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The activation of aldehyde dehydrogenase by diethylstilboestrol and 2,2′-dithiodipyridine

Trevor M. KITSON*
Department of Chemistry, Biochemistry and Biophysics, Massey University, Palmerston North, New Zealand, and Department of Biochemistry, University of Hull, Hull HU6 7RX, U.K.

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1. The activation of sheep liver cytoplasmic aldehyde dehydrogenase by diethylstilboestrol and by 2,2′-dithiodipyridine is described. The effects of the two modifiers are very similar with respect to variation with acetaldehyde concentration, pH and temperature. Thus the degree of activation is maximal when the enzyme is assayed at approx. 1 mM-acetaldehyde, is greater at 25°C than at 37°C, and is greater at pH 7.4 than at pH 9.75. With low concentrations of acetaldehyde both modifiers decrease the enzyme activity. 2. Diethylstilboestrol affects the sheep liver cytoplasmic enzyme in a very similar way to that previously described for a rabbit liver cytoplasmic enzyme. Preliminary experiments show that the same is true for a preparation of human liver aldehyde dehydrogenase. It is proposed that sensitivity to diethylstilboestrol (and steroids) is a common property of all mammalian cytoplasmic aldehyde dehydrogenases.

The last few years have seen the purification of liver aldehyde dehydrogenases from several mammalian species (Feldman & Weiner, 1972; Crow *et al.*, 1974; Eckfeldt *et al.*, 1976; Sugimoto *et al.*, 1976; Hart & Dickinson, 1977; Greenfield & Pietruszko, 1977; Duncan, 1977; MacGibbon *et al.*, 1979; Dickinson *et al.*, 1981). These enzymes show many similarities in terms of size, subunit composition, broad substrate specificity, susceptibility (particularly of the cytoplasmic form) to the anti-alcohol drug disulfiram (see Kitson, 1977), esterase activity etc. An early report (Maxwell & Topper, 1961) described an aldehyde dehydrogenase from rabbit liver that was sensitive to steroidal hormones and diethylstilboestrol. In a recent review (Pietruszko, 1980) it was stated that 'subsequent attempts to demonstrate effects of steroids on enzymes from species other than the rabbit were unsuccessful'. This statement, however, is incorrect; it overlooks our preliminary report (Crow *et al.*, 1974) that the aldehyde dehydrogenases of sheep liver are affected by progesterone and diethylstilboestrol, and the paper by Koivula & Koivusalo (1975) on a steroid-hormone-sensitive phenobarbital-induced cytoplasmic aldehyde dehydrogenase of rat liver. In the work reported below, I present an extended study of the interaction between sheep liver cytoplasmic aldehyde dehydrogenase and diethylstilboestrol, showing how in this respect the sheep and rabbit enzymes are in fact closely similar. The activation of the enzyme by diethylstilboestrol is also compared with the activation produced by the thiol reagent 2,2′-dithiodipyridine (Kitson, 1979, 1982).

* Present address: Department of Chemistry, Biochemistry and Biophysics, Massey University, Palmerston North, New Zealand.

Experimental

Materials

All chemicals were analytical-grade whenever available and were purchased from the usual commercial chemical companies. Sheep liver cytoplasmic aldehyde dehydrogenase, purified from contamination with the mitochondrial enzyme, was prepared as before (Dickinson *et al.*, 1981). Human liver aldehyde dehydrogenase was a generous gift from Ms. K. Newland and Dr. K. E. Crow (unpublished isolation procedure, involving homogenization, centrifugation, fractionation by poly(ethylene glycol) precipitation, ion-exchange chromatography on DEAE-cellulose and affinity chromatography on AMP–Sepharose).

Methods

Protein concentrations were determined as before (Kitson, 1982). Enzyme assay as a dehydrogenase was performed fluorimetrically as described by Hart & Dickinson (1977) or spectrophotometrically with

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an Aminco DW-2a instrument. The standard assay was performed at 25 °C in 33 mM-sodium phosphate buffer, pH 7.4, with 1 mM-NAD$^+$ and 1 mM-acetaldehyde. Where mentioned in the text the temperature, buffer, pH, aldehyde and concentration of aldehyde were all varied as described. Diethylstilboestrol was added in a small volume of ethanol; the same amount of ethanol was added to control assays. Modification of the enzyme by disulfiram and by 2,2′-dithiodipyridine (in the presence of NAD$^+$) was done as previously described (Kitson, 1982). Enzyme assay as an esterase was performed as before (Kitson, 1982). Studies of the pre-steady-state burst of NADH production by native and 2,2′-dithiodipyridine-modified enzyme were conducted both by using the method described before (Kitson, 1982) and with a stopped-flow instrument as described by Hart & Dickinson (1978). Experiments to determine the enhancement of fluorescence of NADH bound to 2,2′-dithiodipyridine-modified enzyme were performed by the method previously described (Dickinson *et al.*, 1981) after rapid passage of the modified enzyme down a column (8 cm × 1 cm) of Bio-Gel P-6 to remove NAD$^+$ and 2-thiopyridone.

Results

Fig. 1 shows the effect of a range of diethylstilboestrol concentration on the activity of sheep liver cytoplasmic aldehyde dehydrogenase in the standard assay (see the Experimental section). In Table 1 the effect of diethylstilboestrol is compared with that of other modifiers of aldehyde dehydrogenase, disulfiram (Kitson, 1978, 1982) and 2,2′-dithiodipyridine (Kitson, 1979, 1982). In the standard assay, 10 µM-diethylstilboestrol approximately doubles the rate. It may be seen that the limiting amount of disulfiram used in the experiments of Table 1 leaves a certain fraction of the enzyme unaffected and that the activity of this remaining enzyme is activated by the subsequent addition of diethylstilboestrol, as would be expected. Adding the disulfiram to the enzyme in the presence of diethylstilboestrol results in the same degree of activation as with the alternative order of mixing, showing that diethylstilboestrol does not protect the enzyme against disulfiram. This result contrasts with the effect of 2,2′-dithiodipyridine, which has been shown to activate the enzyme and also protect it from the subsequent inactivatory effect of disulfiram (Kitson, 1979, 1982). The remaining entries in Table 1 show that under these conditions 2,2′-dithiodipyridine also approximately doubles the rate, but that diethylstilboestrol does not further activate the 2,2′-dithiodipyridine-modified enzyme. Neither does 2,2′-dithiodipyridine further activate the enzyme already in the presence of diethylstilboestrol.

Table 2 records how 2,2′-dithiodipyridine and diethylstilboestrol affect the oxidation of substrates other than acetaldehyde. With 2,2′-dithiodipyridine there is a trend towards less activation with increasing size of the aldehyde, and with the bulkier sub-

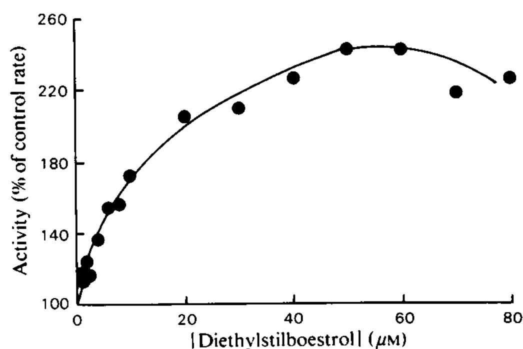

Fig. 1. *Effect of a range of diethylstilboestrol concentrations on sheep liver cytoplasmic aldehyde dehydrogenase*

The enzyme (0.16 µM) was assayed under standard conditions (see the Experimental section) in the presence of various concentrations of diethylstilboestrol. (The limiting solubility of diethylstilboestrol under these conditions appeared to be approx. 80 µM.)

Table 1. *Interactions among various modifiers (diethylstilboestrol, 2,2′-dithiodipyridine and disulfiram) and sheep liver cytoplasmic aldehyde dehydrogenase*

The activity of the enzyme was assayed at pH 7.4 at 25 °C with NAD$^+$ (1 mM) and acetaldehyde (1 mM) after the addition of various modifiers in the order indicated. The results are expressed as percentages of the control rate in the absence of any modifier and are averages of at least two determinations. Concentrations used were: enzyme, 0.156 µM; disulfiram, 0.2 µM; diethylstilboestrol, 10 µM; 2,2′-dithiodipyridine, 1 µM.

Addition	Activity (% of control)
Diethylstilboestrol	203
Disulfiram	18.2
Disulfiram, then diethylstilboestrol added subsequently	32.4
Disulfiram (added to enzyme already in the presence of diethylstilboestrol)	28.8
2,2′-Dithiodipyridine (added to enzyme in the presence of NAD$^+$)	217
2,2′-Dithiodipyridine (added to enzyme in the presence of NAD$^+$), then diethylstilboestrol added subsequently	187
2,2′-Dithiodipyridine (added to enzyme already in the presence of NAD$^+$ and diethylstilboestrol)	203

Table 2. *Effects of 2,2′-dithiodipyridine and of diethylstilboestrol on the oxidation of various aldehydes by sheep liver cytoplasmic aldehyde dehydrogenase*

The enzyme was treated with 2,2′-dithiodipyridine in the presence of NAD^+ (1 mM) at pH 7.4 at 25°C. After 1 min the enzyme-catalysed reaction was initiated by the addition of 0.1 ml of aldehyde solution. The stock solution of acetaldehyde was made in water, those of propanal, benzaldehyde and 4-nitrobenzaldehyde in ethanol/water (1:9, v/v) and that of butanal in ethanol/water (1:1, v/v). Indol-3-ylacetaldehyde solution was prepared as described previously (Kitson, 1982). The results are expressed as percentages of the corresponding control rates with native enzyme. Each value is the average of two to six determinations. In Expt. A the enzyme concentration was 0.166 μM and 2,2′-dithiodipyridine concentration was 1 μM; in Expt. B the corresponding values were 0.046 μM and 0.25 μM. In some cases the rates in the presence of 10 μM-diethylstilboestrol were also compared with the control rates.

			Rate in the presence of modifier (% of control)	
Expt.	Aldehyde	Concn. (mM)	2,2′-Dithiodipyridine	Diethylstilboestrol
A	Acetaldehyde	1	201	—
	Propanal	1	146	—
	Butanal	1	106	—
	Benzaldehyde	0.1	96	—
	Indol-3-ylacetaldehyde	0.1	58	—
B	Acetaldehyde	0.2	135	152
	Indol-3-ylacetaldehyde	0.2	76	126
	4-Nitrobenzaldehyde	0.2	62	172

strates the modified enzyme is rather less active under the conditions used than is the native enzyme. However, diethylstilboestrol activates with these larger aldehydes. Particularly noteworthy is the fact that diethylstilboestrol accelerates the oxidation of 4-nitrobenzaldehyde, a poor substrate that shows a different rate-determining step from other aldehydes (MacGibbon *et al.*, 1977*a*). Thus the effect of diethylstilboestrol cannot be merely to speed up the rate of dissociation of the enzyme–NADH complex (which is thought to be the limiting step in the oxidation of most aldehyde substrates).

It is shown in Table 2 that the degree of activation with acetaldehyde as substrate depends on the aldehyde concentration, and this was further investigated over a wider concentration range. The results for diethylstilboestrol are shown in Fig. 2; a virtually identical result in every detail was obtained when comparing the 2,2′-dithiodipyridine-modified enzyme with the native species. The control Lineweaver–Burk plot curves strongly downwards at high acetaldehyde concentration, as previously reported (MacGibbon *et al.*, 1977*b*), but in the presence of diethylstilboestrol the plot is linear (Figs. 2*a* and 2*b*). At approx. 30 μM-acetaldehyde, diethylstilboestrol has no effect, and in fact it inhibits the reaction at lower aldehyde concentration. The degree of activation is maximal at approx. 1 mM-acetaldehyde, which is the concentration used in the standard assay. At very high acetaldehyde concentrations diethylstilboestrol again tends to have little effect on the rate of the enzyme-catalysed reaction. These variations are clearly depicted in Fig. 2(*c*). A crossing-over of the Lineweaver–Burk plots with and without diethylstilboestrol was also found with the rabbit liver enzyme with glyceraldehyde as substrate (Maxwell & Topper, 1961), and, again with the rabbit liver enzyme, Duncan (1977) reported a change from inhibition to stimulation by diethylstilboestrol as the substrate (acetaldehyde or glucuronolactone) concentration was increased. In contrast there is a steady trend for the degree of activation to increase with decreasing indol-3-ylacetaldehyde concentration as shown in Fig. 3. As with acetaldehyde, the control Lineweaver–Burk plot shows a downward curvature, but also (at very high indol-3-ylacetaldehyde concentration) substrate inhibition is evident.

The esterase activity of sheep liver cytoplasmic aldehyde dehydrogenase (with 100 μM-4-nitrophenyl acetate and 0.42 μM-enzyme at pH 7.4 at 25°C) is decreased in the presence of 10 μM-diethylstilboestrol to 38% of the control rate. In the presence also of 100 μM-NAD^+ the corresponding value is 78%. Likewise, Duncan (1977) reported that inhibition of the esterase activity of the rabbit liver enzyme by diethylstilboestrol was observed at all concentrations of 4-nitrophenyl acetate. 2,2′-Dithiodipyridine modification of the sheep enzyme also results in a decrease in esterase activity (Kitson, 1982).

In their early study of the steroid-sensitive rabbit liver enzyme, Maxwell & Topper (1961) investigated the effect of diethylstilboestrol as a function of temperature and of pH. This has been repeated during the present work with the sheep liver cytoplasmic enzyme. The activity of aldehyde dehydrogenase in the standard assay increases dramatically with temperature (Fig. 4*a*); between 25°C and 35°C the

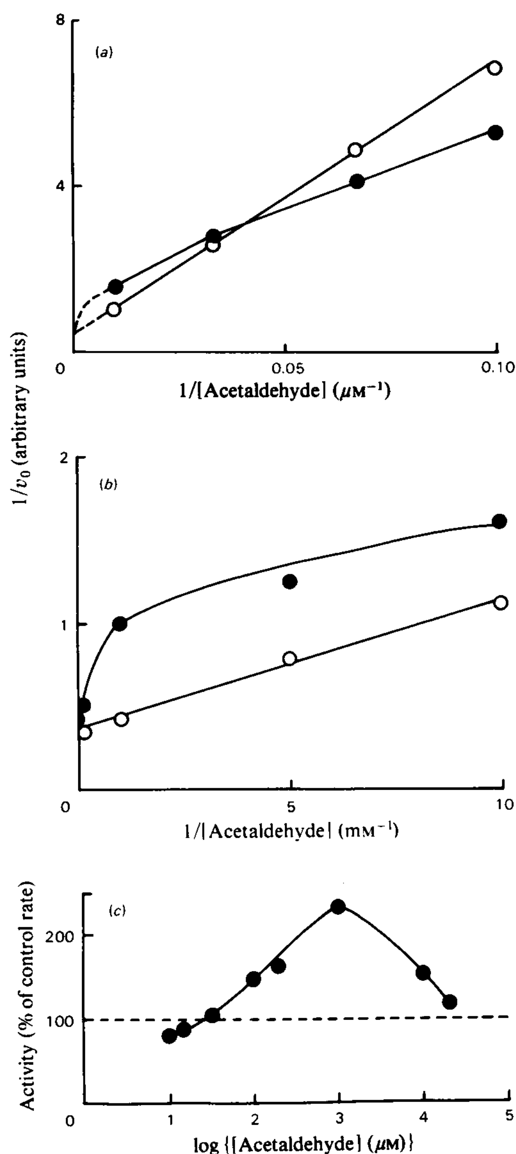

Fig. 2. *Lineweaver–Burk plot showing the effect of diethylstilboestrol on sheep liver cytoplasmic aldehyde dehydrogenase as a function of acetaldehyde concentration*

The activity of the enzyme (0.27 μM) was measured at 25°C and pH 7.4 with 1 mM-NAD$^+$. In (*a*) the results for native enzyme (●) and for enzyme in the presence of 10 μM-diethylstilboestrol (○) are shown for a wide range of acetaldehyde concentration. In (*b*) a scale expansion is given of that part of the diagram in (*a*) shown as broken lines. In (*c*) the activity of the enzyme in the presence of diethylstilboestrol (expressed as a percentage of the control rate) is plotted against the logarithm of the acetaldehyde concentration (μM). A very similar result was obtained with enzyme modified by 2,2′-dithiodipyridine.

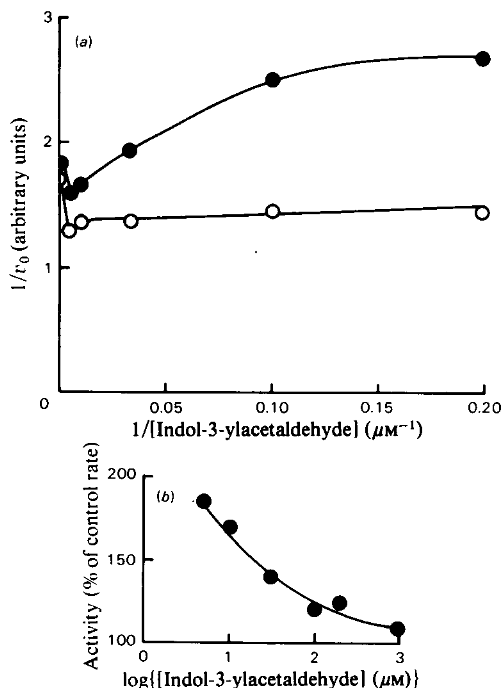

Fig. 3. *Lineweaver–Burk plot showing the effect of diethylstilboestrol on sheep liver cytoplasmic aldehyde dehydrogenase as a function of indol-3-ylacetaldehyde concentration*

The activity of the enzyme (0.27 μM) was measured at 25°C at pH 7.4 with 1 mM-NAD$^+$, either in the absence (●) or the presence (○) of 10 μM-diethylstilboestrol. In (*b*) the activity of the enzyme in the presence of diethylstilboestrol (expressed as a percentage of the control rate) is plotted against the logarithm of the indol-3-ylacetaldehyde concentration (μM).

activity increases by a factor of 4.7-fold. [Maxwell & Topper (1961) quote a doubling of the rate with every 5°C increment in temperature.] At first glance the corresponding temperature-dependence in the presence of diethylstilboestrol is not very different (Fig. 4*a*), but Fig. 4(*b*) accentuates that there is a marked change with temperature of the diethylstilboestrol-stimulated rate relative to the control activity. [Similarly, Maxwell & Topper (1961) found the activation to be maximal at 20°C and insignificant at 40–45°C.] The effect of diethylstilboestrol at 36°C was ascertained with a range of acetaldehyde concentrations. At 10 mM, 1 mM, 100 μM and 50 μM the rates were 121%, 128%, 73% and 75% respectively of the control rate. Again the trend is paralleled by 2,2′-dithiodipyridine; in an experiment at pH 7.4 the enzyme was modified (in the presence of NAD$^+$) by 2,2′-dithiodipyridine at 24°C and

then assayed (with 1 mM-acetaldehyde) at 24 °C and at 35 °C. At the lower temperature it exhibited 208% of the control activity, but at 35 °C it was only 117% as active as the corresponding control.

The results in Table 3 show how the enzyme is affected by diethylstilboestrol at a high pH (9.75) compared with the usual pH of 7.4. It is clear that there is much less stimulation of activity at the higher pH. With the rabbit liver enzyme, diethylstilboestrol still activates strongly at pH 9.5, but much less so at pH 10 (Maxwell & Topper, 1961). The activity of the enzyme modified (at pH 7.4) by 2,2′-dithiodipyridine relative to the control is constant over the pH range 6.4–8.15 (approx. 200%), but then there is a slight trend towards less activation (165%) as the pH rises to 9.56.

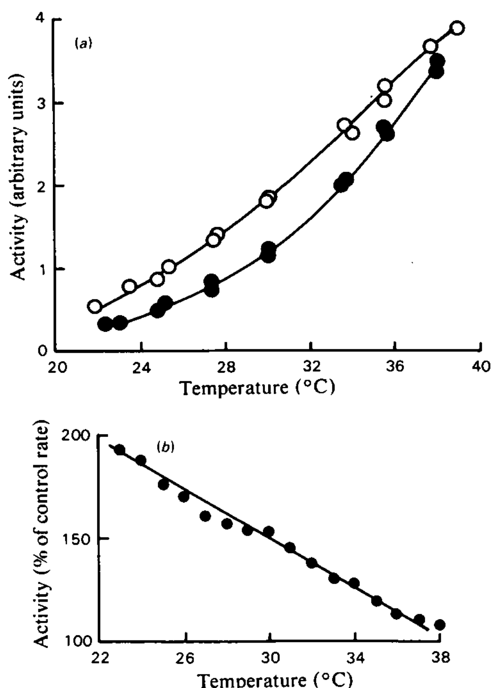

Fig. 4. *Effect of temperature on the activation of sheep liver cytoplasmic aldehyde dehydrogenase by diethylstilboestrol*

The enzyme (0.43 μM) was assayed at pH 7.4 with 1 mM-acetaldehyde and 1 mM-NAD$^+$ in the absence (●) or in the presence (○) of 10 μM-diethylstilboestrol over a range of temperature. From the curves drawn through the points in (*a*), the activation at each temperature was calculated (as a percentage of the control rate) and is plotted against temperature in (*b*).

The amplitude of the burst of NADH production catalysed by the 2,2′-dithiodipyridine-activated enzyme on adding 1 mM-acetaldehyde was measured. With the technique described before (Kitson, 1982) the burst size was found to be 27% of the control with native enzyme (average of eight determinations; range 13–48%). This result was checked by a stopped-flow experiment; Fig. 5(*a*) is re-drawn from the chart recording obtained. The burst size for the modified enzyme was 37% of the control, whereas the steady-state rate was 170%. This decrease in NADH burst size could not be interpreted without first comparing the enhancement of NADH fluorescence provided by the modified and native enzymes. The results of such NADH titrations are shown in Fig. 5(*b*). [After activation the enzyme was passed through Bio-Gel P-6 to remove 2-thiopyridone and NAD$^+$ in case these interfered with the NADH titration. The gel filtration and NADH titration were performed rapidly before the time-dependent loss of activity observed previously (Kitson, 1982) had become significant.] From Fig. 5(*b*) the apparent maximal enhancement of NADH fluorescence by the modified enzyme is 38% of the control value with native enzyme. Obviously the decrease in amplitude of the NADH burst exhibited by 2,2′-dithiodipyridine-activated enzyme is merely a factor of the corresponding decrease in enhancement of NADH fluorescence.

A few experiments with human liver aldehyde dehydrogenase were performed to see how it compares with the sheep liver cytoplasmic enzyme with respect to diethylstilboestrol-sensitivity. The results in Table 4 show there is a close resemblance; diethylstilboestrol activates the human enzyme more at 25 °C than at 37 °C, more with 1 mM-acetaldehyde than with lower concentrations, and more when the

Table 3. *Effect of diethylstilboestrol on sheep liver cytoplasmic aldehyde dehydrogenase at high pH*

The enzyme was assayed at 25 °C with NAD$^+$ (1 mM) and acetaldehyde (1 mM) in the presence and in the absence of diethylstilboestrol (10 μM) in the buffers indicated. At least two results were averaged to give each value shown.

Buffer	Control rate (arbitrary units)	Rate with diethylstilboestrol (arbitrary units)	Rate with diethylstilboestrol (% of control rate)
33 mM-Sodium phosphate, pH 7.4	42.8	77.9	182
33 mM-Glycine/NaOH, pH 9.75	19.2	21.7	113
33 mM-Glycine/NaOH plus 33 mM-Na_2HPO_4, pH 9.75	59.5	69.0	116

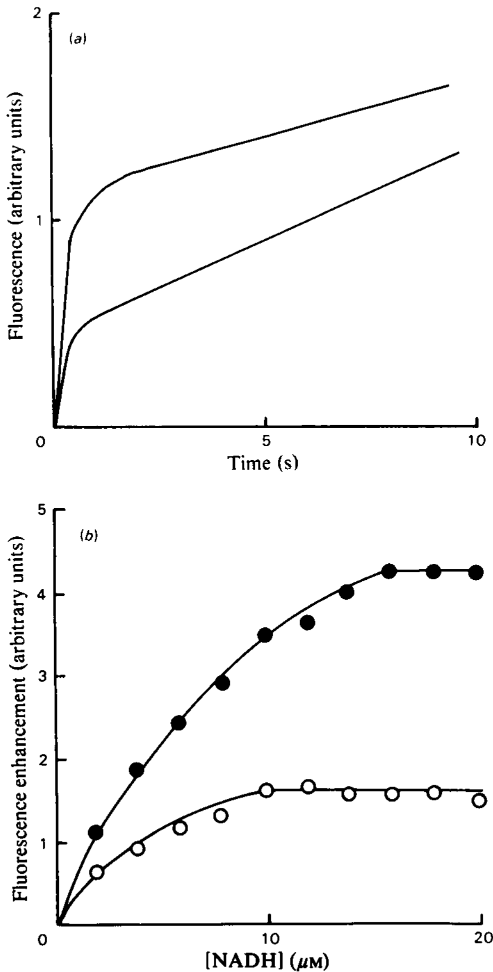

Fig. 5. *Burst of NADH production in the reactions catalysed by native and 2,2'-dithiodipyridine-modified sheep liver cytoplasmic aldehyde dehydrogenase*

In (*a*) the upper curve is a re-drawing of the chart-recording obtained in a stopped-flow experiment when enzyme and NAD^+ were rapidly mixed with NAD^+ and acetaldehyde. Both syringes contained 44mM-sodium phosphate buffer, pH 7.0. After mixing the concentrations were enzyme (1 μM), NAD^+ (0.4mM) and acetaldehyde (1mM). The lower curve is the trace obtained under identical conditions with enzyme that had been pre-modified (in the presence of 0.4mM-NAD^+) with 2,2'-dithiodipyridine (10μM). (*b*) shows the results of fluorescence titrations between NADH and native and 2,2'-dithiodipyridine-modified enzyme. The enzyme (70μM) was treated with the modifier (200μM) in the presence of NAD^+ (1mM) and then rapidly separated from NAD^+, 2-thiopyridone etc. by gel filtration through Bio-Gel P-6. The binding of NADH to the modified enzyme was then examined by fluorescence titration as described previously (Dickinson *et al.*, 1981). The enzyme concentration in the titration was 2.45μM. The results are shown as ○. Unmodified enzyme subjected to the same procedure gave the results shown as ●. (All points have had subtracted the corresponding fluorescence observed when NADH was added to buffer in the absence of enzyme.)

ratio of diethylstilboestrol to enzyme concentration is higher, these results all being similar to those found with the sheep enzyme. The human enzyme samples used in the present study lost 82–83% of their activity when treated with 10μM-disulfiram and so are substantially the same as the previously characterized E1 isoenzyme (Greenfield & Pietruszko, 1977). Pure E1 isoenzyme is inactivated approx. 90% by disulfiram according to Hempel *et al.* (1980).

Discussion

Effect of diethylstilboestrol on aldehyde dehydrogenases from various species

A misapprehension has appeared in the recent literature that only an aldehyde dehydrogenase from rabbit liver is sensitive to steroids. As mentioned in the introduction, statements to this effect (Pietruszko, 1980; Vallari & Pietruszko, 1981) ignore at least two reports of steroid-sensitivity of enzymes from other species (sheep and rat). Duncan (1977) notes that a number of aldehyde dehydrogenases have been obtained in a highly purified state (Feldman & Weiner, 1972; Takio *et al.*, 1974; Sidhu & Blair, 1975*a*,*b*) and observes that they have not been reported to be affected by steroids; however, it would be equally true to say that they have not been reported to be unaffected either. The only firm statements that I can find in the original literature denying an effect of diethylstilboestrol are those by Kraemer & Deitrich (1968) and Blair & Bodley (1969) dealing with preparations of human liver aldehyde dehydrogenase. The former workers found that 100μM-diethylstilboestrol did not affect their enzyme under conditions that were not unequivocally stated but that were probably pH 9.8 and 25°C, with 1mM-NAD^+ and 0.3mM-propanal. From the results reported in the present paper it is obvious that a range of different variables must be investigated before an effect of diethylstilboestrol can be ruled out. Moreover, the enzyme studied by Kraemer & Deitrich (1978) has been equated with the E2 isoenzyme described by Pietruszko *et al.*, (1977), which is mitochondrial in origin, and (for sheep liver) the mitochondrial form is less steroid-sensitive than is the cytoplasmic form (Crow *et al.*, 1974). Blair & Bodley (1969) found that diethylstilboestrol and a number of steroids had no effect

Table 4. *Effect of diethylstilboestrol on the activity of human liver aldehyde dehydrogenase*
The enzyme was assayed with and without diethylstilboestrol (10 μM) at pH 7.4 with 1 mM-NAD$^+$ and the concentration of acetaldehyde shown at the temperature indicated. Expts. A and B refer to preparations of enzyme from two different human livers; in Expt. A the enzyme concentration was approx. 0.05 μM and in Expt. B it was 0.19 μM. Results are the average of two or three separate determinations.

Expt.	Temperature (°C)	Concn. of acetaldehyde (mM)	Rate in the presence of diethylstilboestrol (% of control)
A	25	1	339
	25	0.1	198
	25	0.01	98
B	25	1	199
	33	1	127
	37	1	114

when the enzyme was assayed at 22°C at pH 9.6 with 8.3 mM-acetaldehyde. Again, a different picture may have emerged if a range of pH and aldehyde concentration had been used. The enzyme studied by Blair & Bodley (1969) was inhibited to an extent of only 35% by 40 μM-disulfiram, so that it cannot be the same as the cytoplasmic E1 isoenzyme (Greenfield & Pietruszko, 1977; Pietruszko *et al.*, 1977), which (like the cytoplasmic sheep liver enzyme) is very sensitive to inactivation by low concentrations of disulfiram. On the other hand, the human enzyme used in the present study is both disulfiram- and diethylstilboestrol-sensitive (Table 4). Thus steroid-sensitivity, rather than being a peculiarity of the rabbit enzyme, is a common property of cytoplasmic aldehyde dehydrogenases from rabbit, sheep, human and rat, and presumably all other mammals. There is no longer any justification for referring to 'the steroid-sensitive rabbit liver aldehyde dehydrogenase' (Duncan, 1977) as though it were especially unusual; it is clearly just the normal cytoplasmic form. In fact, the results reported in the present paper show that cytoplasmic aldehyde dehydrogenases from sheep and rabbit liver are really very similar indeed with respect to the effect of diethylstilboestrol and how this varies with temperature, pH, concentration of aldehyde etc.

Comparison of the effects of diethylstilboestrol and 2,2′-dithiodipyridine

There are some differences in the activation of aldehyde dehydrogenase produced by diethylstilboestrol and 2,2′-dithiodipyridine (e.g. in the results found with different substrates: Table 2). The most marked difference is in the susceptibility of the activated enzyme to disulfiram. Either the enzyme–diethylstilboestrol complex rapidly reacts with disulfiram, or disulfiram reacts with the small amount of free enzyme that is presumably present and rapidly displaces the equilibrium. On the other hand, 2,2′-dithiodipyridine-activated enzyme is relatively insensitive to disulfiram (Kitson, 1982), and this is taken as possibly meaning that 2,2′-dithiodipyridine and disulfiram react with the same enzymic thiol groups, in which case they could not be catalytically essential.

Apart from these differences, the effects of diethylstilboestrol and 2,2′-dithiodipyridine are very similar. This applies to the variations seen with temperature, pH and, particularly strikingly, with acetaldehyde concentration (Fig. 2). The effects of the two modifiers are not additive, i.e. enzyme activated by one of them is not further activated by the other (Table 1). Duncan (1977) concluded that the binding of diethylstilboestrol produces a new form of the enzyme with modified kinetic properties; the present results suggest that reaction with 2,2′-dithiodipyridine also produces the same new form of the enzyme.

The molecular basis of the activation of aldehyde dehydrogenase may be a conformational change or may be a change in the aggregation of the enzyme subunits, which normally form a tetramer (MacGibbon *et al.*, 1979). [Weiner's group has shown that the mitochondrial aldehyde dehydrogenase from horse liver is activated approximately 2-fold by Mg^{2+} ions by way of a dissociation of the tetramer (with only two functional active sites) to dimers (each with two functional active sites) (Takahashi & Weiner, 1980; Takahashi *et al.*, 1980).] Workers with rabbit liver aldehyde dehydrogenase have reported that progesterone and diethylstilboestrol probably do not cause dissociation of the enzyme; this was concluded on the basis of no change in electrophoretic mobility (Duncan, 1977) or sedimentation rate (Maxwell, 1962). However, a serious difficulty raised by Maxwell (1962) is that the difference in protein concentrations required for kinetic and ultracentrifugation experiments renders it impossible to rule out dissociation of the enzyme by steroids under conditions used for the

kinetic studies. Fig. 1 of the present work shows that a substantial excess of diethylstilboestrol over enzyme concentration is necessary for a significant degree of activation, and it may be of little use to try and examine the extent of aggregation under conditions (of pH, temperature and concentrations of enzyme, diethylstilboestrol and aldehyde) that are known not to give rise to the maximal effect on the rate of the enzyme-catalysed reaction. During the present work two observations were made that have an indirect bearing on the question of enzyme dissociation. First, no change in amplitude of the burst of NADH production in the reaction catalysed by the 2,2′-dithiodipyridine-modified enzyme was observed (when correction was made for the change in enhancement of NADH fluorescence; see Fig. 5). When the horse liver mitochondrial enzyme dissociates in the presence of Mg^{2+} ions a 2-fold increase in burst size is seen (Takahashi & Weiner, 1980). Secondly, an increase in the number of functioning active sites might mean that a given amount of disulfiram would inactivate a smaller percentage of the initial activity; disulfiram titrations with and without diethylstilboestrol showed that this was not the case. Thus neither of these observations supports the idea that the activators cause dissociation of the enzyme, but of course they do not disprove it.

Significance of steroid-sensitivity

Is there any physiological significance to the action of steroids on aldehyde dehydrogenase? The main factor that argues against this is that concentrations of steroids *in vivo* are unlikely to be high enough to have any effect on the enzyme. Furthermore, as we have seen, the effect of diethylstilboesterol at least is small at 37°C and at low (physiologically reasonable) concentrations of acetaldehyde. On the other hand it is intriguing that naturally occurring steroidal aldehydes are substrates of the enzyme. For example, an enzyme isolated from horse liver that catalyses the oxidation of 3α,7α,12α-trihydroxy-5β-cholestan-26-al is probably identical with one of the normal aldehyde dehydrogenases (Okuda *et al.*, 1973). Steroids with the 20β-hydroxy-21-oxo side chain ('isocorticosteroids') are also substrates of horse liver aldehyde dehydrogenase (Martin & Monder, 1978). The latter compounds are very similar in structure to compounds such as progesterone and deoxycorticosterone, which inhibit rabbit liver aldehyde dehydrogenase (Maxwell & Topper, 1961). If the aldehyde-binding site of aldehyde dehydrogenase is 'designed' to accommodate the steroid side chain of the isocorticosteroid, then compounds such as deoxycorticosterone and progesterone might compete for this site and inhibit the reaction, though this would not explain the activation observed with some other steroids and diethylstilboestrol.

[Instead of, or as well as, acting directly on aldehyde dehydrogenase as they do *in vitro*, steroid hormones may affect the activity of the enzyme *in vivo* by a process of induction. Thus a cytosolic aldehyde dehydrogenase of mouse liver is induced to a 2-fold extent in the pregnant animal (Petersen *et al.*, 1977), and castration of male rats also results in induction of hepatic cytosolic aldehyde dehydrogenase (Messiha *et al.*, 1980).]

In conclusion, the demonstration that steroid-sensitivity is a common property of several cytoplasmic aldehyde dehydrogenases (and not unique to the rabbit) means that this factor certainly warrants further study, particularly towards elucidating whether it has physiological significance in ethanol metabolism and alcoholism.

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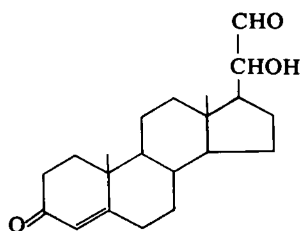
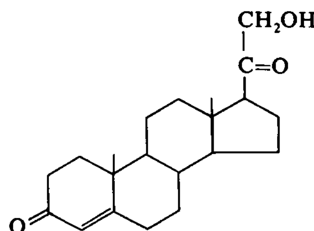


An isocorticosteroid



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