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# Bovine lens aldehyde dehydrogenase

## Kinetics and mechanism

Hong-Hoi TING* and M. James C. CRABBE†
*Nuffield Laboratory of Ophthalmology, University of Oxford, Walton Street, Oxford OX2 6AW, U.K.*

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Bovine lens cytoplasmic aldehyde dehydrogenase exhibits Michaelis–Menten kinetics with acetaldehyde, glyceraldehyde 3-phosphate, *p*-nitrobenzaldehyde, propionaldehyde, glycolaldehyde, glyceraldehyde, phenylacetylaldehyde and succinic semialdehyde as substrates. The enzyme was also active with malondialdehyde, and exhibited an esterase activity. Steady-state kinetic analyses show that the enzyme exhibits a compulsory-ordered ternary-complex mechanism with $NAD^+$ binding before acetaldehyde. The enzyme was inhibited by disulfiram and by *p*-chloromercuribenzoate, and studies with mercaptans indicated the involvement of thiol groups in catalysis.

Bovine lens aldehyde dehydrogenase catalyses the irreversible oxidation of a variety of aldehydes to their corresponding acids, with $NAD^+$ as the specific cofactor. The purification of the enzyme and the characterization of some of its physical properties have been described in the preceding paper (Ting & Crabbe, 1983).

Detailed initial-steady-state-velocity studies as a function of acetaldehyde and $NAD^+$ concentrations have been carried out on cytoplasmic aldehyde dehydrogenase from human liver (Vallari & Pietruszko, 1981), horse liver (Eckfeldt & Yonetani, 1976) and yeast (Clark & Jakoby, 1970). Similar studies with glyceraldehyde as substrate were also carried out on the enzyme from human liver (Sidhu & Blair, 1975). All these results showed intersection patterns of straight lines in double-reciprocal space, indicative of a sequential mechanism with the formation of a ternary complex.

The study of the steady-state kinetics of both human and horse liver cytoplasmic enzymes in the presence of dead-end inhibitors (substrate analogues) and by product inhibition indicated that they essentially follow an ordered pathway with the initial formation of an E·$NAD^+$ binary complex (Eckfeldt & Yonetani, 1976; Vallari & Pietruszko, 1981), but the human enzyme also shows a minor degree of 'randomness', i.e. both E·$NAD^+$ and E·aldehyde binary complexes were formed before the ternary complex, at higher acetaldehyde concentrations (Vallari & Pietruszko, 1981). However, in similar studies of the human liver enzyme with glyceraldehyde as substrate the mechanism has been shown to be essentially following a random-equilibrium pathway (Sidhu & Blair, 1975).

Using competitive substrates, Bradbury & Jakoby (1971) showed that the yeast enzyme's reaction mechanism was ordered, but aldehyde, rather than nucleotide, was the first substrate to bind.

Weiner (1979) has proposed that the carbonyl carbon atom of the aldehyde is attacked by the enzyme nucleophile, which is believed to be an active thiol group, to form a thiohemiacetal. On removal of the hydride to $NAD^+$, a thioester results, which then undergoes hydrolysis to form the acid product.

NADH release from the enzyme was found to be the rate-limiting step in human, horse and sheep liver cytoplasmic enzymes (Vallari & Pietruszko, 1981; Eckfeldt & Yonetani, 1976; MacGibbon *et al.*, 1977) by transient kinetic analysis with stopped-flow apparatus.

In the present paper we report the results of steady-state kinetic studies on the bovine lens aldehyde dehydrogenase, which, although a dimer, shows many kinetic similarities to other, tetrameric, aldehyde dehydrogenases.

## Materials and methods

### *Materials*

Nucleotides and aldehyde substrates were prepared as described in the preceding paper (Ting & Crabbe, 1983). Chloral hydrate was obtained from BDH Chemicals (Poole, Dorset, U.K.) and recrystallized from chloroform at below 0°C. The uncorrected melting point was 59–60°C, and the i.r. spectrum was identical with that of pure chloral hydrate (Pouchert, 1975). Disulfiram (tetraethyl-

* Present address: Dyson Perrins Laboratory, University of Oxford, South Parks Road, Oxford OX1 3QY, U.K.
† To whom correspondence should be addressed.

thiuram disulphide) was purchased from Sigma Chemical Co. (Poole, Dorset, U.K.) and recrystallized from ethanol/water before use. Chlorpropamide [1-(*p*-chlorobenzenesulphonyl)-3-propylurea] (Pfizer, Sandwich, Kent, U.K.) was obtained from the Pharmacy of the Radcliffe Infirmary, Oxford, in tablet form. Ophthalmic acid was kindly given by Dr. Stephen Waley, William Dunn School of Pathology, University of Oxford.

All other substrates and inhibitors were the highest grade obtained from Sigma Chemical Co. and used without further purification.

*Enzyme assay*

Aldehyde dehydrogenase activity was determined spectrophotometrically by monitoring NADH production at 340nm in 0.05M-sodium pyrophosphate buffer, pH8.4, at 37°C with a Perkin-Elmer 555 double-beam spectrophotometer. A molar absorption coefficient of $6.22 \times 10^3 M^{-1} \cdot cm^{-1}$ was used for NADH. Each assay was run against a blank containing all constituents except substrate. All assays contributing data to one experimental set were performed on the same day. Reaction progress curves were linear for at least 5min.

Esterase activity was determined by monitoring the conversion of *p*-nitrophenyl acetate into *p*-nitrophenol spectrophotometrically at 400nm in 0.05M-sodium pyrophosphate buffer, pH8.4, or 0.05M-sodium phosphate buffer, pH7.4; 5% (v/v) acetone was present in all assays. A molar absorption coefficient of $9.8 \times 10^3 M^{-1} \cdot cm^{-1}$ was used for *p*-nitrophenol at pH7.4 (Vallari & Pietruszko, 1981) and $18.3 \times 10^3 M^{-1} \cdot cm^{-1}$ for the *p*-nitrophenolate anion at pH 8.4. Each assay was run against a blank containing the same constituents as the test but without enzyme.

Stock solutions of pure $NAD^+$ (100%; Boehringer, Lewes, East Sussex, U.K.) were assayed chemically by the KCN method (Pinder *et al.*, 1971). The concentration of NADH (100%; Boehringer) was calculated from its absorbance at 340nm. The acetaldehyde concentration in stock solution was calculated by measuring the enzymic production of $NAD^+$ with yeast alcohol dehydrogenase in the presence of excess NADH at pH7.0. Stock solution concentrations of *p*-nitrophenyl acetate were determined by measuring the increase in absorbance at 400nm after hydrolysis in 0.1M-NaOH by using a molar absorption coefficient of $18.3 \times 10^3 M^{-1} \cdot cm^{-1}$ for the *p*-nitrophenolate anion (Kezdy & Bender, 1962).

One unit of enzyme activity is defined as that amount of enzyme catalysing the formation of 1$\mu$mol of product measured/min.

Time-dependent inhibition was analysed by the procedure described by Crabbe *et al.* (1975). Enzyme was incubated with inhibitor in a final volume of 0.2ml in the cuvette. At appropriate time intervals, substrate and nucleotide were added to give a final volume of 1ml. The final concentrations of all constituents, except inhibitor, were the same for each experiment. Substrate or nucleotide protection effects were studied by pre-mixing the enzyme with substrate or nucleotide for 30s before incubation with inhibitor, and the rest of the constituents for the assay were added after appropriate time intervals.

*Enzyme purification*

This was as described in the preceding paper (Ting & Crabbe, 1983).

*Data analysis*

Data points were fitted to the best line in Eadie–Hofstee or Lineweaver–Burk space by utilizing a least-squares linear-regression computer program. Data were also analysed by using the methods of Crabbe (1982) and Eisenthal & Cornish-Bowden (1974). Statistical analyses were as described previously (Crabbe *et al.*, 1980).

When error bars do not appear on Figures, the standard deviations were within the region described by the plotted points.

*Equilibrium constant*

The equilibrium constant of the reaction was calculated from the reduction potentials of the half-equations:

$$NAD^+ + 2H^+ + 2e^- \rightarrow NADH + H^+ \qquad (E'_0 = -0.32\,V)$$

$$Acetate + 2H^+ + 2e^- \rightarrow acetaldehyde \qquad (E'_0 = -0.6\,V)$$

under standard conditions (1atm, 25°C and with 1mol of reactants) at pH7.0.

## Results and discussion

*Effects of mercaptans and thiol-blocking reagents*

In all these experiments, 2-mercaptoethanol was removed from enzyme solutions by dialysis. Enzyme activity increased in the presence of exogenous mercaptans. Glutathione exhibited a maximum effect at 5mM, whereas 2-mercaptoethanol exhibited further activation as its concentration was increased from 5mM to 20mM. Ophthalmic acid (glutamylbutyrylglycine), a naturally occurring analogue of glutathione (Waley, 1956), did not inhibit the enzyme activity at concentrations up to 2.5mM with or without the presence of mercaptans, assayed at both pH8.4 and pH7.4.

Iodoacetate was ineffective at 0.25mM, but iodoacetamide inhibited 59% of the enzyme activity at 0.15mM. Arsenate did not inhibit at 0.25mM with or without the presence of external mercaptans.

Arsenite, however, inhibited the enzyme by 8% at 0.2 mM, and the inhibition was increased to 50% in the presence of 7.1 mM-2-mercaptoethanol.

Both *p*-chloromercuribenzoate and disulfiram were strong inhibitors of purified bovine lens aldehyde dehydrogenase. However, 17% of the enzyme activity still remained even when a large excess of disulfiram was added to the assay (enzyme concentration in the assay medium 0.0037 $\mu$M, assuming $M_r$ 114000). Fig. 1 shows *p*-chloromercuribenzoate acting as a time-dependent inhibitor of the enzyme. The mixing of a saturating concentration of substrate with the enzyme before incubation had a protective effect, whereas premixing with the co-substrate, $NAD^+$, only decreased the apparent pseudo-first-order deactivation constant, and the semi-logarithmic plot still reached the same asymptote as that when $NAD^+$ was absent from the incubation mixture.

The data were also plotted in accordance with the procedure described by Crabbe *et al.* (1975). An arbitrary line was extended to the *y*-axis when $V_0/(V_0-V_t) = 1$, since, when $[I] \rightarrow \infty$, $V_t \rightarrow 0$ and $V_0/(V_0-V_t) = 1$. This secondary plot obtained was also non-linear.

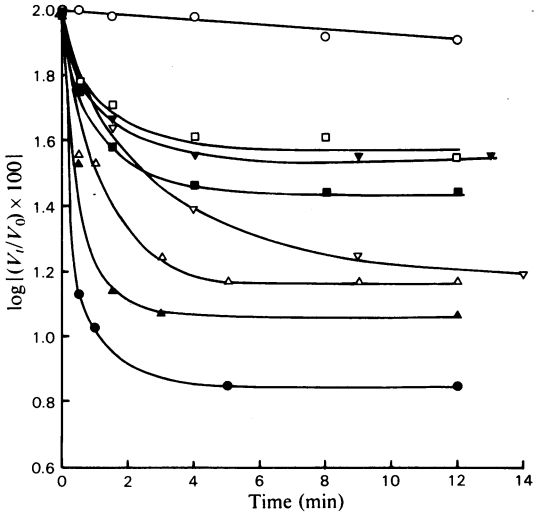



Fig. 1. *Time-dependent inhibition of bovine lens aldehyde dehydrogenase by p-chloromercuribenzoate*

Semi-logarithmic plots of log $(V_t/V_0)$ against time: ○, 0 $\mu$M-*p*-chloromercuribenzoate; □, 2.77 $\mu$M-*p*-chloromercuribenzoate; ■, 3.46 $\mu$M-*p*-chloromercuribenzoate; △, 4.16 $\mu$M-*p*-chloromercuribenzoate; ▲, 4.85 $\mu$M-*p*-chloromercuribenzoate; ●, 5.54 $\mu$M-*p*-chloromercuribenzoate; ▼, premixed with 44 mM-propionaldehyde, 4.16 $\mu$M-*p*-chloromercuribenzoate; ▽, premixed with 0.67 mM-$NAD^+$, 4.16 $\mu$M-*p*-chloromercuribenzoate.

Complete inhibition with *p*-chloromercuribenzoate and disulfiram could not be found even when the inhibitor concentration was much higher than that of the enzyme (400-fold). This suggests that the disulfiram-reactive thiol groups might not be essential for covalent interaction with the aldehyde substrate during catalysis. This phenomenon has also been reported by Kitson (1978) and Dickinson *et al.* (1981) in studies of inhibition of sheep liver cytoplasmic aldehyde dehydrogenase by disulfiram.

The lack of inhibition with iodoacetate is consistent with our observation that sodium acetate did not inhibit the reaction.

### *Steady-state kinetics*

*Substrate specificity.* The purified lens enzyme, like purified aldehyde dehydrogenase from other sources, was non-specific towards its aldehyde substrates. $K_m$ and $V_{max.}$ values for these aldehydes were obtained with the same batch of purified enzyme preparation, under saturating concentration of the nucleotide. The substrate concentration ranges were at least 250-fold, with 10 mM as the highest substrate concentration for acetaldehyde, propionaldehyde, glycolaldehyde, glyceraldehyde and succinic semialdehyde, 2.5 mM for malondialdehyde and *p*-nitrobenzaldehyde (owing to the high intrinsic absorbance of these two substrates), 1.8 mM for glyceraldehyde 3-phosphate, and 0.563 mM for phenylacetaldehyde, owing to its solubility.

Substrate inhibition occurred with malondialdehyde when its concentration exceeded 1 mM in the assay mixture, and deviation from Michaelis–Menten-type kinetics was observed when the results were plotted in Eadie–Hofstee space. Kinetic constants for this substrate were only estimated from the results obtained below 1 mM substrate concentration. The other substrates studied showed no significant deviation from Michaelis–Menten behaviour. $K_m$ and $V_{max.}$ values obtained from non-linear-regression analysis for the substrates are shown in Table 1. The enzyme was inactive when $NADP^+$ was substituted for $NAD^+$.

The purified lens aldehyde dehydrogenase also showed esterase activity with *p*-nitrophenyl acetate. The results obtained at pH 8.4 showed non-linearity when plotted in Eadie–Hofstee space, and substrate inhibition was observed at concentrations above 100 $\mu$M. Both substrate inhibition and non-linearity disappeared at pH 7.4, when $K_m = 4.3\ \mu$M and $k_{cat.} = 0.26\ s^{-1}$.

Preincubation of the enzyme with $NAD^+$, acetaldehyde or propionaldehyde inhibited the hydrolysis of *p*-nitrophenyl acetate, as has been found for the purified rabbit liver enzyme (Duncan, 1977).

*Initial-velocity studies.* Initial-steady-state-velocity studies were performed as a function of acetaldehyde and $NAD^+$ concentrations. Double-

Table 1. *Kinetic constants obtained for various substrates of bovine lens aldehyde dehydrogenase*

| | $K_m$ (mM) | $V_{max.}$ (unit/mg of enzyme) | $k_{cat.}$ ($s^{-1}$) (assuming $M_r$ 114000) | $10^{-3} \times k_{cat.}/K_m$ ($M^{-1} \cdot s^{-1}$) |
|---|---|---|---|---|
| Glyceraldehyde 3-phosphate | 0.372 | 0.147 | 0.28 | 0.751 |
| *p*-Nitrobenzaldehyde | 0.0034 | 0.120 | 0.23 | 67.65 |
| Propionaldehyde | 0.0218 | 0.974 | 1.85 | 84.86 |
| Glycolaldehyde | 0.69 | 0.63 | 1.20 | 1.74 |
| Glyceraldehyde | 0.39 | 0.68 | 1.29 | 3.31 |
| Acetaldehyde | 0.4 | 0.977 | 1.86 | 4.65 |
| Malondialdehyde | 0.13 | 0.628 | 1.19 | 9.15 |
| Phenylacetaldehyde | 0.00352 | 0.730 | 1.39 | 394.89 |
| Succinic semialdehyde | 3.54 | 0.400 | 0.76 | 0.215 |

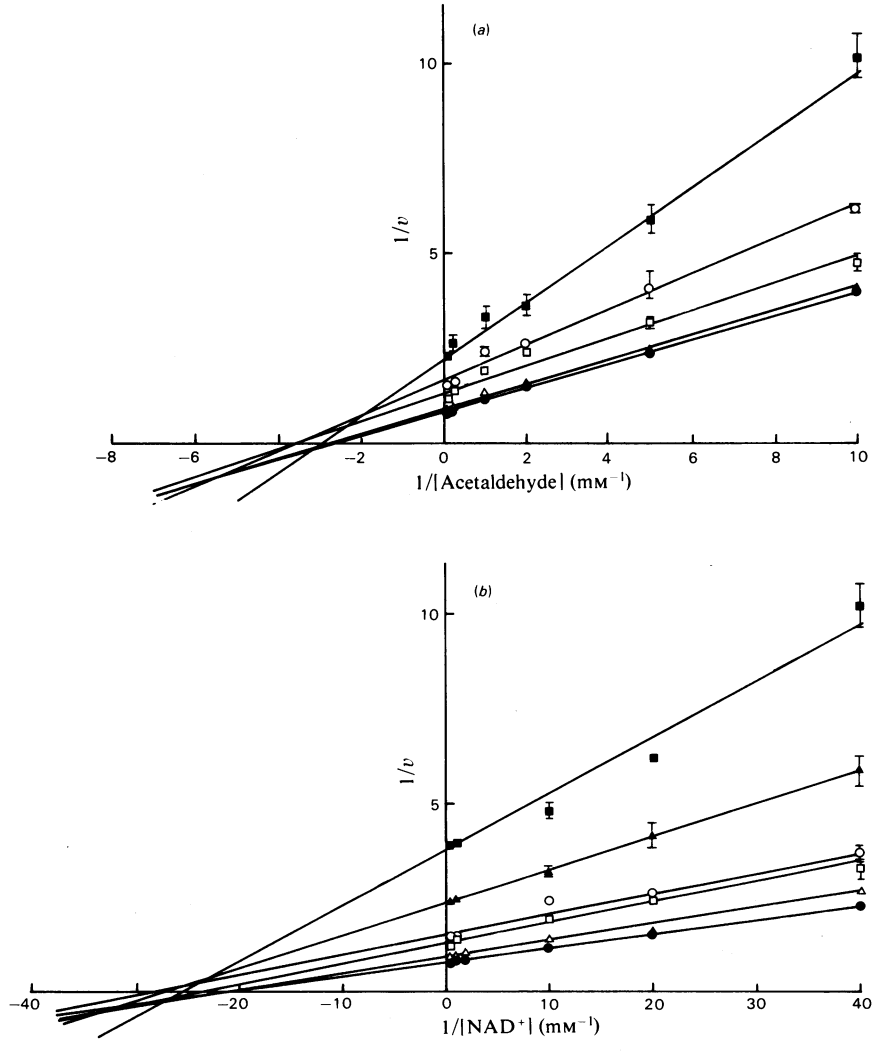



Fig. 2. *Steady-state kinetics of purified bovine lens aldehyde dehydrogenase*
For details see the text. (*a*) Plot of $1/v$ versus 1/[acetaldehyde] for aldehyde dehydrogenase at different [$NAD^+$] values: ●, 2mM; △, 1mM; □, 0.1mM; ○, 0.05mM; ■, 0mM. (*b*) Plot of $1/v$ versus 1/[$NAD^+$] for aldehyde dehydrogenase at different [acetaldehyde] values: ●, 10mM; △, 5mM; □, 1mM; ○, 0.5mM; ▲, 0.2mM; ■, 0.1mM.

reciprocal plots of $1/v$ against 1/[acetaldehyde] at a series of concentrations of $NAD^+$ gave a series of straight lines intersecting near the $-1/$[acetaldehyde] axis (Fig. 2*a*). When the observed experimental data were analysed by a non-linear-regression kinetics program (Crabbe, 1982), all series of data fitted well with the Michaelis-Menten equation.

Similar results were obtained when a double-reciprocal plot of $1/v$ against $1/[NAD^+]$ at a series of concentrations of acetaldehyde was analysed (Fig. 2*b*). These observations can be described by the following equation (Cleland, 1963):

$$v = V_{max.}[A][B]/K_{ia}K_b + K_b[A] + K_a[B] + [A][B]$$

The values of the constants in the equation were calculated from slope and intercept replots as $V_{max.} = 1.206 \pm 0.006$ unit/mg, $K_a = 0.053 \pm 0.006$ mM, $K_b = 0.365 \pm 0.025$ mM and $K_{ia} = 0.028 \pm 0.003$ mM.

*Product-inhibition studies.* The equilibrium constant for the reaction was calculated as $3 \times 10^9$. Sodium acetate did not inhibit the reaction up to a concentration of 250 mM. NADH was a competitive inhibitor with respect to $NAD^+$ when the fixed [acetaldehyde] was either saturating or non-saturating. The $K_{i(slope)}$ values obtained in all cases were similar, with a value of 0.083 mM.

No significant inhibition by NADH was observed (up to 0.25 mM) when its inhibition with respect to acetaldehyde was tested at a fixed concentration of $NAD^+$ at 0.2 mM. At 0.05 mM-$NAD^+$ the inhibition pattern was essentially non-competitive (Fig. 3): $K_{i(slope)} = 0.23 \pm 0.05$ mM and $K_{i(intercept)} = 0.26 \pm 0.12$ mM. The constant $K_{i(slope)}$ for NADH inhibition with respect to $NAD^+$ indicates that the lens enzyme follows an ordered mechanism.

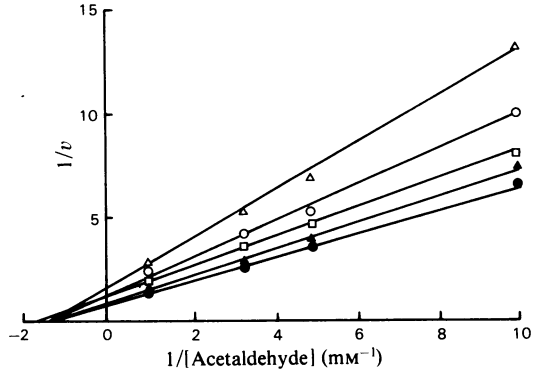



Fig. 3. *NADH product inhibition of bovine lens aldehyde dehydrogenase with respect to acetaldehyde* ($[NAD^+] = 0.05$ mM)

For details see the text. [NADH] values: ●, 0 μM; ▲, 51 μM; □, 101 μM; ○, 152 μM; △, 253 μM.

*Substrate analogues*

*ADP-ribose.* The inhibition of the enzyme by ADP-ribose, with respect to $NAD^+$, at fixed acetaldehyde concentration was competitive (Fig. 4*a*) ($K_{ii} = \infty$). The $K_{i(slope)}$ obtained from the secondary plot of slope versus inhibitor concentration was $0.484 \pm 0.065$ mM, which is also the dissociation constant of ADP-ribose from the E·ADP-ribose complex.

ADP-ribose was essentially non-competitive with respect to acetaldehyde at fixed $NAD^+$ concentration (0.2 mM) (Fig. 4*b*). In this case the values obtained were $K_{is} = 1.313 \pm 0.542$ mM and $K_{ii} = 1.911 \pm 0.951$ mM.

The dissociation constant of ADP-ribose from the E·ADP-ribose complex ($K_I^{ADP\text{-}Rib}$) was calculated from the equations:

$$K_{is} = K_I^{ADP\text{-}Rib}\left(1 + \frac{[A]}{K_{ia}}\right) \quad (1)$$

$$K_{ii} = K_I^{ADP\text{-}Rib}\left(1 + \frac{[A]}{K_m^A}\right) \quad (2)$$

in which [A] = 0.2 mM, $K_{ia}$ and $K_m^A$ were substituted by the values found before. $K_I$ values found from $K_{is}$ and $K_{ii}$ are $0.23 \pm 0.1$ mM (S.D) and $0.34 \pm 0.17$ mM respectively.

ADP (1 mM) was not inhibitory under the same conditions.

*Chloral hydrate.* The enzyme could not use chloral hydrate as a substrate. However, when it was used as substrate analogue for acetaldehyde, the inhibition patterns were different from those reported by Eckfeldt & Yonetani (1976) on horse liver aldehyde dehydrogenase $F_1$ and by Vallari & Pietruszko (1981) on human liver aldehyde dehydrogenase $E_1$.

A primary reciprocal plot of $1/v$ against 1/[acetaldehyde] at fixed $NAD^+$ concentration showed a competitive pattern at low concentrations of chloral hydrate (i.e. only $K_{is}$ effect), but an intercept effect appeared at higher inhibitor concentrations (0.5 mM and 1.0 mM) (Fig. 5). The replots showed that there was a hyperbolic slope effect and a parabolic intercept effect.

The primary plots of $1/v$ versus $1/[NAD^+]$ at a fixed concentration of acetaldehyde (10 mM) were non-linear. This non-linearity increased with higher concentrations of chloral hydrate and became more significant at the region of lower $NAD^+$ concentrations.

*Thio-NAD*$^+$. Thio-$NAD^+$ was found to be an extremely poor alternative substrate to $NAD^+$ for this enzyme. The rate of increase of absorbance at 400 nm, due to the enzymic formation of thio-

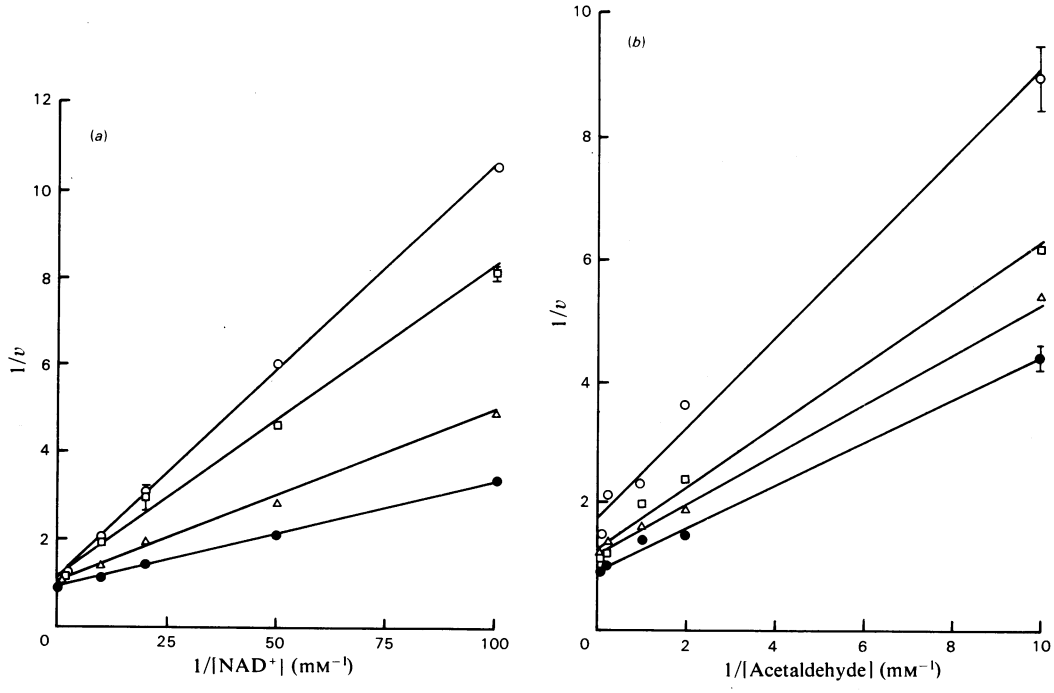



Fig. 4. *ADP-ribose as a substrate analogue of purified bovine lens aldehyde dehydrogenase*
For details see the text. (*a*) ADP-ribose inhibition of aldehyde dehydrogenase with respect to [$NAD^+$]: ●, 0 mM; △, 0.4 mM; □, 1 mM; ○, 1.5 mM. (*b*) ADP-ribose Inhibition of aldehyde dehydrogenase with respect to [acetaldehyde]: ●, 0 mM; △, 0.4 mM; □, 1 mM; ○, 1.5 mM.

NADH, was only 1/265 times that of the formation of NADH, when both were measured under the same assay conditions at pH 8.4.

Since the velocity of the aldehyde dehydrogenase reaction with thio-$NAD^+$ is not a significant contribution in the presence of $NAD^+$, thio-$NAD^+$ was used as a dead-end inhibitor. The results showed that thio-$NAD^+$ was competitive with respect to $NAD^+$ and non-competitive with respect to acetaldehyde. All the inhibition constants obtained were of lower magnitude than the $K_{ia}$ or $K_a$ obtained for $NAD^+$, indicating a relatively tighter binding between the thio-nucleotide and the enzyme. The inhibition constants obtained from replots were as follows: with respect to $NAD^+$, $K_{ii} = \infty$, $K_{is} = 0.75\,\mu M$; with respect to aldehyde, $K_{ii} = 14.5\,\mu M$, $K_{is} = 13.5\,\mu M$.

The true inhibition constant $K_I$ and $K_I''$ can be obtained by substituting in eqns. (1) and (2) shown above: $K_I = 0.60\,\mu M$; $K_I'' = 0.56\,\mu M$.

These constants are similar to that obtained from the $K_{is}$ (with respect to $NAD^+$), $0.75\,\mu M$.

Dead-end inhibitors ADP-ribose and chloral hydrate were used as substrate analogues in order to confirm the ordered mechanism. ADP-ribose was only competitive with respect to $NAD^+$ and non-competitive with respect to acetaldehyde. The $K_{i(slope)}$ and $K_{i(intercept)}$ obtained when acetaldehyde

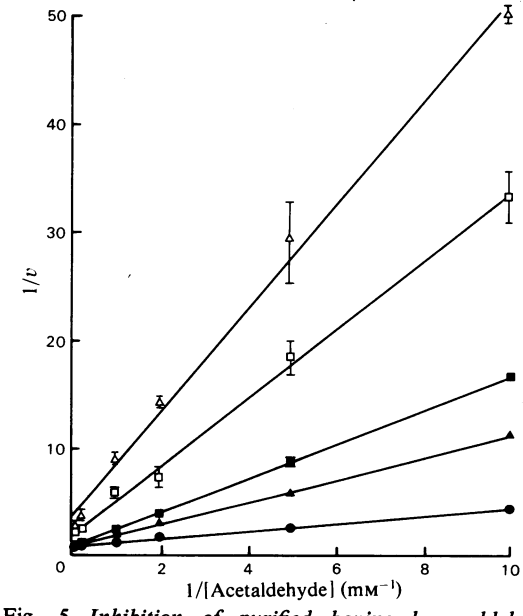



Fig. 5. *Inhibition of purified bovine lens aldehyde dehydrogenase by chloral hydrate*
For details see the text. Chloral hydrate inhibition of aldehyde dehydrogenase with respect to [acetaldehyde]: ●, 0 mM; ▲, 0.05 mM; ■, 0.1 mM; □, 0.5 mM; △, 1 mM.

Table 2. *Summary of inhibition constants for bovine lens aldehyde dehydrogenase*

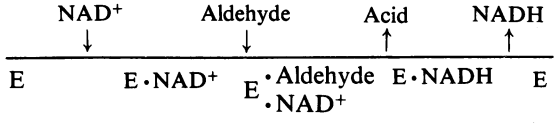
| Inhibitor (mM) | Varied substrate (mM) | Fixed substrate (mM) | Form of inhibition* | $K_{i(slope)}$ (mM) | | | $K_{i(intercept)}$ (mM) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | Measured† | Predicted‡ | Calculated§ | Measured† | Predicted | Calculated |
| Acetate (250) | $NAD^+$ (0.2) | Acetaldehyde (1, 10) | NI | — | — | — | — | — | — |
| Acetate (250) | Acetaldehyde (0.05–10) | $NAD^+$ (0.2) | NI | — | — | — | — | — | — |
| NADH (0–0.184) | $NAD^+$ (0.025–0.5) | Acetaldehyde (0.5, 5, 10) | C | 0.083 | $K_i$ | 0.083 | — | — | — |
| NADH (0–0.235) | Acetaldehyde (0.1–1) | $NAD^+$ (0.05) | NC | 0.23 | $K_i\left(1+\frac{[A]}{K_{ia}}\right)$ | 0.18 | 0.26 | $K_i\left(1+\frac{[A]}{K_a}\right)$ | 0.18 |
| ADP-ribose (0–1.5) | $NAD^+$ (0.01–1) | Acetaldehyde (10) | C | 0.484 | $K_i^{ADP\text{-}Rib}$ | 0.484 | — | — | — |
| ADP-ribose (0–1.5) | Acetaldehyde (0.1–10) | $NAD^+$ (0.2) | NC | 1.313 | $K_i^{ADP\text{-}Rib}\left(1+\frac{[A]}{K_{ia}}\right)$ | 2.74 | 1.911 | $K_i^{ADP\text{-}Rib}\left(1+\frac{[A]}{K_a}\right)$ | 2.74 |
| Chloral hydrate (0–0.1) | $NAD^+$ (0.1–1) | Acetaldehyde (1) | UC | — | — | — | 0.044 | $K_i^{Choral}\left(1+\frac{[B]}{K_b}\right)$ | 0.127 |
| Chloral hydrate (0–1) | Acetaldehyde (0.1–10) | $NAD^+$ (1) | C | 0.03 | $K_i^{Choral}$ | 0.03 | — | — | — |
| Thio-$NAD^+$ (0–0.0147) | $NAD^+$ (0.05–2) | Acetaldehyde (10) | C | 0.00075 | $K_i^{Thio\text{-}NAD^+}$ | 0.00075 | — | — | — |
| Thio-$NAD^+$ (0–0.0294) | Acetaldehyde (0.1–10) | $NAD^+$ (1) | NC | 0.0135 | $K_i^{Thio\text{-}NAD^+}\left(1+\frac{[A]}{K_{ia}}\right)$ | 0.01819 | 0.0145 | $K_i^{Thio\text{-}NAD^+}\left(1+\frac{[A]}{K_a}\right)$ | 0.01810 |

* NI, no inhibition; C, competitive inhibition; NC, non-competitive inhibition; UC, uncompetitive inhibition.
† $K_{i(slope)}$ and $K_{i(intercept)}$ were obtained from replots and linear-regression analysis.
‡ Predicted for compulsory-ordered mechanism.
§ Calculated from predicted; agreement between measured and calculated values support the mechanism.

was the varied substrate were similar to those predicted from an 'Ordered' mechanism. The measured and predicted results for an 'Ordered' mechanism is shown in Table 2. The close resemblance of the calculated and predicted results suggests not only that the mechanism is 'Ordered Sequential' but also that $NAD^+$ is the substrate that binds first.

The non-parallel and intersecting pattern of inhibition by ADP-ribose versus acetaldehyde in this study strongly favours $NAD^+$ to be the first binding substrate. However, when chloral hydrate was used as substrate analogue (with respect to the aldehyde) the expected uncompetitive inhibition by chloral hydrate versus $NAD^+$ was not apparent. The pattern seemed to be masked by a non-specific chloral hydrate effect on the enzyme protein, as was found for the human liver aldehyde dehydrogenase $E_1$ (Vallari & Pietrusko, 1981).

We therefore suggest that the dimeric bovine lens aldehyde dehydrogenase follows an ordered ternary-complex mechanism, as detailed in Scheme 1. The assignment of NADH as the final product is in accord with other dehydrogenases with ternary complexes.




Scheme 1.

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