Biochemical Pharmacology, Vol. 44, No. 12, pp. 2425–2426, 1992.
Printed in Great Britain.

0006-2952/92 $5.00 + 0.00

Activation of aldehyde dehydrogenase at physiological temperatures

(*Received* 1 *June* 1992; *accepted* 9 *September* 1992)

Abstract—The release of NADH from the enzyme·NADH complexes was rate limiting at 37°, for the oxidation of propionaldehyde by sheep liver cytosolic aldehyde dehydrogenase. Marked substrate activation was observed at this temperature as was activation by *p*-(chloromercuri)benzoate. Activation of enzymic activity may be of importance *in vivo*.

Acetaldehyde has many deleterious metabolic effects [1], and it is believed that alcoholic liver disease which afflicts approximately one-third of heavy drinkers is associated with elevated levels of this toxic metabolite. Cytosolic aldehyde dehydrogenase (ALDH) clearly has a role in metabolising acetaldehyde. For example, it is this class 1 enzyme which is highly susceptible to disulfiram, the compound used in therapy against alcoholism [2]. Also some Caucasians who have a mutation in class 1 ALDH show the flushing syndrome, typical of a reduced ability to metabolise acetaldehyde [3].

The sheep liver cytoplasmic enzyme oxidises propionaldehyde (a less volatile substrate than acetaldehyde, and hence more convenient to use) by an iso-ordered bi bi mechanism [4, 5] as given in scheme I where *E indicates a conformationally rearranged form of the enzyme. At 25° isomerisation of the binary enzyme·NADH complexes and NADH release (step 2) are rate limiting [5, 6] and like many other mammalian aldehyde dehydrogenases the steady-state rate of the sheep liver enzyme is activated by a number of effectors including aldehyde substrates [4], *p*-(chloromercuri)benzoate [7] and diethylstilbestrol [8].

$$E + NAD^+ \rightleftharpoons E \cdot NAD^+ + ald \rightleftharpoons E \cdot NAD^+ \cdot ald$$
$$\rightleftharpoons E \cdot NAD^+ \cdot acyl \rightarrow {}^*E \cdot NADH \quad (1)$$
$${}^*E \cdot NADH \rightleftharpoons E \cdot NADH \rightleftharpoons E + NADH \quad (2)$$

(Scheme I)

However, the importance of such activation effects *in vivo* has been called into question by studies at physiological temperatures first by Kitson and Crow [8], who observe no activation with diethylstilbestrol at 37° and more recently by Henehan and Tipton [9], who did not observe substrate activation with the human erythrocyte aldehyde dehydrogenase at this temperature.

With sheep liver cytosolic aldehyde dehydrogenase, we show, however, that the rate-limiting step in the mechanism was unchanged at 37°, and that activation effects were of importance at physiological temperatures.

Materials and Methods

NADH (grade III), NAD^+ (grade III) and *p*-(chloromercuri)benzoate were obtained from the Sigma Chemical Co. (St. Louis, MO, U.S.A.) and propionaldehyde solutions were prepared as described by Hill *et al.* [6].

Sheep liver cytosolic aldehyde dehydrogenase was prepared as described by Hill *et al.* [6]. The enzyme active site concentration was determined as described by Blackwell *et al.* [5] using a k_{cat} value of 0.56 sec^{-1} [5]. Steady-state enzyme assays were performed as described by Hill *et al.* [6] on a Hewlett–Packard 8452 diode array spectrophotometer. All nucleotide displacement experiments were performed on a HI-TECH Scientific stopped-flow spectrofluorimeter essentially as described by Blackwell *et al.* [5], and data were analysed by the methods described by Blackwell *et al.* [5]. Enzyme modification experiments with *p*-(chloromercuri)benzoate were performed as described by Motion *et al.* [7].

Results and Discussion

Figure 1A shows a double-reciprocal plot for the oxidation of propionaldehyde by the enzyme at 37° and pH 7.6. The plot is clearly triphasic at this temperature. First, there was a linear region at low aldehyde concentrations (up to 120 μM) for which a k_{cat} value of 0.93 sec^{-1} per active site was determined by extrapolation to infinite propionaldehyde concentration. Second, there was a definite region of substrate activation, as the propionaldehyde concentration was increased to 5 mM. Finally, at even higher propionaldehyde concentrations a marked substrate inhibition occurred (Fig. 1B). At 25° only the linear region and the region of substrate activation are observed [4]. The triphasic behaviour is similar to that

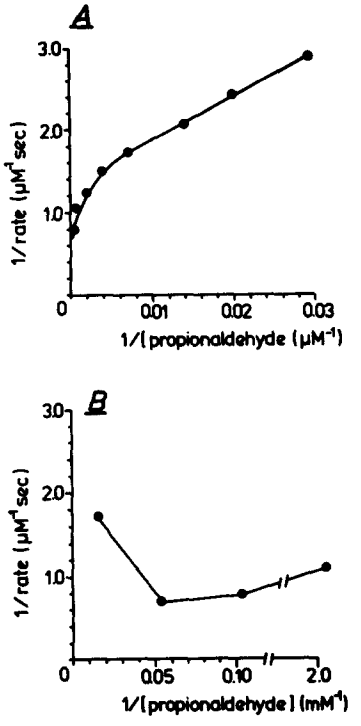

Fig. 1. Double-reciprocal plot for the steady-state oxidation of propionaldehyde by the enzyme at 37°. (A) Enzyme (0.8 μM) was assayed in 35 mM sodium phosphate buffer, pH 7.6, in the presence of 1 mM NAD^+ and various concentrations of propionaldehyde at 37°. Note the inhibited value at very high propionaldehyde concentrations (the point on the vertical axis). (B) Expansion of the plot shown in A at high propionaldehyde concentrations.

reported by Henehan and Tipton [9] for human erythrocyte ALDH at 25°. Thus, for the sheep liver cytoplasmic enzyme increasing the reaction temperature does not remove the substrate activation. The k_{cat} value of 0.93 sec^{-1} per active site at 37° represents an approximate 6-fold increase over the non-activated k_{cat} value of 0.16 sec^{-1} per active site determined at 25° [5]. Also determined from the linear section of the plot was a K_m value for propionaldehyde of 43.5 μM, which was substantially higher than the K_m value of approximately 2.0 μM determined at 25° [4].

At 37° and pH 7.6, the displacement of NADH from the enzyme was biphasic, with a rate constant for the fast process (λ_f) of 6.0 sec^{-1} and for the slow process (λ_s) of 1.1 sec^{-1}. For an iso-ordered bi bi mechanism such as that shown in scheme I provided NADH release is rate limiting [6] then:

$$k_{cat} = \lambda_f\lambda_s/(\lambda_f + \lambda_s).$$

The k_{cat} calculated in this way was 0.93 sec^{-1}, the same as that determined from extrapolation of the linear portion (low aldehyde concentrations) of the double-reciprocal plot (Fig. 1A). Clearly, the rate of release of NADH from the enzyme (step 2, scheme I) was rate limiting at 37°, as previously reported at 25°. For an NAD^+ concentration of 1 mM, the maximum extent of the substrate activation at 37° (Fig. 1A) was less than the 3-fold activation observed at 25° [4] being only 1.75-fold at a propionaldehyde concentration of approximately 5 mM. However, increasing the NAD^+ concentration above 1 mM, progressively removed the inhibitory effect of the highest aldehyde concentrations. In this way the substrate activation effect was eventually increased to a maximum of 2.8-fold at a propionaldehyde concentration of 48 and 6.8 mM NAD^+; a level of substrate activation which was similar to that observed at 25° [4]. The substrate inhibition, observed at very high propionaldehyde concentrations, may arise from the formation of a dead-end aldehyde·enzyme complex [9].

Modification of a single thiol group on the sheep liver cytosolic enzyme (2 μM) in the presence of NAD^+ (1 mM) by a 2-fold excess of *p*-(chloromercuri)benzoate resulted at 37° in a 2.5-fold activation of the rate of propionaldehyde oxidation, at low (100 μM) non-activating concentrations of the substrate, similar to the activation observed at 25° [7].

At physiological temperatures the mechanism of sheep liver cytoplasmic aldehyde dehydrogenase follows scheme I with isomerisation of the enzyme·NADH complex and release of NADH rate limiting. Activation of the steady-state rate by both high concentrations of propionaldehyde and stoichiometric amounts of *p*-(chloromercuri)benzoate was observed at these temperatures. The main effect of the higher temperature was a predictable increase in the values of the various rate constants and K_m values. This latter fact may account for previous failures to observe activation at elevated temperatures at the substrate concentrations reported in the literature.

It is unlikely that the *in vivo* concentrations of aldehydes in the liver could reach the levels which are found to activate the enzyme based on the results shown in Fig. 1. However, the enzyme may encounter aldehydes in the cytosol (for example aldehydes of biogenic origin [10]) which cause effects similar to those seen with propionaldehyde but at much lower concentrations. Furthermore, thiol reagents, such as *p*-(chloromercuri)benzoate and dithiodipyridine may have physiological counterparts which cause activation *in vivo*. The *in vivo* activity of the enzyme could be quite different from that which the *in vitro* k_{cat} values determined from non-activated kinetics would indicate.

Activation of the enzymic activity by diverse compounds occurs at 37° *in vitro* by a common mechanism (activation of NADH release) and such activation may also be of importance *in vivo*.

Acknowledgements—We gratefully acknowledge the receipt of a post-doctoral fellowship (J.P.H.) and a Massey University Summer Scholarship (R.L.K.).

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