The Action of Chelating Agents on Human Liver Aldehyde Dehydrogenase

By RAMESHWAR S. SIDHU and A. HUNTLY BLAIR

Department of Biochemistry, Dalhousie University, Halifax, N.S. B3H 4H7, Canada

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Human liver aldehyde dehydrogenase was inhibited by aromatic chelating agents. However, structurally related compounds with much lower metal-complexing ability displayed affinities for enzyme essentially equal to those of their respective chelating analogues. Inhibition was competitive with respect to the coenzyme. It is suggested that hydrophobic interactions between the inhibitors and the coenzyme-binding site of the enzyme are responsible for the observed effects on activity.

Inhibition of enzymes by chelating agents can result from co-ordination with a metal atom specifically bound to the active protein or required for formation of an enzyme-substrate complex. Alcohol dehydrogenases from horse liver (Vallee, 1960; Drum *et al.*, 1969), rat liver (Arslanian *et al.*, 1971) and yeast (Vallee & Hoch, 1955), which are sensitive to compounds such as 1,10-phenanthroline and 2,2'-dipyridyl, contain tightly bound essential zinc. Spectral and X-ray-crystallographic studies of horse liver alcohol dehydrogenase have indicated that 1,10-phenanthroline acts by complex-formation at zinc sites (Vallee & Coombs, 1959; Drum & Vallee, 1970; Spallholz & Piette, 1972; Brändén *et al.*, 1973). Early studies of the effect of 1,10-phenanthroline and other inhibitors on partially purified preparations of bovine liver and yeast aldehyde dehydrogenases were interpreted to indicate that both proteins are zinc metallo-enzymes (Schwarcz & Stoppani, 1960; Stoppani *et al.*, 1966). Yeast aldehyde dehydrogenase was later purified to homogeneity (Steinman & Jakoby, 1967), but analytical data for zinc have not been reported. The present paper deals with the manner in which human liver aldehyde dehydrogenase is affected by a selected group of compounds with a wide range of metal-complexing behaviour.

Materials and methods

Chemicals. Acetaldehyde was purchased from Matheson, Coleman and Bell (East Rutherford, N.J., U.S.A.). β -NAD⁺ and benzoic acid were obtained from Sigma Chemical Co. (St. Louis, Mo., U.S.A.). 2,9-Dimethyl-1,10-phenanthroline, 2,2'-dipyridyl and quinoline were purchased from Eastman Organic Chemicals (Rochester, N.Y., U.S.A.), 4,4'-dipyridyl hydrochloride was from K & K Laboratories (Plainview, N.Y., U.S.A.) and 1,10-phenanthroline was from Fisher Scientific Co. (Fair Lawn, N.J., U.S.A.). Inhibitor stock solutions, adjusted to an appropriate pH, were prepared just before use.

Purification of aldehyde dehydrogenase. On the basis of the procedures of Blair & Bodley (1969), the enzyme was purified from normal liver obtained at autopsy and stored at -20°C before use. Initial extraction and fractionation with $(\text{NH}_4)_2\text{SO}_4$ were carried out with 1.0mM-EDTA added to the extraction medium that was described previously (Blair & Bodley, 1969). The dialysed 40–55% saturation- $(\text{NH}_4)_2\text{SO}_4$ fraction was chromatographed directly on DEAE-cellulose, as described (Blair & Bodley, 1969), with the addition of 1.0mM-EDTA and 1.0mM-GSH to all buffers. Eluted fractions corresponding to the single activity peak were pooled, concentrated by $(\text{NH}_4)_2\text{SO}_4$ precipitation and stored at -20°C (specific activity 0.35–0.45 unit/mg).

Enzyme activity. Dehydrogenase activity was determined spectrophotometrically at 22°C by measuring the formation of NADH at 340 nm as described previously (Blair & Bodley, 1969). One unit of activity is defined as that amount of enzyme catalysing the formation of $1\text{ }\mu\text{mol}$ of NADH/min under the specified conditions (Blair & Bodley, 1969). Reaction mixtures for inhibition studies are specified in Fig. 1 legend.

Data processing. Initial-velocity data were fitted by a least-squares method to rate equation (1) for linear competitive inhibition (Cleland, 1963). The calculations were done by a CDC 6400 Computer using the FORTRAN program described by Cleland (1963).

$$v = \frac{VA}{K_m^A(1 + I/K_i) + A} \quad (1)$$

A is the substrate concentration; I is the inhibitor concentration; K_m^A is the Michaelis constant associated with the substrate A ; K_i is the inhibition constant associated with the inhibitor I ; v and V are the initial velocity and maximal velocity respectively.

Results and discussion

Human liver aldehyde dehydrogenase, in common with other oxidoreductases, was found to be inhibited

Fig. 1. Double-reciprocal plot with NAD^+ as the variable substrate and 1,10-phenanthroline as the inhibitor

Reaction mixtures contained 33mM-sodium pyrophosphate, pH9.5, 0.045 unit of enzyme, 2.0mM-acetaldehyde and NAD^+ as indicated; 1,10-phenanthroline was absent (●) or present at (0.25mM) (○) or 0.5mM (Δ). The total volume was 1 ml. The lines are drawn from the calculated fit to eqn. (1).

Table 1. Inhibition of human liver aldehyde dehydrogenase by metal-chelating agents and related compounds

Reaction mixtures and experimental conditions were as indicated in Fig. 1, except for the specified inhibitor. Inhibition constants (K_i) were calculated from conventional double-reciprocal plots as described in the text.

Inhibitor	K_i (mM)
1,10-Phenanthroline	0.13 ± 0.01
2,9-Dimethyl-1,10-phenanthroline	0.14 ± 0.02
2,2'-Dipyridyl	1.0 ± 0.1
4,4'-Dipyridyl	1.3 ± 0.2
Quinoline	0.23 ± 0.02
Benzoic acid	2.3 ± 0.2

by certain chelating agents. 1,10-Phenanthroline, which forms strong complexes with transition metals such as zinc, gave competitive inhibition with respect to NAD^+ , as shown in Fig. 1. Accordingly, selected analogues, with various structures and metal-complexing properties, were investigated to assess the contribution of chelation to the inhibitory effects. Activity measurements were initiated immediately on addition of the enzyme to the other components of the reaction systems, i.e. inhibitor, substrate and

buffer. Under these conditions the compounds listed in Table 1 all gave linear inhibition, competitive with respect to NAD^+ . Sodium azide, ethylenediamine and EDTA inhibited only slightly or not at all at concentrations up to 50mM. Previous work showed that diethyldithiocarbamate and diethylamine interact only weakly at relatively high concentrations (10mM) (Blair & Bodley, 1969). Time-dependent effects arising from prolonged exposure of the enzyme to an inhibitor were not investigated.

Slope inhibition constants (K_i) for the inhibitors in Table 1 were not correlated with ability to form metal chelate complexes. Since there is steric hindrance in 2,9-dimethyl-1,10-phenanthroline, its 1:1 zinc complex is 1000-fold less stable than that formed with 1,10-phenanthroline (Martell & Sillén, 1964). This suggests that inhibition of dehydrogenase activity by this analogue should be weaker than by 1,10-phenanthroline, if inhibition depends on chelation. Closely similar values were obtained experimentally, as shown in Table 1. Analogous reasoning may be applied in the case of 2,2'-dipyridyl and its analogue 4,4'-dipyridyl, which does not chelate zinc because the potential donor ring nitrogen atoms are too far apart to form a bidentate complex. The inhibition constant for 4,4'-dipyridyl should be much higher than that for 2,2'-dipyridyl if chelation of zinc or another transition metal causes inhibition of the dehydrogenase reaction. However, closely similar K_i values were obtained for the two dipyridyls (Table 1). Quinoline and benzoic acid are also unable to form stable complexes with zinc, but inhibited enzymic activity. Moreover, the inhibition constant for benzoic acid did not differ greatly from that for the dipyridyls (Table 1).

The finding of similar inhibition constants for agents differing widely in their ability to form zinc complexes fails to provide experimental support for chelation of essential metal in this enzyme as the underlying basis of inhibition. In accord with the conclusion drawn from inhibition studies, analysis of human aldehyde dehydrogenase by atomic absorption spectrophotometry did not show significant amounts of zinc. Also, added ZnCl_2 did not increase activity at concentrations up to 1.0mM, above which it became inhibitory.

The competitive nature of inhibition by these compounds suggests that the NAD^+ -binding site has an affinity for aromatic ring structures in general, based on hydrophobic interactions. Such a hydrophobic area in the region of the NAD^+ -binding site has been postulated to explain inhibition of yeast alcohol dehydrogenase (Anderson *et al.*, 1966) by nitrogen bases.

In a previous study of yeast aldehyde dehydrogenase, the effectiveness of inhibitors decreased in order of decreasing stability of their zinc complexes (Schwarcz & Stoppani, 1960; Stoppani *et al.*, 1966).

This correlation may be misleading, as shown by the present investigation. Other properties of the inhibitors, governing interaction with the enzyme protein, may vary in a manner parallel to chelating ability. For example, it was noted that compounds in which aromatic rings are attached to a common C-C bond and can rotate (e.g. dipyridyls) were 5-10-fold less inhibitory towards the human enzyme than those that have fused aromatic ring structures (e.g. quinoline and the phenanthrolines).

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