7.4, 25°C, 1mM-NAD^+, 1mM-acetaldehyde) brings about a rapid and substantial inactivation within the time of mixing. However, adding disulfiram to the enzyme already in the presence of 4-nitrophenyl acetate has little inactivatory effect, although prior modification of the enzyme abolishes the esterase and dehydrogenase activities to similar extents (Kitson, 1982*a*).

It appears from the work referred to above that a high concentration of aldehyde interferes with the binding of 4-nitrophenyl acetate, and that 4-nitrophenyl acetate interferes with the reaction between enzyme and disulfiram. Logically, this implies that a high aldehyde concentration should inhibit the reaction of the enzyme with disulfiram, and it is an investigation of this possibility that is the subject of the present paper. Also reported is the effect of high aldehyde concentrations on the rate of reaction of the enzyme with another thiol-group modifier, 2,2′-dithiodipyridine, which has received previous attention (Kitson, 1984*a*, and references cited therein).

Experimental

Cytoplasmic aldehyde dehydrogenase from sheep liver, purified by the method of Dickinson *et al.* (1981), was dialysed before use against several changes of N_2-saturated 50mM-sodium phosphate buffer, pH7.4, containing 0.3mM-EDTA at 4°C.

The effect of disulfiram on the dehydrogenase activity of the enzyme in the presence of NAD^+ (1mM) and various concentrations of acetaldehyde or propionaldehyde was examined as follows. The enzyme-catalysed reaction was initiated by mixing enzyme, buffer (33mM-sodium phosphate, pH7.4), NAD^+ and aldehyde at 25°C in a volume of 3ml. After the rate of production of NADH at 340nm had been recorded for a minute or two, disulfiram was added as 15μl of an ethanol solution to give a concentration of 1μM. The disulfiram solution was added to the cuvette as rapidly as possible on a glass nail and then the rate of the enzyme-catalysed reaction was again recorded. Some similar experiments were performed with 5,5′-dithiobis-(1-methyltetrazole) in place of disulfiram.

The early time course of the release of 2-thiopyridone from the reaction of 2,2′-dithiodipyridine and aldehyde dehydrogenase under various conditions was studied as follows. Enzyme (0.2ml) was mixed with 50mM-sodium phosphate buffer, pH 7.4 (2.3ml), and either water (0.5ml) or an equal volume of an aqueous solution of acetaldehyde, propionaldehyde, propan-1-ol, acetone or chloral hydrate. The final concentration of chloral hydrate was 10mM and of the other compounds mentioned 100mM. After equilibration of the mixture at 25°C the initial absorbance at 343nm was recorded. 2,2′-Dithiodipyridine was then added as rapidly as possible on a glass nail as 15 μl of an ethanol solution to give a concentration of 10 μM. The resulting increase in absorbance at 343nm was monitored over the next 2–2½min.

Results and discussion

Most previous work on the effect of disulfiram on sheep liver cytoplasmic aldehyde dehydrogenase has been carried out with 1mM-acetaldehyde. It is well established that adding disulfiram to the enzyme in the presence of 1mM-acetaldehyde and 1mM-NAD^+ brings about an immediate inactivation (Kitson, 1982*a*). The results of a typical experiment under these conditions are shown by trace *G* in Fig. 1. The same result is found with 20mM-acetaldehyde (trace *F*), but with 40mM-acetaldehyde there is a short but definite tailing off in the rate of the enzyme-catalysed reaction before the final extent of inactivation becomes established (trace *E*). With propionaldehyde at 100 μM and 1mM the inactivation is again over within the time of mixing (traces *D* and *C* respectively), but with 20mM-propionaldehyde the delay in onset of the inactivated rate is obvious (trace *B*). In trace *A*, with 40mM-propionaldehyde, the effect becomes very marked. The addition of disulfiram to an assay under these conditions has virtually no effect initially; only during the next 5–6min does the rate of the enzyme-catalysed reaction gradually fall to a constant lower value. These results suggest that aldehydes compete weakly with disulfiram for the site at which disulfiram reacts, the competition only becoming significant at relatively high aldehyde concentration.

5,5′-Dithiobis-(1-methyltetrazole) is a thiol-group modifier that has an effect on aldehyde dehydrogenase similar in many ways to that of disulfiram, although it is not as effective an inactivator (Kitson, 1984*b*). The broken line in Fig. 1 shows the rate obtained by pre-modification of the enzyme with 5,5′-dithiobis-(1-methyltetrazole) (2 μM) before it was assayed with 20mM-propionaldehyde. Trace *H* shows that on addition

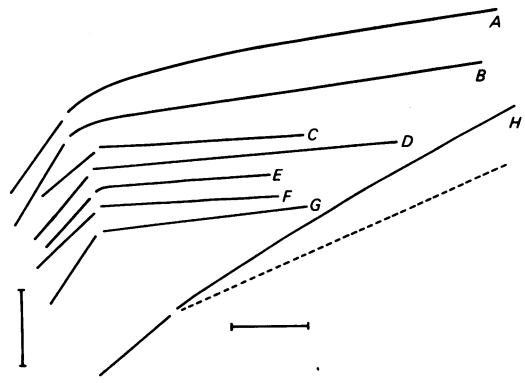

Fig. 1. *Slowing of the inactivation of cytoplasmic aldehyde dehydrogenase by disulfiram in the presence of high concentrations of aldehydes*

The Figure shows spectrophotometer traces obtained with enzyme, NAD^+ (1mM) and various concentrations of acetaldehyde or propionaldehyde. The break in each trace marks the point at which disulfiram (1 μM) was added or, in the case of trace *H*, 5,5′-dithiobis-(1-methyltetrazole) (2 μM). Traces *A*, *B*, *C* and *D* were obtained with 40mM-, 20mM-, 1mM- and 100 μM-propionaldehyde respectively, traces *E*, *F* and *G* with 40mM-, 20mM- and 1mM-acetaldehyde, and trace *H* with 20mM-propionaldehyde. The broken line shows the rate obtained by adding 5,5′-dithiobis-(1-methyltetrazole) (2 μM) to the enzyme before the addition of 20mM-propionaldehyde. The enzyme concentration was 0.29 μM, except for trace *H*, where it was 0.27 μM. The horizontal bar represents 100s, and the vertical bar represents an A_{340} change of 0.1 (traces *A*, *B*, *C*, *E* and *F*), 0.04 (traces *D* and *G*) or 0.2 (trace *H*).

of the modifier to an established assay system there is a gradual slow fall in the rate of the enzyme-catalysed reaction. Therefore it appears that high aldehyde concentration tends also to block the action of 5,5′-dithiobis-(1-methyltetrazole), as it does that of disulfiram.

Under some conditions 2,2′-dithiodipyridine causes a temporary activation of cytoplasmic aldehyde dehydrogenase (Kitson, 1984*a*). Its reaction with the enzyme can be conveniently monitored in the spectrophotometer by measuring the increase in A_{343} as 2-thiopyridone is released. (This is not the case with the modifiers above, where the reaction is monitored indirectly by observing the effect on the enzyme-catalysed reaction.) Fig. 2 shows that 100mM-acetaldehyde markedly slows the reaction of enzyme with 2,2′-dithiodipyridine (compare trace *C* with trace *A*) and that 100mM-propionaldehyde has a similar though smaller effect (compare traces *B* and *G* with traces *A* and *E*). (A control experiment showed that the presence of 100mM-acetaldehyde de-

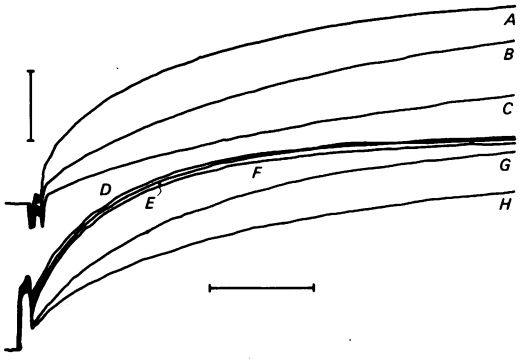

Fig. 2. *Slowing of the reaction of cytoplasmic aldehyde dehydrogenase with 2,2'-dithiopyridine in the presence of high concentrations of aldehydes*

The Figure shows the increase in A_{343} as 2-thiopyridone is released during the reaction of enzyme and 2,2'-dithiodipyridine (10 μM). The upper set of traces was obtained with an enzyme concentration of 2.20 μM, the lower with 2.32 μM. The reaction mixtures contained: traces *A* and *E*, water (controls); traces *B* and *G*, propionaldehyde (100 mM); trace *C*, acetaldehyde (100 mM); trace *D*, propan-1-ol (100 mM); trace *F*, acetone (100 mM); trace *H*, chloral hydrate (10 mM). The horizontal bar represents 30 s, and the vertical bar an A_{343} change of 0.04.

creases the absorbance of a 12 μM solution of 2-thiopyridone by 3.5%, which does not significantly alter the interpretation of the results in Fig. 2.) Unlike propionaldehyde, propan-1-ol and acetone have no effect on the reaction of enzyme with 2,2'-dithiodipyridine (see traces *D* and *F*), and so the effect of propionaldehyde is not just due non-specifically to the presence of an organic solvent. Chloral hydrate (10 mM) has a pronounced slowing effect (trace *H*). The more potent effect of chloral hydrate than that of propionaldehyde parallels its more effective inhibition of the esterase activity of aldehyde dehydrogenase. [MacGibbon *et al.* (1978) give values of K_i of 4.2 mM and 287 μM respectively for propionaldehyde and chloral hydrate.]

An attempt was made to see whether 4-nitrophenyl acetate slows the reaction of the enzyme with 2,2'-dithiodipyridine. However, this experiment was found to be impossible, as both 4-nitrophenyl acetate and the 4-nitrophenoxide ion have an appreciable absorbance at 343 nm.

Previous work has suggested that disulfiram and 2,2'-dithiodipyridine initially react with different groups from a particular pair of closely positioned thiol groups, and that in each case the remaining group of the pair then displaces the label, leading eventually to the same enzymic disulphide (Kitson, 1984*a*; Kitson & Loomes, 1985). Thus, although both modifiers react at broadly the same site, they are not seen as initially modifying precisely the same reactive group. This difference may be reflected in the results observed in the present work; that is, although high aldehyde concentration slows the reaction of both modifiers, in the case of disulfiram propionaldehyde is more effective than acetaldehyde, whereas the reverse is true for 2,2'-dithiodipyridine. Blackwell *et al.* (1983) suggest that aldehydes may bind to a reactive thiolate anion in the P2 binding domain to form a hemithioacetal. If this is so, one would expect the tightness of binding to decrease in the order: chloral > acetaldehyde > propionaldehyde > acetone (according to the susceptibility of these carbonyl compounds to nucleophilic attack), which is in line with their relative effect on the reaction of enzyme and 2,2'-dithiodipyridine (Fig. 2). Thus the presence of a high concentration of aldehyde may affect specifically that cystine residue which is first attacked by 2,2'-dithiodipyridine. The inhibition of the reaction with disulfiram at the other thiol group of the pair (more so by the larger propionaldehyde than by acetaldehyde) would then be a steric effect.

The conclusion to be drawn from this work is that high concentrations of aldehydes interfere with the reaction of thiol-group modifiers with cytoplasmic aldehyde dehydrogenase. This is consistent with the picture of the enzyme painted by Blackwell *et al.* (1983). The P2 site in their model appears not only to be a low-affinity aldehyde-binding site and the esterase active site, but also the site attacked by thiol-group modifiers such as disulfiram and 2,2'-dithiodipyridine. Furthermore, it is possibly the diethylstilboestrol-binding site too, since this compound and 2,2'-dithiodipyridine affect the enzyme in very similar ways (Kitson, 1982*b*). This leaves the interesting question whether the various properties of this site are all purely fortuitous, or whether they indicate a genuine modifier site capable of being occupied by some unknown agent *in vivo*, and thereby exercising a controlling influence on the enzyme activity.

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