INACTIVATION OF PHOSPHOENOLPYRUVATE CARBOXYKINASE BY ACETALDEHYDE

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SUMMARY: Preincubation with acetaldehyde at 37°C inactivates rat liver phosphoenolpyruvate carboxykinase. The inactivation is dependent upon the acetaldehyde concentration and the pH and duration of preincubation, and is prevented but not reversed by glutathione. The binding of the substrate ITP appears to be affected in the inactivation process. This effect of acetaldehyde might contribute to inhibition of gluconeogenesis resulting from ethanol metabolism.

Although PEP[2] carboxykinase (EC 4.1.1.32) is recognized as an important rate-limiting enzyme in gluconeogenesis, it has been difficult to establish its precise regulatory function because, unlike other "control" enzymes, few effectors of physiological importance have yet been discovered. Many inhibition studies have therefore used non-physiological compounds such as quinolinate and hydrazine. These inhibitors cause characteristic decreases in the levels of PEP, 2-phosphoglycerate and 3-phosphoglycerate in perfused rat liver (1,2). While similar decreases have been reported in rat liver perfused with ethanol (3) and following ethanol administration *in vivo* (4), it has never been suggested that PEP carboxykinase activity might be affected by ethanol or a product of its metabolism, despite the fact that gluconeogenesis is known to be inhibited by ethanol (5).

The profound effects of acetaldehyde, the first oxidation

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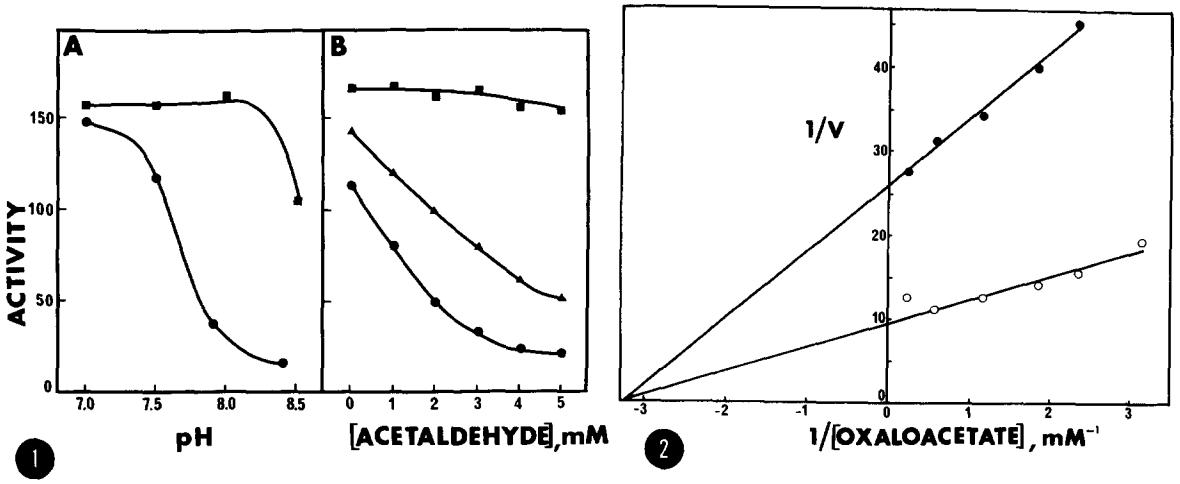
[2]Abbreviation: PEP, phosphoenolpyruvate.

product of ethanol, on the formation of gluconeogenic substrates by liver mitochondria have been demonstrated in this laboratory (4,6) and others (7). Experiments reported in this communication show that incubation of rat liver cytosol fraction with acetaldehyde results in a loss of activity of the rate-limiting gluconeogenic enzyme PEP carboxykinase.

METHODS: Livers from 24 h-starved male rats (Wistar, Castle Hill) were homogenized in 4 volumes of 0.25 M sucrose containing 10 mM Na HEPES, pH7.4 (4°C). After centrifugation at 120,000 g for 1 h, the supernatant was dialyzed extensively against 50 mM tris-HCl, pH8.2 (4°C) before being preincubated and assayed for PEP carboxykinase activity. Preincubations were performed in small stoppered tubes with essentially no air space above the liquid, to minimize the evaporation of acetaldehyde. A typical preincubation mixture contained 0.5 ml of dialyzed supernatant (6.9 mg protein) and 0.05 ml of 55 mM acetaldehyde (5 mM final concentration). PEP carboxykinase activity was determined on 0.5 ml aliquots at 37°C using the incubation conditions of Nordlie and Lardy (8), the PEP formed being estimated by the method of Zorzoli et al. (9). Acetaldehyde (British Drug Houses) was redistilled before use.

RESULTS: The acetaldehyde concentrations studied were comparable to those measured in blood following ethanol administration (10). While such levels had no direct inhibitory effect on PEP carboxykinase, preincubation of dialyzed liver supernatant in the presence of acetaldehyde caused a decrease in enzyme activity which was dependent upon the preincubation pH. Fig. 1A shows that 5 mM acetaldehyde caused 26% inactivation at pH7.5, while at pH8.0, approximately 80% (range: 70-90%) of activity was lost. Variations in the acetaldehyde concentration and duration of preincubation also affected the degree of inactivation (Fig. 1B). The enzyme was stable to acetaldehyde at 2°C, becoming increasingly sensitive with higher incubation temperature. Several other compounds associated with ethanol metabolism were tested under identical conditions to acetaldehyde. Neither ethanol itself (50 mM) nor acetate, citrate or 3-hydroxybutyrate (sodium salts, 10 mM) had any effect on enzyme activity.



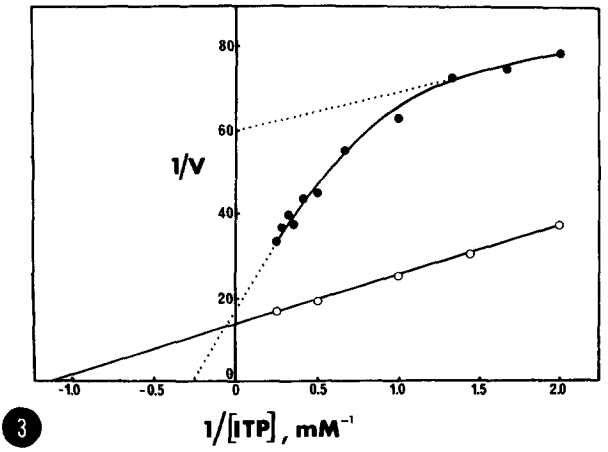


Fig. 1A. The effect of preincubation pH on the activity of rat liver PEP carboxykinase treated for 1 h in the absence (■) or presence (●) of 5 mM acetaldehyde. The buffer was 50 mM tris-HCl.
1B. The effect of acetaldehyde concentration on the activity of rat liver PEP carboxykinase treated for 0 min (■), 30 min (▲) or 60 min (●) at 37°C in 50 mM tris-HCl, pH8.0. Activity is expressed as nmoles PEP formed/6 min/mg protein.

Fig. 2. Double reciprocal plot showing the effect of oxaloacetate concentration on rat liver PEP carboxykinase activity before (o) and after (●) preincubation for 30 min at pH8.0 and 37°C with 5 mM acetaldehyde. The concentration of ITP was 2 mM. 1/V is expressed as min.(μmoles PEP)$^{-1}$.

Fig. 3. Double reciprocal plot showing the effect of ITP concentration on rat liver PEP carboxykinase activity before (o) and after (●) preincubation for 30 min at pH8.0 and 37°C with 5 mM acetaldehyde. For each point the Mg^{2+} concentration was 3.0 times that of ITP. The oxaloacetate concentration was 5 mM. 1/V is expressed as min.(μmoles PEP)$^{-1}$.

TABLE I: Protection by ITP and Glutathione against Inactivation of Phosphoenolpyruvate Carboxykinase by Acetaldehyde.

Addition	Concentration (mM)	Percent Inactivation
None	-	80
ITP	0.02	69
	0.06	57
	0.20	16
	0.30	6
Glutathione	1.0	51
	2.0	26
	3.0	20
	5.0	24

Preincubations were for 1 h at 37°C and pH8.0 in the presence of 5 mM acetaldehyde.

As previously shown by Marco and Sols (11), the K_m of the crude enzyme for oxaloacetate was determined to be 0.31 mM, taking into account the spontaneous decomposition of oxaloacetate under the conditions of the experiment. The same value was obtained for acetaldehyde-inactivated enzyme (Fig. 2), with only the V_{max} being altered. Inactivation had a more complicated effect on the K_m for ITP. Fig. 3 shows that for the native enzyme a value of 0.9 mM was observed. The inactivated enzyme gave a curved double reciprocal plot, so that at high ITP concentrations, the K_m for ITP appeared to be higher than for the native enzyme, whereas at lower concentrations both the K_m and the V_{max} were apparently decreased. The nature of the enzyme assay prevented the testing of still lower ITP concentrations.

The presence of ITP together with acetaldehyde during pre-

incubation afforded some protection against inactivation, as Table I shows. Although some protection was observed at ITP concentrations below 0.1 mM, results were quite variable, with large differences in the degree of protection observed in different experiments. Oxaloacetate gave little or no protection up to a concentration of 2 mM.

Glutathione in the preincubation medium also partially protected PEP carboxykinase against acetaldehyde inactivation. In the experiment described in Table I, glutathione reduced the effect from 80% inactivation to about 20%. However, dialysis of acetaldehyde-treated enzyme at 4°C for 6 h against a large volume of 50 mM tris-HCl (pH8.0 at 37°C) failed to restore activity, whether or not 5 mM glutathione was present in the dialysis buffer.

DISCUSSION: The time-dependence of the inactivation of PEP carboxykinase by acetaldehyde suggests that a slow conformational change in the enzyme might be required to expose the acetaldehyde-sensitive function. Grisolia et al. (12) have recently reported similar time-dependent inactivation of alcohol and retinol dehydrogenases by acetaldehyde, though considerably higher concentrations were required. Acetaldehyde reacts readily with thiol groups to form thiohemiacetals (13), a reaction thought to be responsible for the decrease in brain cholinesterase activity observed following ethanol oxidation (14). Acetaldehyde might react similarly with PEP carboxykinase thiol groups. The enzyme is known to be inactivated by thiol reagents (15) and it has been suggested that loss of activity of the enzyme from tryptophan-treated rats might involve the oxidation of thiol groups (16). The protective effect of glutathione against acetaldehyde supports the involvement of a thiol group.

Since the K_m of the enzyme for oxaloacetate is not altered by acetaldehyde inactivation, and the presence of oxaloacetate during preincubation does not protect the enzyme against inactivation, it is unlikely that oxaloacetate binding is affected by acetaldehyde. Conversely, the protection afforded by ITP, and the unusual effect on the K_m for ITP resulting from inactivation, both indicate that ITP binding is affected during the process.

Inhibition of gluconeogenesis by ethanol is generally attributed to an effect on the cell NAD-NADH redox state (5,17), which becomes more reduced in both cytosol and mitochondria following ethanol administration in vivo (18). However, doubts have been expressed that this is the only factor involved (19, 20). The results presented above suggest that diminished activity of PEP carboxykinase, mediated by acetaldehyde, could play a role in this phenomenon.

REFERENCES:

1. Veneziale, C.M., Walter, P., Kneer, N., and Lardy, H.A. (1967) Biochemistry, 6, 2129-2138.
2. Ray, P.D., Hanson, R.L., and Lardy, H.A. (1970) J. Biol. Chem., 245, 690-696.
3. Williamson, J.R., Scholz, R., Browning, E.T., Thurman, R.G., and Fukami, M.H. (1969) J. Biol. Chem., 244, 5044-5054.
4. Baxter, R.C. (1971) Ph.D. Thesis, University of Sydney.
5. Kreisberg, R.A. (1967) Diabetes, 16, 784-790.
6. Baxter, R.C., and Hensley, W.J. (1971) Proc. Aust. Biochem. Soc., 4, 21.
7. Cederbaum, A.I., Lieber, C.S., and Rubin, E. (1974) Arch. Biochem. Biophys., 161, 26-39.
8. Nordlie, R.C., and Lardy, H.A. (1963) J. Biol. Chem., 238, 2259-2263.
9. Zorzoli, A., Turkenkopf, I.J., and Mueller, V.L. (1969) Biochem. J., 111, 181-185.
10. Batt, R.D. (1974) N.Z. Med. J., 79, 556-560.
11. Marco, R., and Sols, A. (1970) in Metabolic Regulation and Enzyme Action (FEBS Symposium 1969), pp. 63-76 (Sols, A., and Grisolia, S., eds.) Academic Press, New York.
12. Grisolia, S., Guerri, C., and Godfrey, W. (1975) Biochem. Biophys. Res. Commun., 1112-1117.
13. Lienhard, G.E., and Jencks, W.P. (1966) J. Amer. Chem. Soc., 88, 3982-3995.
14. Martin, G.J., Moss, J.N., Smyth, R.N., and Beck, H. (1966) Life Sci., 5, 2357-2362.
15. Chang, H.C., and Lane, M.D. (1966) J. Biol. Chem., 241, 2413-2420.

16. Foster, D.O., Lardy, H.A., Ray, P.D., and Johnston, J.B. (1967) Biochemistry, 6, 2120-2128.
17. Madison, L.L., Lochner, A., and Wulff, J. (1967) Diabetes, 16, 252-258.
18. Baxter, R.C., and Hensley, W.J. (1969) Biochem. Pharmacol., 18, 233-236.
19. Cahill, G.F. (1964) Adv. Enz. Reg., 2, 137-148.
20. Zakim, D. (1968) Proc. Soc. Exp. Biol. Med., 129, 393-397.