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Effects of acetaldehyde upon catalysis by human erythrocyte transketolase*

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Ethanol use appears to lower blood and tissue thiamin concentrations when used chronically in humans [1–4] or rats [5, 6], or following a single dose in rabbits [7]. The mechanism by which ethanol promotes thiamin loss from tissues is unknown but could include a diminished affinity of enzymes for the cofactor thiamin diphosphate (pyrophosphate) (TDP), a mechanism consistent with the observation that a single intoxicating dose of ethanol depresses erythrocyte transketolase activity and increases its activation by TDP added *in vitro*. These effects on transketolase are maximal 6 hr before the maximal depression of the whole blood thiamin concentration [7]. A reduced erythrocyte transketolase activity and increased activation *in vitro* by TDP can be observed also when thiamin deficiency occurs in the absence of ethanol use [8–10], but is not the subject of this report. In the case of continued ethanol use [1, 5, 11], the activation of transketolase *in vitro* by TDP can be reduced to well below the value expected for the same level of thiamin deficiency in the absence of ethanol use.

Ethanol itself must reach very high concentrations to reduce the activity of transketolase. A 50% reduction of rat brain or liver transketolase *in vitro* requires approximately 5 M or 3 M ethanol respectively [5], supralethal concentrations for animals. Therefore, it is likely that any effect of ethanol, given *in vivo*, upon tissue transketolase is mediated by the direct metabolite acetaldehyde which effects a 50% reduction of transketolase activity in crude rat brain or liver homogenates at 100–200 mM [5].

We have tested the mechanism postulated above by measurement of the effect of acetaldehyde upon catalysis by purified human erythrocyte transketolase.

Methods

Materials. Acetaldehyde (analytical grade) was obtained from Merck Chemicals, Darmstadt, West Germany; glycerol 3-phosphate dehydrogenase and triose phosphate isomerase were a mixture from Boehringer Mannheim, Penzberg, West Germany; all other biochemicals were from the Sigma Chemical Co., St Louis, MO, U.S.A.

Transketolase preparation and assays. Human erythrocyte transketolase was purified 5000-fold to a specific activity of 2 units/mg, resolved of TDP by the method described previously [12], and used within 5 hr. Recovery of enzyme activity after reconstitution with TDP was 22–78% in three preparations, and the residual activity without addition of TDP ranged from 0 to 15% of that with TDP. Dilutions of acetaldehyde were kept ice-cold prior to incubation with resolved enzyme preparations, TDP and Mg^{2+} at 30°. The coupled spectrophotometric assay [13] was as modified previously [12], with 0.8 mM D-xylulose 5-phosphate, 10 mM D-ribose-5-phosphate and final TDP concentrations in the range of 10 to 1000 nM.

Analysis of data. To estimate the binding of TDP to resolved transketolase, the Michaelis–Menten equation was fitted to the kinetic data; values of apparent K_m and V_{max} were calculated by fitting proportionally-weighted, non-linear, least-squares regressions to the hyperbolic form of the equation [14]. The same program was used to analyze several sets of data, each with a different V_{max} but having a common K_m value. The fit to the common K_m value was compared to the fit to independent K_m values by use of the F statistic [15].

Results and discussion

Acetaldehyde decreased the activity of transketolase more when it reacted with apoenzyme than when it reacted with holoenzyme. In either case, the activity was reduced but not abolished by 50 mM acetaldehyde which was therefore chosen to test its effect upon the apparent K_m value for the interaction of TDP and transketolase.

In the absence of acetaldehyde, the apparent K_m value for TDP and human erythrocyte transketolase was 17.2 ± 1.0 nM (mean of three resolved preparations), comparable to the value obtained previously for resolved, purified transketolase preincubated with TDP [12]. Incubation of the resolved transketolase preparation with 50 mM acetaldehyde prior to addition of TDP resulted in a marked reduction of V_{max}; for example, in one preparation from 23.8 to 4.9 nmol/min/ml (Fig. 1). A similar reduction was observed in two other preparations. However, the acetaldehyde-treated enzyme preparation exhibited very little change in apparent K_m value for TDP, the value being 21.9 ± 1.4 nM (mean of three resolved preparations; 21.3 nM from the data illustrated in Fig. 1). When the resolved enzyme preparation was reconstituted with various concentrations of TDP for 60 min followed by 50 mM acetaldehyde for 15 min prior to assay, the V_{max} decreased only a little (e.g. from 23.8 to 20.1 nmol/min/ml in Fig. 1) and the apparent K_m value for TDP was 22.5 ± 1.1 nM (mean of three resolved preparations). To test whether acetaldehyde altered the K_m value for TDP, all nine sets

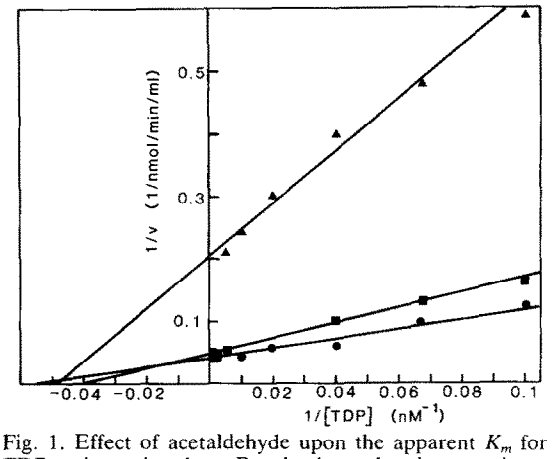

Fig. 1. Effect of acetaldehyde upon the apparent K_m for TDP and transketolase. Resolved transketolase was incubated at 30° with acetaldehyde for 15 min followed by TDP and Mg^{2+} for 60 min and then the activity was assayed (▲); or first with TDP and Mg^{2+} for 60 min followed by acetaldehyde for 15 min and then the activity was assayed (■); or with TDP and Mg^{2+} for 60 min and then water in place of acetaldehyde after which the activity was assayed (●). Final concentrations were: acetaldehyde, 50 mM; TDP, 10–1000 nM; and Mg^{2+}, 1.4 mM. Activities were determined at 37°. The lines are drawn using the K_m and V_{max} values determined by fitting the hyperbolic form of the Michaelis–Menten equation to each set of data independently (see Methods).

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of data (three resolved enzyme preparations, each tested under the three conditions above, 51 experimental measurements) were analyzed by fitting to them nine separate lines, each having an independent V_{max} but constrained to have a common K_m value which proved to be 19.9 ± 0.8 nM. This constrained fit with a single K_m was compared to the fit with each set of data having an independent K_m by means of an F test [15]. This provided no evidence for any significant lack of fit ($0.1 < P < 0.2$), indicating that acetaldehyde treatment does not affect the K_m of transketolase for TDP.

Following ethanol use *in vivo*, the activation of erythrocyte transketolase by additional TDP added *in vitro* cannot be due to an acetaldehyde-induced reduction of TDP binding, but could follow loss of thiamin from the erythrocyte and generation of apotransketolase by whatever mechanism. The fact that the apparent concentration of apotransketolase, measured by activation by TDP added *in vitro*, is less in the presence of ethanol use than in isolated thiamin deficiency without ethanol use [5], suggests that ethanol use does affect transketolase. Indeed, we have found that acetaldehyde does not decrease the maximum velocity of transketolase (Fig. 1), and that that decrease is much greater when acetaldehyde is allowed to react with apotransketolase than with holotransketolase (Fig. 1). Therefore, adequate thiamin nutrition should afford some protection against this effect of acetaldehyde, although adequate thiamin nutrition is difficult to achieve when ethanol is consumed regularly [3, 16].

Acetaldehyde is not a substrate for transketolase [17], but it could be considered an analogue of the aldose phosphate acceptor substrate. To test whether acetaldehyde competes at the site for binding of that substrate, the K_m value for ribose 5-phosphate was measured using unresolved holotransketolase, and was 1.06 ± 0.04, 1.18 ± 0.05, and 1.13 ± 0.08 mM at respective acetaldehyde concentrations of 0, 50, and 100 mM. Again the total data could be fitted to lines constrained to have a common K_m value which proved to be 1.12 ± 0.03 mM, and the constrained fit was not worse than the fit of a series of lines having independent K_m values, indicating that acetaldehyde does not affect the K_m value of transketolase for ribose 5-phosphate. Therefore, we must assume that acetaldehyde simply inactivates transketolase by reacting with some unspecified group or groups important to its catalytic activity, and that in the apoenzyme these groups are more reactive or more exposed than in the holoenzyme.

The effect of acetaldehyde upon the activity of purified human erythrocyte transketolase is greater for the apoenzyme than for the holoenzyme, but the concentration used in these experiments is unlikely to be achieved in whole blood *in vivo*. Additionally, many other proteins will compete with transketolase for reaction with acetaldehyde. Nevertheless, it is conceivable that persistent low concentrations of acetaldehyde within the erythrocyte could have a cumulative effect upon apotransketolase over a period of time, and that the concentration of acetaldehyde within the liver cell could be sufficient to affect apotransketolase readily following an intoxicating dose of ethanol, particularly in individuals whose acetaldehyde dehydrogenase is entirely the variant having a low affinity for acetaldehyde [18].

Transketolase is the only TDP-dependent enzyme in human erythrocytes, and erythrocyte TDP is the principal form in which thiamin is found in whole blood [19]. Therefore, any account of the loss of thiamin from blood following single [7] or repeated [1, 6] doses of ethanol should consider the binding of TDP to transketolase. Acetaldehyde inactivates transketolase without affecting the binding of TDP to the residual active enzyme. However, it may be that the inactive enzyme has little or no affinity for TDP and the loss of thiamin from blood may be directly attributable to transketolase inactivation. Nevertheless, we cannot at this stage rule out other mechanisms for the ethanol-stimulated loss of thiamin from tissues *in vivo*, such as a decrease in the thiamin concentration gradient between plasma and intracellular compartments, perhaps by an effect upon the phosphorylation or dephosphorylation of thiamin.

In summary, acetaldehyde did not affect the K_m values for the interactions of TDP and ribose 5-phosphate with human erythrocyte transketolase, but did inactivate the enzyme so as to decrease the velocity of the holoenzyme 1.3-fold and that of the apoenzyme 4-fold.

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Department of Biochemistry
University of Queensland
St. Lucia
Queensland 4067; and the
Princess Alexandra Hospital
Wooloongabba
Queensland 4102, Australia

T. M. SUNETHRA ATUKORALA*
RONALD G. DUGGLEBY
PETER F. NIXON†

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* Present address: Department of Biochemistry, Faculty of Medicine, University of Colombo, Colombo 8, Sri Lanka.

† Requests for reprints should be addressed to P. F. Nixon at the University of Queensland.